Studies of Fulvic and Humic Acids
1. Determination of the Steady States for Slow Processes in 0.1 M NaCl Aqueous Solution at 25 °C

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Humic substances in natural waters are dynamic systems. High-precision EMF methods were used to measure pH as a function of time and total concentration of humic substances after sequential additions of NaOH. After each addition of base, the time derivative of pH asymptotically approached a constant value, a steady state. We observed phenomena typical for dynamical systems, such as oscillations.

The steady-state rates of slow, pH dependent reactions were measured for humic acids extracted from soil for $2.5 < \text{pH} < 8.5$ and total concentrations of humic substances within 6 mg DOC dm^{-3} and 116 mg DOC dm^{-3} and for fulvic acids from a lake water for $2.5 < \text{pH} < 8.5$ with 28 mg DOC dm^{-3} (original sample) and 7 mg DOC dm^{-3} (diluted sample). Simple models were suggested to explain the observations.

Humic substances play an important role in natural waters and in soil.[1-3] They interact with many materials such as clays and metal(hydr)oxides.[4,5] Their properties have been summarised and discussed by Perdue and Gjessing[6] and also by Frimmel and Christman.[7] The solution chemistry of humic and fulvic acids has been described by equilibrium models, reviewed by Tipping and Hurley.[8] Sjöberg's group in Umeå used classical coordination models for metal–humate complex formation in a bog water.[9] More sophisticated models have been developed by Allard's group in Linköping,[10] by de Wit et al.[11] and by Buffle et al.[12,13] Humates are observed to be very heterogeneous, and they are charged polymers.[14,15] Models for handling the polyelectrolytic behaviour were developed by de Wit et al.[11] and Marinsky[16] and Ephraim.[10] Morel et al.[17,18] suggested an oligoelectrolytic model which can handle both different molecular sizes and polyelectrolytic behaviour in connection with acid–base reactions and metal complexation. Österberg and Mortensen[19,20] showed that neutron scattering from humic acids solutions obeyed a power-law, indicating a fractal structure. Their analysis yielded a fractal dimension of 2.3 and the average diameters of two observed clusters 1720 and 700 Å, respectively. This information can be used to describe the structure of the polymers of humus.[21,22]

Application of models on humates in natural water

Natural aquatic systems are dynamic. Therefore, it is necessary to study the properties of organic material as a function of time. Taugbol et al.[23] have extended Birkenes[24] hydrogeochemical model to include interactions of metal ions and protons with dissolved organic compounds and applied it to data from a catchment area at Svartberget in Northern Sweden. One problem with such models is the lack of information on the reactions of the dissolved organic compounds. So far, no experimental kinetic study of the fundamental processes of humates in aquatic system has been reported, at least to our knowledge.[25-27]

We found that humic acids could be modelled as apparent quasi equilibria for pH < 6.5, but more precise experiments revealed slow relaxations to a state with a constant time derivative of pH (cf. Fig. 1). It is not possible to model high-precision acid–base titrations of humic substances without considering the changes on time-scales of minutes and hours. It can take several hours for a perturbed system of humic acids in water to reach a new approximately constant pH value. The slow changes could be due to alterations of the conformation or aggregation of humates or precipitation/dissolution reactions. We will refer to these states as steady states.
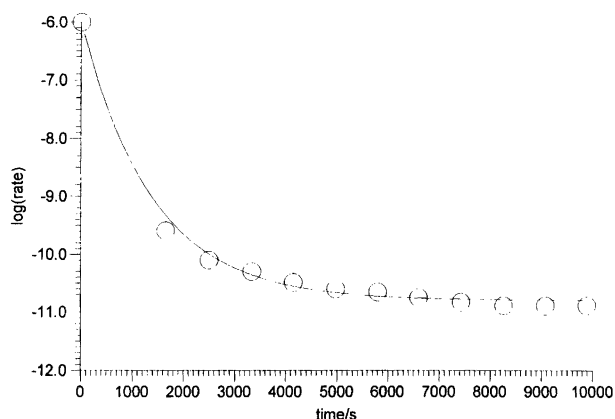
Fig. 1. Log(rate) as a function of the time. The rate decreases to a steady-state value after approximately 3 h. The solid curve corresponds to the relaxation time of 23 min.
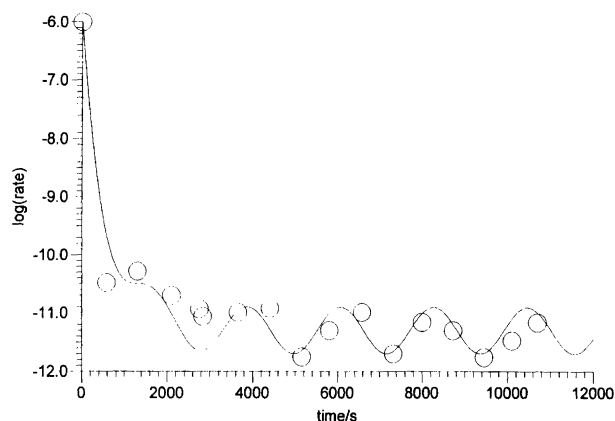

Fig. 2. Log(rate) as a function of the time. The solid curve corresponds to the relaxation time of 11 min and an oscillation with the period of 37 min.

For pH < 6.5 the changes were about 0.2 mV h^{-1} but for pH > 6.5 the changes were on the order of magnitude 10 mV h^{-1}. Slow reactions of humates have been identified earlier as hysterisis of acid base titrations.[28] In the present study we have used 0.100 M NaCl as an ionic medium to get high enough precision in the measurements. We have started to collect data at ionic strengths down to 2 mM, which is, however, much more difficult.

Two pK_a values, 4.0 and 5.4, were sufficient to explain our data. However, like Driscoll,[29] we detected some extra acid–base capacity at pH ≈ 2.5 and also at pH > 6.5. In our case, these effects could possibly be due to a release or uptake of protons by slow reactions. For field data apparent pK_a values may be appropriate.

Since humic substances seem to be dynamic systems, we are not surprised to observe phenomena like oscillations.[30–32] Certainly, that could explain some of the difficulties we had with our intended equilibrium measurements in the beginning of this project.

Objectives of this study

The main objective of the present study is to determine the steady-state rates for slow, pH dependent reactions of humic substances after additions of sodium hydroxide. We have chosen two different kinds of samples, one extracted from soil with a high concentration of humic acids and one lake water with a high concentration of fulvic acids. The idea is to compare the reactions of humic substances of different molar mass. We added portions of a sodium hydroxide solution to the humate solution and observed the changes of pH for 3–4 h. In natural waters, slow transformations of organic substances may be caused by changes of the temperature and by mixing of water with different concentrations of DOC and alkalinity. Our experiments do not consider microbiological activity, photochemical reactions[33] and redox reactions[34] which are all important in natural waters.

Symbols, units and activity scale

All symbols describe physical quantities. Concentrations are expressed in mol dm^{-3}. The concentrations of H^+ will be written as h pH $= -\log(\gamma_H[H^+])$, where γ_H is the activity coefficient. D will be used for an aggregate of humus. The fractions of D will be referred to as D1, D2, D3 etc. The activity coefficients for the low molecular species are assumed to be constant. This is justified here, since the sodium chloride is at least in 50-fold excess. Thus, in this study we assumed that $\gamma = 1$. Therefore, the constants reported are valid in 0.100 M NaCl. The concepts fulvic and humic are operationally defined.[13] We refer to smaller molecular sizes with the former and to larger molecules with the latter word. The rates have the units (mol dm^{-3}) s^{-1}. The standard deviation will be denoted by σ.

Experimental

Chemicals and analyses. Strong acids and bases were prepared and analysed as described by Li *et al.*[35]

We have used humic substances from two sources:

1. We have taken samples of water directly from Lake Snuggan 15 km North of Stockholm, Sweden. In one experiment we diluted the water four times. The water from Lake Snuggan contains a mixture of fulvic and humic acids with the former in excess. The water contained 0.12 mM Ca^{2+}, 0.02 mM K^+, 0.05 mM Mg^{2+}, 0.17 mM Na^+, 0.11 mM SO_4^{2-} and 0.2 mM Cl^-, as measured by ICP.

2. We prepared solutions with humus extracted from the soil at Kungsängen, 5 km south of Uppsala, Sweden. This humic acid sample is a generous gift from the late professor Lindqvist. The methods of preparation and analyses are described by Lindqvist.[36] These solutions are first dialyzed against 10 mM EDTA solution for a few hours and then against distilled water for 48 h. The cutoff of the dialysis tubes was 8000 Da. In this way we tried to prepare metal ion free solutions of the high

molecular humic acids. About half of the iron and aluminium could be removed in this way, i.e. 1–10 μM.

Measurements. For each experiment 2 mg soil humus was accurately weighed and dissolved in 5 ml distilled water. After dialysis the humus solution was diluted to 10, 50 or 200 ml in calibrated glassware. The lake water was filtrated through a 0.45 μm filter. One lake sample was passed through a column with proton-saturated cation exchanger and one lake sample was diluted to 1/4 of the original concentration. Sodium chloride and hydrogen chloride were then added to both types of humates to make a solution with 0.1000 M NaCl and 0.5–2.0 mM HCl. The experiments were made as titrations adding 0.1000 M NaOH solution from a buret to the solution containing humic substances. The titration vessel was either coated with paraffin or it was made of Teflon. The experiments were carried out in the dark under $N_2(g)$ atmosphere at 25.00 ± 0.02 °C. The nitrogen gas was purified by bubbling through three flasks containing Cr^{2+}, 10% NaOH and 10% H_2SO_4, respectively. The nitrogen gas was finally saturated with water vapour by bubbling through a 0.100 M NaCl solution.

We measured EMF of the following cell:

$$-Ag(s), AgCl(s)| \,|studied\ solution|ME + \qquad (1)$$
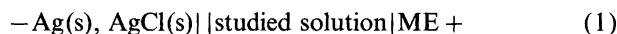
ME is either a glass electrode or a hydrogen electrode. The hydrogen electrodes were only used for calibration purpose. The solution in the salt bridge and in the studied solution both contained 0.1 M NaCl.

EMF data were automatically recorded by a small laboratory computer and then transferred to a personal computer. We used a digital voltmeter no. 3456a from Hewlett Packard. The EMF values were stable within ± 3 μV for several hours. Thus, the precision of the data is very high. However, the absolute accuracy of the EMF measurements is probably only about 0.1 mV. We used glass electrodes and reference electrodes from Metrohm. The electrode system was calibrated with strong acid and also compared with standard hydrogen electrodes. The pH was calculated from:

$$E = E^0 + 59.155 \log h + jh \qquad (2)$$

with pH $= -\log(h\gamma)$. The activity coefficient γ is a constant within our error limits and set equal to unity in 0.100 M NaCl solution. The liquid junction potential is approximated by the function jh, where j is a constant. We found typically $E^0 = 362.5$ mV and $j = -462$ mV M^{-1}. We doubled the rate of the magnetic stirrer but this did not change the EMF value. Therefore, we conclude that diffusion did not influence the measurements. Altogether 12 000 data points were measured and treated.

Data treatment and results

We are not sure which reactions cause the slow changes of pH, but probably the state of aggregation and the
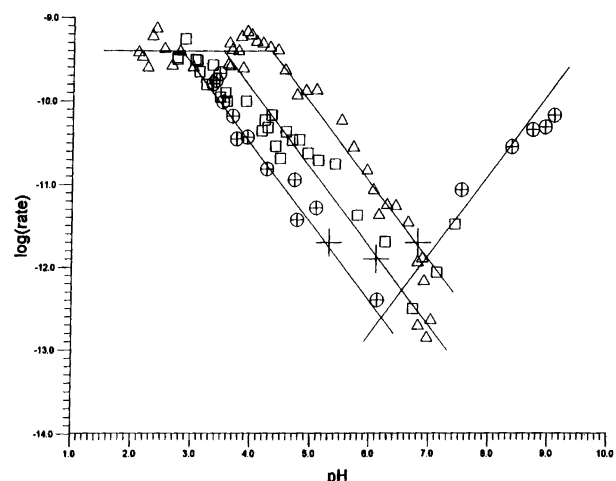

Fig. 3. Log(rate) as a function of pH for different DOC. The solid lines represent the calculated model. The model with integer slopes is shown [cf. eqns. (6)–(12)]. The crosses indicate changes of the sign of the reaction rate. Protons are consumed for pH < pH marked with a cross and produced at higher pH values. $\oplus$, 6; $\square$, 23; $\triangle$, 116 mg DOC dm^{-3}.

conformation are important. The apparent reaction rates were calculated from the changes of the proton concentrations with time. These changes can be calculated from eqn. (3), which was obtained by derivation of eqn. (2), neglecting the time derivative of the liquid junction potential:

$$rate = dh/dt = (dE/dt)h/25.68 \qquad (3)$$

For pH $\geqslant 7$ we assumed OH^- to be a reactant and calculated the reaction rates from eqn. (5):

$$rate = d[OH^-]/dt = (dh/dt)kw/h^2$$

with dh/dt from eqn. (3) $\qquad (4)$

where we have used with $\log kw = 13.78$.[37] Since we have collected the data points at very small time intervals, we can use the quotient below to approximate the derivative:

$$dE/dt \approx (\Delta E/\Delta t) \qquad (5)$$

The log(rate) values for the steady states were plotted against pH as shown in Figs. 3 and 4.

Interpretation of the data. Besides the presented data, we also collected some data by adding hydrochloric acid to humate solutions. Those data indicated reversible reactions for pH < 6.5, but the precision was still not adequate to confirm or falsify the reversibility. To explain the present data, we suggest both reversible and irreversible reactions. In a few solutions we observed more complex behaviour as illustrated in Fig. 2. Such data were exluded from the present calculations. The steady-state rates were obtained by a simple curve-fitting methods, cf. Fig. 1. The rate constants were obtained by
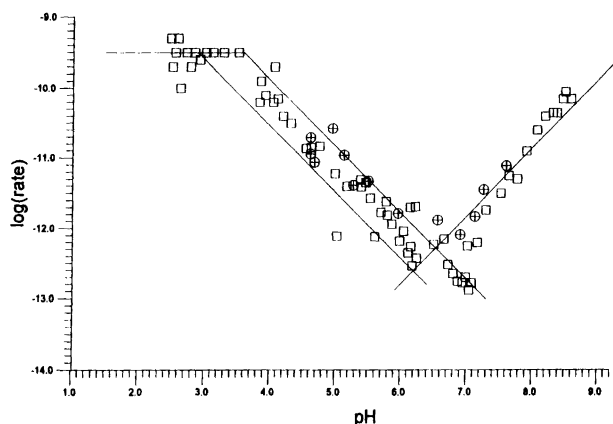
Fig. 4. Log(rate) as a function of pH for different DOC. The solid lines represent the model. The model with integer slopes is shown. [cf. eqns. (6)–(12)]. $\oplus$, 7; $\square$, 28 mg DOC dm^{-3}.

least-squares fitting of straight lines to the data (Figs. 3 and 4). Below we suggest a model referring to previously known processes.[3,26]

A model for acid regions with pH < 4. In the acid region, the steady-state reaction rates do not seem to vary with pH or the total concentration of humate. We assume that the consumption of protons is due to dissolution of small particles. The particles could have been precipitated when hydrochloric acid was added before starting the titration. We assume that precipitation/dissolution reactions of humic substances are the rate determining reactions. They are written as:

$$D(s) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D(aq) \tag{6}$$

with the rate equation

$$\text{rate} = k_1 D(s) - k_2 D(aq) \tag{7}$$

Fast reactions of D(aq) with protons may cause the observed change of pH with time, since D(s) and D(aq) probably have different acid–base properties.

We did not observe any dependence of the rate on D(aq). Therefore, we assume that the dissolution of small solid particles determines the reaction rate. From the data in Figs. 3 and 4 we obtained $\log k_1 = -6.7 \pm 0.2$ for 1 g of humic material and the slope 0.0 ± 0.1. It is not possible to determine k_2 with the present data. The errors given are 3σ.

A model for the pH region 4 < pH < 6.5. Like Sposito and Holtzclaw[38] we found that the protonation reactions are dependent on the total concentration of humate. The polymerization of humic substances is supposed to change slowly when hydroxide ions are added. At present we do not know the nature of the products in eqn. (8).

We suggest a very simple model, which can explain our present data:

$$D + H^+ \underset{k_4}{\overset{k_3}{\rightleftharpoons}} D1 + D2 \tag{8}$$

The forward reaction describes a particle D, which by reaction with a proton is split into two smaller fragments D1 and D2. The rate equation is written as

$$\text{rate} = k_3[D][H^+]^x - k_4[D1][D2] \tag{9}$$

We could only observe the forward reaction in the present study. Two different types of protonated sites on the surfaces of the humates were observed. One type of site with the observed $pK_a = 4.0$ probably corresponds to surface carboxylate groups. The other type of site with $pK_a = 5.4$ is assumed to be directly related to the polymerization reaction. In more concentrated solutions of humic acid, we observed a smaller amount of the second type of site, which thus could be involved in hydrogen bonds. Protons were consumed in acid solutions, but produced at higher pH-values. These changes are consistent with an apparent pK_a and hydrogen bond formation, cf. Fig. 4. The rate data could be fitted to the following equation:

$$\log(\text{rate}) = \log k_3 + \log[D] - x\,pH \tag{10}$$

with $\log k_3 = -6.5 \pm 0.2$ and $x = 1.2 \pm 0.1$. The errors are 3σ. The same values of the parameters were obtained for soil humus and for lake humus. It was not possible to calculate the slopes of the individual lines due to poor data. With a fixed slope of -1 we obtained $\log k_3 = -7.4 \pm 0.2$, which also was determined by a graphical method. Both models could explain the data well, but the model with $x = 1.2$ gave a slightly better fit with the data. Only the data points for consumptions of protons were used in the calculations (cf. Figs. 3 and 4). The very low rates at pH 6.5 could be caused by a more or less complete breakdown of the aggregates.

A model for the region with pH > 6.5. For solutions with pH > 6.5 the production of protons could possibly be due to the disintegration of humic acid. The products D3 and D4 are unknown. The data in alkaline solutions could be explained by a reaction with OH$^-$:

$$D + OH^- \overset{k_5}{\longrightarrow} D3 + D4 \tag{11}$$

with the rate equation

$$\text{rate} = k_5[OH^-][D] \tag{12}$$

Least-squares calculations gave $\log k_5 = -19.7 \pm 0.4$ for the soil humus and $\log k_5 = -20.2 \pm 0.2$ for the lake humus. We tried to determine the slope, but were unable to do so. We found that the reactions for pH > 7 involved

a large consumption of hydroxyl ions to obtain a steady state.

Conclusions

The present experimental steady-state data are consistent with simple models involving processes such as protonation, precipitation, dissolution, polymerisation, and disintegration of humates. Conformational changes could also be important. We assume that the protonation is rapid and that the other reactions are relatively slow. We found that the humus from two completely different sources could be explained by the same model parameters within experimental error. The data for the lowest concentration of lake humus is uncertain. Therefore, with the present data we can not confirm the dependence on the total concentration of lake humus. For a few pH-values oscillations were observed, cf. Fig. 2.

Discussion

The measured effects are very small. The buffer capacities range from 50 to 1000 μM. EMF changes of 0.1–1 mV as a function of time during the relaxation were observed for pH < 6.5, while bigger changes occur for higher pH-values. The first item to discuss is whether we have measured the properties of humates, some property of the measuring system or some impurity. It was necessary to know E^0 to within 0.1 mV or better. We also found that it was necessary to determine E^0 of the EMF cell with the humates present. The EMF could change by 3–4 mV when little humate was added to a sodium chloride solution. Therefore, we added hydrogen chloride in the beginning of the titration and determined E^0 from a few acid points. Measurements with other methods would be valuable to check the present results. Fluorescence measurements can be used for direct measurements of humates or their complexes.[14,39]

Buffle[13] suggests the name 'titration window' for the pH-region 3 < pH < 5, where the apparent quasi-equilibria are reached within a few minutes. This is in agreement with our observations. However, a closer look reveals slow reactions, which can give substantial changes of the system in a few days. On a short timescale we can determine the apparent protonation quasi-equilibria for a certain distribution of polymers in the laboratory, taking into account the electrostatic effects. There are now good models for such data, and also many reports with experimental data.[6,8,38]

In the field, we have to regard changes related to different timescales. Owing to changes of aggregation and conformation, the available surface area of the humates and thereby their properties in solution will change with time. This will for example occur when different natural waters are mixed or when the temperature changes. Therefore, it is necessary to account for the kinetic effects when modelling natural waters containing refractory organic matter. In this study, a first attempt has been made to model the slow processes of humates. Further experiments are necessary to construct a more complete model. Finally, multivariate analysis seems to be a powerful tool to extract information from measurements on humic substances.[40]

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