Eur Biophys J (1992) 21, 163–167

European
Biophysics Journal

# Fractal dimension of humic acids

## A small angle neutron scattering study

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Received December 20, 1991/Accepted in revised form May 7, 1992

**Abstract.** Small-angle neutron scattering experiments have been made on solutions of humic acid aggregates with an acidity corresponding to pH 5.0 and at 0.1 M ionic strength. We observe power-law decay of the intensity over one decade of the scattering vector, $Q$, indicating that the aggregates are fractal. We explain the normalized intensity in the entire $Q$-range by assuming that the humic acid particles can be described by building units of a radial size, $\leq 25$ Å, aggregated into clusters with an average radius of 400–500 Å. For humic acids obtained from two different sources, we determine the fractal dimension, $D = 2.3 \pm 0.1$. For small values of $Q$, the measured data of one of the samples extend into the Guinier range giving an average radius of gyration of $320 \pm 20$ Å.

**Key words:** Humic acid – Neutron scattering – Fractal dimension

## Introduction

Humic acids, which are present in soils and all natural water systems, play a central part in the biosphere since they are the key compounds for the transport of nutrients to the plant kingdom; see, e.g., the review by Konova (1966). Owing to their amphipathic character they bind both hydrophilic and hydrophobic compounds, involving 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 Kahn 1972; Wershaw and Goldberg 1972).

Many suggestions have been made regarding the size and shape of humids acids (see e.g., the reviews in Hayes et al. 1989), but, as yet, their structure is not known in detail. In solution, humic acids are supposed to be polydispersed, especially in the acid range (Wershaw and Pinckney 1973), but very little is known about the mechanism of aggregation. In order to gain an increased understanding of the aggregation process and of the structure of the resulting clusters, humic acids have been studied in solution using small-angle neutron scattering (SANS). As our main finding we report the fractal nature of humic acids. This indicates that the essential geometric properties of the humic acid system are independent of length scale, and that the particles in solution are invariant over a certain range of scale transformation.

## Experimental

One sample of humic acid was purchased from the International Humic Substances Society, Golden, Colorado, USA (I). The other sample was prepared by a gentle procedure developed by Lindquist (1982) from soil collected from an area outside Uppsala, Sweden (II). The samples were dissolved in a 0.01 M acetate buffer of pH 5.0 containing 0.5 mM EDTA with an ionic strength of 0.10 M (NaCl), and then they were dialysed against the same buffer. Then, finally, the samples were dialysed for 36 h against a $D_2O$ solvent containing the same buffer. A pH of 5.0 was chosen in order to facilitate $H^+$-catalysis. (This pH corresponds to the pH of many natural water system in Scandinavia where the soils are exposed to acid rain falls.) The concentration of humic acid was determined via elementary analysis of C, assuming 52% C per dry weight humic acid.

The small angle neutron scattering data were recorded at the SANS facilities at Risø, Denmark (K. Mortensen, unpublished work). The samples were studied using incident wavelengths of 6.0, 7.8, and 13.8 Å and using distances, sample to detector, of 300 and 600 cm. 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; then they were divided by the corresponding corrected $H_2O$ spectra (May et al. 1982). Regarding the data of sample II, which extend into the Guinier region, the radially aver-

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aged spectra of the different angular ranges were combined and deconvoluted with the wavelength and primary beam profiles using the resolution function (Skov-Pedersen et al. 1990) and a modified version of Glatter's computer program (Glatter 1977). In order to enable the aggregation process to attain a steady state or "equilibrium" (cf. Schaefer et al. 1984) the samples studied were stored at 20° for $\geq 48$ h before they were subjected to the neutron beam. The measurements were done at 11° in 2 mm ($D_2O$) and 1 mm ($H_2O$) quartz cuvettes.

## Results

Figure 1 shows the SANS-data obtained from the two different preparations of humic acids. Several solutions of each sample were studied using concentrations of 1–4 mg/ml of humic acid; no concentration dependent deviation due to interparticle scattering was noted. It follows from Fig. 1 that the intensity, $I(Q)$, for both sets of data obey a power-law decay in $Q$:

$$I(Q) \approx Q^{-D} \tag{1}$$

which is a general criterion for fractals (Sinha et al. 1984; Martin and Hurd 1987). As shown by Fig. 1, this power-law behaviour covers about one decade in the scattering vector $Q$ corresponding to the range $1/\xi \leq Q \leq 1/a$, where $Q = 4\pi(\sin\theta)/\lambda$; $\xi$ is the cut-off distance to the Guinier range (it is related to $R_g$, a generalized radius of gyration for the largest cluster(s), see (6) below; $a$, the characteristic dimension of each individual scatterer; $2\theta$, the scattering angle; and $\lambda$, the wavelength. From Fig. 1, we distinguish at small $Q < 1/\xi$, the Guinier region, but, at large $Q > 1/a$, there is no clear cross-over to an asymptotic region of the Porod type (see e.g., Auvray and Auroy 1991). It should be noted that the desmearing of the sample II data mainly affected the data at low $Q$ and that there was only a limited change of the slope in the power-law region (cf. Wignall et al. 1988). The slopes of the two sets of data displayed in Fig. 1 yield, in the power-law region, an apparent fractal dimension, $D$, of 2.0 (a), and 2.1 (b), respectively.

We also analysed the data by considering the possible effects of the cut-off lengths. Then we first assumed that the normalized intensity, $I(Q)/I(0)$, can be written in the form:

$$I(Q)/I(0) = P(Q)\,S(Q) \tag{2}$$

where $P(Q)$ is the particle structure factor. $S(Q)$ is the interparticle structure factor, which describes the correlation between particles in a cluster. Although the size and shape of the "monomer" are unknown, spheroids of the size 15–30 Å in diameter have been identified from electron microscopic pictures of the related fulvic acid (Schnitzer and Kodama 1975). Therefore, as a first approximation, a spherical shape with a radius of $a$ was assumed for the monomer. The function $P(Q)$ can then be calculated by the equation (Guinier and Fournet 1955):

$$P(Q) = [3(\sin(Qa) - Qa\cos(Qa))/(Qa)^3]^2. \tag{3}$$

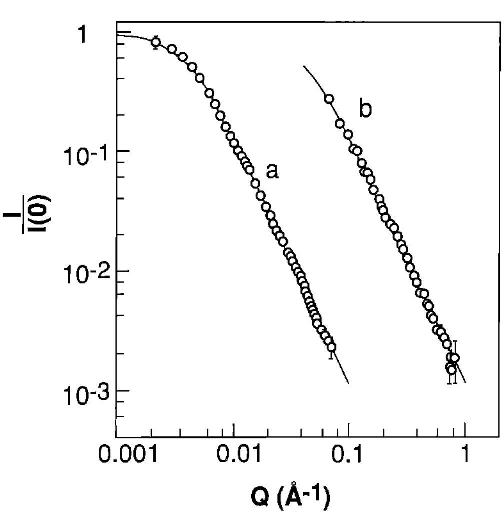

**Fig. 1.** Small-angle neutron scattering from humic acid solutions: (**a**) Sample II, 2.9 mg/ml; (**b**) Sample I, 3.6 mg/ml (b); the normalized intensity, $I(Q)/I(0)$, plotted against $Q$ in a log-log diagram (o). For clarity, curve b has been shifted one decade along the $Q$-axis. The I(0)-value for the data of Sample II was obtained via a Guinier plot and it was tentatively obtained for those of Sample I via a curve-fitting procedure. Curve a has been calculated from (2)–(4) using $\xi_2 = 200$ Å, $a = 20$ Å, and $D = 2.30$; an equally good fit is obtained using (5) and $\xi_1 = 385$ Å, and $D = 2.30$. Curve b has been calculated for $D = 2.30$ and $\xi_1 = 385$ Å using (5); please note, that for the Sample I data (b) $\xi_1$ is only correct in magnitude, see the text. For the data labelled a, the mean incident wavelength was 7.8 Å and the distances, sample to detector, were 300 and 600 cm; for those of b, the wavelengths were 6.0 and 13.8 Å and the distance, sample to detector, was 300 cm. The statistical error is less than the plotted circles except at low $Q$-values, where error bars are indicated for every third point

In order to determine $S(Q)$ the following equation is currently used in the literature (Teixeira, 1988):

$$S(Q) = 1 + \{D\Gamma(D-1)\cdot\sin[(D-1)\tan^{-1}(Q\xi_2)]\}/ (Qa)^D[1 + 1/Q^2\xi_2^2]^{(D-1)/2} \tag{4}$$

where $\Gamma$ is the gamma function of argument $(D-1)$. It should be noted, however, that the parameter, $\xi_2$, does not easily correlate with the cluster size. This is due to the fact that $\xi_2$ was introduced as a length defined through a convenient choice of exponential decay of the fractal correlation function (cf. Sinha et al. 1984). Therefore, we also analyzed our data using the approximation of Fisher and Burford (1967), (cf. Martin and Ackerson 1985; Cabane 1991):

$$I(Q)/I(0) \approx (1 + 2\xi_1^2 Q^2/3D)^{-D/2}. \tag{5}$$

This equation has the advantage of simplicity and it has the right limiting behaviour: for small values of $Q$, it reduces to the Guinier approximation and for large $Q$ it approaches the power-law of Eq. (1).

The parameters of Eq. (5) were conveniently evaluated by comparison of the experimental data, $\log[I(Q)/I(0)]$ versus $\log Q$, with normalized graphs, $\log[I(Q)/I(0)]$ versus $\log \boldsymbol{Q}$ at constant $D$, where $\boldsymbol{Q} = \xi_1 Q$ (cf. Sillén 1956). For the data of sample II, the result was $\xi_1 = 385$ Å and $D = 2.30$. From Eqs. (2)–(4) we obtained for the same data $\xi_2 = 200$ Å, $D = 2.30$, and $a = 20$ Å, and, as shown by

Fig. 1 a, the data are well described by these parameters. It follows from this result that regarding the fractal dimension, $D$, there is excellent agreement between (4) and (5). The parameter, $\xi_1$, which in the Guinier range, corresponds to the average radius of gyration, $\bar{R}$, deviates from the value of $\bar{R} = 320 \pm 20$ Å, obtained via a Guinier plot and via the distance distribution function, $p(r)$ (Glatter 1977); this deviation of about 17% may be ascribed to the fact that the intensity calculated by (5) also depends on $\xi_1$ beyond the Guinier region. From the parameter, $\xi_2$, of (4) we calculate a generalized radius of gyration, $R_g$, using the equation (Teixeira 1988):

$$R_g^2 = D(D+1)\xi_2^2/2. \qquad (6)$$

The result, $R_g = 390$ Å, which is in good agreement with $\xi_1$, shows that it is $R_g$ rather than the parameter $\xi_2$ which compares with $\xi_1$. Here, it is interesting to note that a sphere corresponding to the $R_g$-value has a diameter of 1006 Å, a value in agreement with the maximal distance within the largest clusters obtained from the $p(r)$-curve: $1100 \pm 100$ Å. One advantage of (4) is that it involves $a$, the radius of the monomer and that it can be combined in (2) with the structure factor, $P(Q)$, and, thus, be used beyond the cut-off for $Qa > 1$ (Chen and Teixeira 1986), (for (5) this would require an additional term and another fitting parameter (cf. Martin et al. 1986)). Since the cut-off for the small length region, $Qa > 1$, is not defined in the measured range (Fig. 1), we have not accounted for the region at large $Q$, where the intensity becomes dominated by surface scattering (cf. Martin et al. 1986). As a result, within our $Q$-range, the normalized intensity depends little on the characteristic dimension of each individual scatterer and for this dimension we only give an upper limit, $a \leq 25$ Å.

As shown by Fig. 1 b, the data recorded for Sample I mainly involves the power-law region of $Q$, and none of the cut-off regions are well defined. Thus, using (5) we only estimate a lower limit for $\xi_1$; and, via the curve-fitting procedure with normalized model functions we obtained $D = 2.3$ and $\xi_1 \geq 385$ Å. It should be noted that a higher value of $\xi_1$ is quite likely, since the intensities of the Sample I data (Fig. 1 b) are tentatively normalized with $I(0)$ close to its lower limit. However, a change in $I(0)$, within a relatively large range, will not affect the value of $D = 2.3 \pm 0.1$, and for Sample I this is the main outcome of the study.

One important question is whether or not polydispersity will affect the power-law scattering observed (cf. Schmidt 1982). This might occur when the polydispersity is characterized by a relatively large polydispersity exponent, which leads to percolation or gelation (Martin 1986). In the case of a percolation distribution of clusters the scattering exponent will depend on both the fractal dimension and the polydispersity exponent; for a detailed analysis of this particular subject see Martin and Ackerson (1985). However, the two different samples of humic acids studied in this work did not show any sign of macroscopic gel formation (after storage for several weeks) nor any indication of cluster-cluster interactions from their small-angle neutron scattering (cf. Kinning and Thomas 1984). Therefore, our present samples appear to belong to regimes with polydispersity exponents $<2$; and, thus, the scattering exponent depends only on the fractal dimension (Martin 1986). It should also be noted that the prolonged dialysis of the samples and the fact that the aggregation was allowed to proceed for 48 h at room temperature before data collection would favor large clusters and limit the number of particle sizes.

## Discussion

It has been pointed out repeatedly that small-angle scattering is the method of choice for analysing fractal systems in solution (Martin and Hurd 1987; Teixeira 1988). This is especially true for SANS, since this method enables the use of favorable contrast by modifying the solvent or solute by isotopic substitution. Also, it is important to choose a collimation which keeps the smearing of the scattered beam at a minimum. Certain systems where the Guinier region is outside the measured range require such a collimation (cf. Fig. 1 b) since mathematical desmearing is difficult or even impossible to accomplish (Wignall et al. 1988).

Small-angle scattering has previously been used in studies of humic acids, see for instance the X-ray studies by Wershaw et al. (1967); by Lindqvist (1970); and by Wershaw and Pinckney (1973). However, no attempts were made to analyse the data in some detail, such as for instance for a power-law dependence. Furthermore, these studies involved slit-smeared data obtained by a Kratky camera, through which it is difficult to reach the Guinier range for particles of the present size. In addition, it is not clear from these early studies which kinetic stage of the humic acid aggregation the data represented. On the other hand, these early studies might give a hint regarding the size of the "monomer". Thus, for certain humic acid solutions, a radius of gyration of the order of $\leq 10$ Å has been reported (Wershaw and Pinckney, 1973). Whether or not this corresponds to the "monomer" of the present clusters is not known, but our data are compatible with a monomer of the size $a \leq 25$ Å.

The results presented in the previous section have indicated that the structure of humic acid clusters can be described as a fractal. This result as well as other observations regarding the kinetics of the aggregation process, such as $H^+$-catalysis and time dependence (Österberg and Mortensen, unpublished work) suggest that humic acids at pH 5 behave as random colloid aggregates and show properties very similar to kinetic growth processes. Based on their study on colloidal silica, Schaefer et al. (1985) have discussed the factors that favor kinetic growth processes. There are two main models which predict the fractal structure of colloidal aggregates: diffusion limited aggregation with $D = 2.50$ and cluster aggregation with $D = 1.78$ (cf. th review by Martin and Hurd 1987). Thus, the present $D$-value, 2.3, seems to agree with the diffusion limited aggregation rather than the cluster aggregation. In diffusion limited aggregation the clusters grow intirely from monomers, which approach the cluster with a random-walk trajectory; the monomer will be found, if it comes within one lattice constant (i.e., $a$) of the growing cluster. The random walk of the approaching monomer favors growth in the peripheral parts of the cluster; thus, open, ramified geometries develop.

A model of aggregation, perhaps, more closely related to the present system is a kind of cluster-cluster aggregation that yields $D = 2.1$. In this reaction-limited aggregation there is a residual barrier between monomers and this suggests that monomers will be excluded from the dense, central part of the cluster (Schaefer et al. 1985). Such a residual barrier would be expected to exist among humic acid particles at pH 5, since then the carboxylate groups are only partly neutralized. However, in the reaction-limited model it is the short range attraction that eventually causes the aggregation. For humic acids, short range attraction might involve the formation of hydrogen bonds, since at pH 5 protons are slowly consumed during the aggregation process (Wahlberg, O., unpublished work); it may also involve the association of hydrophobic residues, say of the quinone and/or phenol type, perhaps leading to charge transfer complexes (Lindqvist 1982); furthermore, bound water molecules may function as an entropy source for the aggregation reaction by being released at the sites of binding.

The fractal dimension reported for silica aggregates was 2.1 (Schaefer et al. 1984; Aubert and Channell 1986), thus, in excellent agreement with the theory for reaction-limited aggregation (Weitz et al. 1985). Here, it must be kept in mind that the experimental $D$-values were determined from the slope of the intensity curve. In the present study the slope yields lower values: we found $D$-values of 2.0 and 2.1 from the slope (Fig. 1) compared to $D = 2.3$ from (4) and (5). Thus, our result obviously falls within the range of the slow, reaction-limited process and such a fractal would favour an open structure (see, e.g., Fig. 13 in Martin and Hurd 1987).

In agreement with an open structure is an electron microscopic study of fulvic acid (present in humic substances and being related to humic acids), see Schnitzer and Kodama (1975). At acid pH, fulvic acid consists of elongated, irregularly shaped structures, 500–1000 Å in diameter, formed by small spheroidal aggregates. Each spheroid is supposed to be 15 to 30 Å in diameter. From a recent review on this subject, by Chen and Schnitzer (1989), it appears that electron microscopy of humic acids and of fulvic acids yields very similar pictures. Thus, the diameters of the structures indicated from the electron micrographs are of the same order or magnitude as those, 824 and 1006 Å, which can be calculated from the $\bar{R}$ and $R_g$-values of sample II by assuming a spherical shape. From the size of the spheroids it is quite likely that the "monomer" of humic acids might be of the same magnitude as the one indicated in this study, which is $a \leq 25$ Å.

In summary, our results show that humic acids obtained from two entirely different sources and via two different methods of preparation yield essentially the same fractal dimension, $D = 2.3$. The chemically complex humic acid system can be described by assuming that the particles in solution consist of building units of a radial size, $\leq 25$ Å, aggregated into clusters with an average radius of 400–500 Å. This result could be useful for future studies involving the mechanism of aggregation of humic acids as well as their interactions with various inorganic and biological systems.

*Acknowledgements.* Financial support from the Swedish Agricultural Sciences Research Council is gratefully acknowledged.

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