

PARTICLE SIZE OF HUMIC ACID

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Abstract

The largest humic acid particles that can exist in solutions of the same acidity as most Scandinavian soil and water systems (but at a higher concentration, 1–4 mg/mL) are ≈ 110 nm in their largest dimension; these particles, thus, are of about the same magnitude as large viruses. This is shown by small-angle neutron scattering at 10 °C, pH 5, and 0.10 M ionic strength (NaCl). The mean radii of gyration of humic acids obtained from three different soil samples were found to be of the same magnitude: 21.4, 29.7, and 28.3 nm; the mean molecular masses were 500, 2000, and 1500 kDa. The radius of gyration of the cross section for the particles in solution was found to be constant, 2.1 nm; thus, the humic acid particles might essentially vary only in regard to length. A contrast variation study of one of the samples was found to be consistent with the particles in solution having a cross section with less than average scattering density in its central part. This supports the idea that the particles might contain a central "core" with a different structure than the peripheral parts.

HUMIC ACIDS, which are present in soil and all natural water systems, play a central role in our environment, since they are the key compounds for the transport of nutrients to the plant kingdom (for a review, see Kononova, 1966). Due to their amphipathic character, they bind both hydrophilic and hydrophobic compounds, including not only essential metal ions and organic compounds but also toxic metal ions as well as herbicides and pesticides (Gorbunova et al., 1971; Schnitzer and Khan, 1972; Wershaw and Goldberg, 1972). Nevertheless, as yet the structure of humic acids is not known in detail. Furthermore, we know neither the size and shape of the largest particles, nor the size distribution for the particles in solution. Using small-angle neutron scattering, we have started a general program intended to analyze the size and shape of humic acids under various environmental conditions. First, we chose to analyze the particle sizes existing at pH 5, since this acidity corresponds to many of the natural water systems in Scandinavia, where the soils are exposed to acid rain.

Humic acids have previously been studied by small-angle x-ray scattering (Wershaw et al., 1967; Lindqvist, 1970; Wershaw and Pinckney, 1973), but these studies involved slit-smear data obtained by a traditional Kratky camera, through which it is difficult to reach the Guinier range for particles of a size ≥ 100 nm. For pH 5, Wershaw and Pinckney (1973) reported a radius of gyration, R , of 4.74 nm and for pH 3.5 they reported R values ≥ 6.7 nm. An earlier study by Wershaw et al. (1967) showed larger variations among different samples, and R values from 3.8 to 13.7 nm were reported. Likewise, there are large

variations among the molecular masses reported for large humic acid particles; for instance, Stevenson et al. (1953) reported a value of 4×10^4 and Wershaw et al. (1967) reported values as high as 1×10^6 . It should be noted that the size of the humic acid particles in solution appears to vary with both the concentration and the pH. For example, relatively low molecular weight particles, 1.0 to 10 kDa, have been indicated from natural water systems at concentrations generally ranging from 0.02 to 0.06 mg/mL humic acids (Thurman et al., 1982). However, at higher concentrations in the range of our study, 0.5 to 4 mg/mL, no apparent change in size was noted after a fivefold dilution (Wershaw et al., 1967). Regarding the pH dependence, the degree of aggregation appears to increase as the pH decreases from 6 to 3; and, at pH 7 and low ionic strength, humic acids are supposed to be essentially monomeric (Wershaw and Pinckney, 1973).

The aim of this particular study has been to make an attempt to analyze the size of the very largest humic acid particles that may exist in aqueous solution under conditions supposed to prevail in most Scandinavian soil and water systems.

Materials and Methods

Two samples of humic acid were purchased from the International Humic Substances Society, Golden, CO; one of those was a standard soil sample obtained in 1990 (I) and the other was a reference soil sample obtained in 1984 (III). The third humic acid sample was prepared by a gentle procedure developed by Lindqvist (1982) from soil collected from an area outside Uppsala, Sweden (II). The samples were generally dissolved in a 0.01 M acetate buffer of pH 5.0 containing 0.5 mM EDTA having an ionic strength of 0.10 M NaCl. They were then dialyzed for 36 h against solvents of D₂O or H₂O, or against mixed D₂O–H₂O solvents containing the same buffer.

The small-angle neutron scattering (SANS) data were recorded at the SANS facilities (K. Mortensen, unpublished data) at Risø, Denmark. For the analysis of the radii of gyration and the forward scattering, the incident wavelengths were 0.78 and 2.0 nm and the distances, sample to detector, were 300 and 600 cm. For the analysis of the cross-sectional radius, the wavelength was 0.6 nm and the distance, sample to detector, was 300 cm. For each setting, the source to sample distance was the same as the sample to detector distance. The neutron beam was passed through two circular slits, one source slit with a diameter of 16 mm and one sample slit with a diameter of 7 mm. Scattering intensities were measured by a two-dimensional position-sensitive detector. Sample scattering data were corrected for the contribution of the buffer, empty cell, and background noise, normalized to the monitor counts, and then divided by the corresponding corrected H₂O spectra (May et al., 1982). The smearing effects were neglected since, within the angular range measured, they are small (<5%) (cf., Skov Pedersen et al., 1990). All the measurements were done at 10 °C in 2-mm (D₂O) or 1-mm (H₂O) quartz cuvettes. It should be noted that the Guinier region is most accurately obtained in D₂O due to the low intensity of the buffer background.

Results and Discussion

Figure 1A shows the Guinier plots obtained from data recorded from solutions of the three different humic acid samples in D₂O. In the measured range, 1 to 4 mg/mL,

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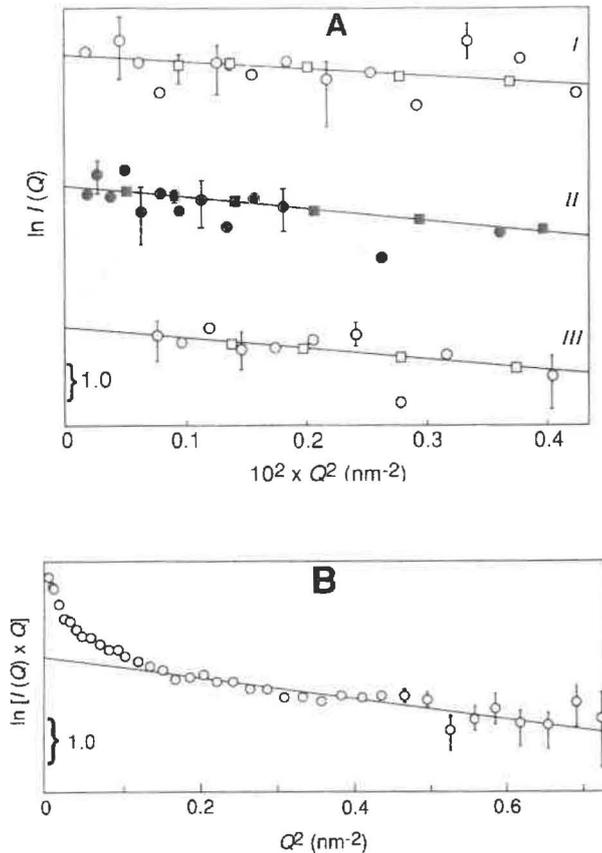


Fig. 1. Experimental small-angle neutron scattering of humic acids in D_2O in an acetate buffer corresponding to pH 5.0 and 0.1 M ionic strength (NaCl). (A) The intensity, $I(Q)$, is plotted as $\ln I(Q)$ vs. Q^2 , for three different samples, I, II, and III; the scattering vector, Q , $= 4\pi(\sin\theta)/\lambda$, where 2θ is the scattering angle and λ the wavelength. The mean incident wavelength was 0.78 nm for the data indicated by squares (\square , \blacksquare) and 1.7, 2.2, and 1.7 nm for the data indicated by circles (\circ , \bullet) for Samples I, II, and III, respectively. In the range $RQ \leq 1.0$, using the 0.78-nm data, the radius of gyration, R , and the forward scattering were obtained from the best-fitting straight line. The 1.7- to 2.2-nm data are plotted to show consistence with the 0.78-nm data and that there are no indications for larger particles beyond the size indicated by the 0.78-nm data. The statistical error is indicated for every third point for the 1.7- to 2.2-nm data and for every 0.78-nm point (in general less than the plotted squares). (B) Cross-section plot, $\ln [I(Q) \times Q]$ vs. Q^2 , for data recorded for a solution of sample II. The mean incident wavelength was 0.6 nm and the sample to detector distance was 300 cm. From the best-fitting straight line through the distal points, in the range $0.8 \leq R_s(Q) \leq 1.7$, the radius of gyration of the cross section, R_s , was determined.

the normalized intensity, I/c , was independent of the concentration, c , so that interparticle scattering could be neglected. It should be noted that, for $Q \leq 0.8 \text{ nm}^{-1}$, all three samples yielded a similar concave scattering curve when $\ln I(Q)$ was plotted against Q^2 , (cf., Wer-shaw et al., 1967; Lindqvist, 1970). Here, $Q = 4\pi(\sin \theta)/\lambda$, where 2θ is the scattering angle and λ is the wavelength. From the slopes of the straight lines in Fig. 1A, the mean radii of gyration (Guinier and Fournet, 1955; Glatter and Kratky, 1982) were determined; the results were 21.4 nm for Sample I, 29.7 for sample II, and 28.3

nm for Sample III. Extrapolation of Fig. 1A lines to zero angles yielded the forward scattering, $I(0)$. Then, using the procedures described by Jacrot and Zaccari (1981), we estimated the mean molecular masses to be 500 kDa for Sample I, 2000 kDa for Sample II, and 1500 kDa for Sample III. This estimation requires a pre-knowledge of the match point (in a contrast variation study [see, e.g., Stuhrmann, 1982], it was found to correspond to the scattering density of 49% D_2O); the partial specific volume, v , (estimated at 0.60 mL/g via the density [Wer-shaw et al., 1967]); the scattering length (obtained via the conditions at the match point using the v value, see, e.g., Eq. [6] in Sjöberg et al. [1985]); and the concentration of the humic acids (obtained via elementary analysis of C assuming 52% C per unit dry weight humic acid).

As the first step in analyzing the shape of the particles, we determined the radius of gyration of the cross section, R_q , (Pilz, 1982) and the distance distribution function, $p(r)$, (Glatter, 1977). As shown by Fig. 1B, the plot of $\ln [I(Q)Q]$ vs. Q^2 shows polydispersity in the proximal angular range (Pilz, 1982), but its slope becomes constant for increasing values of Q^2 , indicating that the various particles in solution might have the same cross section. In D_2O , the R_q value was 2.1 nm. By tentatively assuming, as a first approximation, a circular cross section, we obtained a value of 3.0 nm for its radius. From the $p(r)$ we determined a maximum distance within the largest particles, D_{max} , of 110 ± 20 nm. Although a detailed shape analysis does not seem feasible due to polydispersity, the constant R_q value, as well as the $p(r)$ curve, does not exclude the possibility of a fiberlike molecule, perhaps involving some bends and branching.

By analyzing SANS data recorded for Sample II in a contrast variation study (see, e.g., Stuhrmann, 1982), using solutions of different H_2O - D_2O compositions, we obtained a series of R_q values. In a Stuhrmann plot (Ibel and Stuhrmann, 1975), where R_q is plotted against $\Delta\rho^{-1}$, the plotted points could be fitted to a straight line with a positive slope ($\Delta\rho$ is the excess scattering density of the solute compared with that of the solvent). A positive slope in a Stuhrmann plot may indicate that there is less than average scattering density close to the center and more than average scattering density in the peripheral parts of the particles. One explanation for such a distribution might be that the center is more hydrophobic and the outer parts more hydrophilic than the average composition of the particles (see, e.g., Österberg et al., 1988). Thus, this part of the study supports and is in agreement with the idea that the humic acid particles may contain a central "core" with a different structure than the peripheral parts (Lindqvist, 1970; cf. Wer-shaw et al., 1986).

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