Notes

Particle Sizes of Standard Humic Substances Calculated as Radii of Gyration, Maximum Diameter and Hydrodynamic Radii

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Abstract

Average particle size and particle size distribution of humic substances under solution conditions is useful physicochemical information for investigating the role of humic substances in geochemical processes. In this study, the average particle sizes of standard humic acid (HA) and fulvic acid (FA) samples from the Japanese Humic Substance Society (JHSS) were evaluated by small angle X-ray scattering (SAXS). The scattering intensity in the observed range of scattering angles was higher in the HAs than in the FAs. The distance distribution functions, $p(r)$, involved in the structural characteristics of the particles when dispersed in solution were obtained by a Fourier inverse transform of the scattering intensity. The radii of gyration, R_G, calculated from the $p(r)$ were 10.1, 7.3 and 1.4 nm for the Dando HA, Inogashira HA and the two FAs, respectively. Concurrently the hydrodynamic radii (R_H) of these standard samples were calculated from Einstein's equation using already-known molecular weights and intrinsic viscosity for comparison with R_G values. The values of R_G in the HAs were considerably larger than R_H values, probably due to higher sample concentrations in the SAXS measurement. Larger ratios of R_G to R_H (R_G/R_H) in the HAs than in the FAs indicate a relatively elongate shape of the HAs as compared to the FAs. This was also confirmed by a ratio of maximum diameter of averaged particles (D_{max}) and R_G (D_{max}/R_G) obtained by the SAXS analysis. Smaller particle HAs, which were confirmed in a dilute solution system by high performance size exclusion chromatography (HPSEC), can aggregate in the more concentrated system used for the SAXS measurement. The elongate shape of the larger HA particles may result from hydrophobic interaction between smaller HA particles. In contrast, FAs with low ratios of D_{max}/R_G and R_G/R_H were dispersed as stable single small particles in the solution. Larger contents of acidic functional groups in the FA chemical structure allow single FA particles to disperse by electrostatic repulsion. The different dispersion behavior of HAs and FAs in solution can be attributed to their chemical structures.

Keywords: SAXS, distance distribution function, radius of gyration, intrinsic viscosity, hydrodynamic radius

Introduction

Humic substances are ubiquitous in both terrestrial and aquatic environments on the Earth's surface and interact with minerals, microorganisms, xenobiotics and metals, which control their reactivity and behavior. These interactions are strongly controlled by colloidal sizes of the humic substances. As sizes of humic substances in solution are rather variable depending on solution conditions such as pH, ionic strength and concentration, data from various available analyses are useful in evaluating their average sizes and size distributions. Scattering techniques are important analytical methods for determining the colloidal size and shape of solutes because they can provide absolute size information.

Small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS) are mainly used for determination of the sizes and shapes of macromolecules, synthetic polymers, and natural polymers in

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highly polydispersed solution systems. Scattering from a single particle in a solution corresponds to the electron density difference between the particle and the solvent, which provides information on the size and shape of the particle. The scattering intensity of an X-ray from a particle is greatest at the direction of the incident beam and decreases as the scattering angle increases, due to interference among scattering beams. The size and shape of the particle can be estimated from the scattering pattern (Kajiwara and Hiiragi, 1996). Small angle X-ray scattering is suitable for observing particles with dimensions from 1 to 100 nm. The SAXS method has also been applied to humic substances to obtain size and shape information (Wershaw et al., 1967, Kawahigashi et al., 1995, Pranzas et al., 2003). The reported sizes of humic substances in solutions were at a scale of several nanometers, which is detectable by the SAXS technique. Mathematical inversion of the scattering data is also able to provide physicochemical information such as the shape, aggregation and dissociation dynamics as well as physical sizes (Kajiwara and Hiiragi, 1996).

The size of the standard humic substances of Japanese Humic Substances Society has not been determined by scattering analysis. Here we attempt to determine their particle sizes using SAXS and compare the results with molecular sizes calculated from published data.

Materials and Methods

Humic substances samples

The HAs and FAs used were standard samples certified by the Japanese Humic Substances Society. These samples were prepared from the surface horizons of the Dando (Dystric Cambisol) and Inogashira (Umbric Andosol) mineral soils, according to the IHSS method (Kuwatsuka et al., 1992, Watanabe et al., 2004).

Ten mg powder samples of the humic substances were mixed with 5 mL of 0.1 M NaOH and fully dispersed by sonication. After complete dissolution of the samples, the solutions were made up to 10 mL using 0.1 M $Na_2B_4O_7$ solution. The pH of the sample solution was 10.4. The buffer solution used and the pH condition have been shown to be suitable for SAXS analyses of humic substance samples in their well dispersed condition as was confirmed by scattering angle dependence of the intensity (Kawahigashi et al., 1995).

Small angle X-ray scattering (SAXS)

The SAXS measurements were performed at 25°C in the range of the scattering vector q ($=4\pi \sin(\theta/2)/\lambda$) of 6×10^{-2} to 2.0 nm^{-1} using a SAXS spectrometer installed at the BL-10C port in the Photon Factory of the National Laboratory for High Energy Accelerator Research Organization (Tsukuba, Japan). Here, θ and λ are the scattered angle and the X-ray wave length, respectively. The sample-to-detector distance and the X-ray wave length (λ) were 0.188 m and 0.149 nm, respectively. The humic solution was filled into a quartz cell with 1 mm thickness and subjected to the X-ray beam under vacuum conditions. The SAXS data, as scattering intensity, $I(q)$, from the sample, were corrected by subtracting scattering of the cell and solvent. The structural analysis based on the total scattering curve was carried out by calculating the distance distribution function, $p(r)$, given by the Fourier inversion of the scattering intensity $I(q)$ as

$$p(r) = \frac{1}{2\pi^2}\int_0^\infty qrI(q)\cdot\sin(qr)\,\mathrm{d}q$$

where r is the distance between every pair of scattering points.

The radius of gyration, R_G, was estimated by the equation:

$$R_G{}^2 = \frac{\int_0^{D\mathrm{max}} p(r)r^2\,\mathrm{d}r}{2\int_0^{D\mathrm{max}} p(r)\mathrm{d}r}$$

where $D_{\max}$ is the maximum diameter of the particle estimated from the analyzed condition $p(r) = 0$ for $r > D_{\max}$. The R_G value is a common parameter determining the size of polymer molecules. If a polymer chain is considered to be composed of segments, R_G can be expressed as the mean of the distance from the center of gravity to each segment. The ratio of $D_{\max}$ to R_G provides a shape factor for a polymer molecule. For example, a spherical particle is indicated by a ratio of two ($D_{\max}/R_G = 2$).

Results and Discussion

SAXS

Divergence of scattering intensity in the very small angle region ($q<0.2$ nm^{-1}) was observed for both the Dando and Inogashira HA samples, indicating stable dispersion of the humic acid particles in the suitable solvent (Fig. 1). Although the scattering intensities of both FAs were much smaller than those of the HAs,

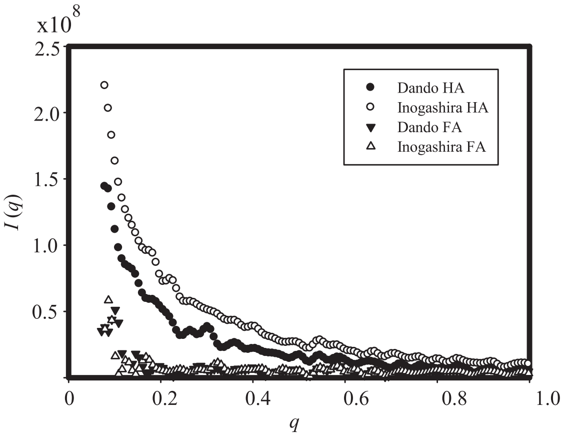

Fig.1 Scattering intensity of humic substances in the small angle region as a function of $q(=4\pi \sin(\theta/2)/\lambda)$ vector.

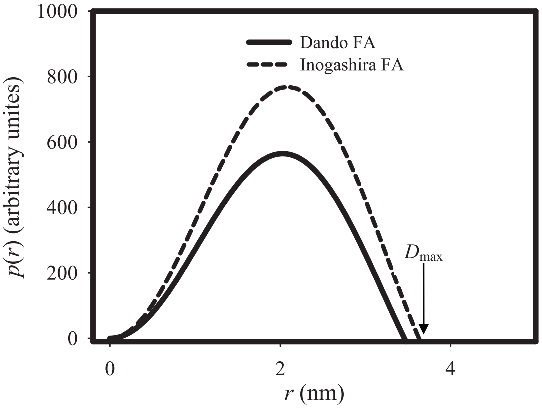

Fig.2 Distance distribution function, $p(r)$, of fulvic acids.

the intensity was sufficiently greater than that of the solvent to estimate values of R_G. The smaller scattering intensities of both FAs probably correspond to the lower electron density and smaller sizes of the FAs.

The $p(r)$ function relates to the electron density distribution of solutes in solution, referring to particle size in the solution. Both FAs show the Gaussian-like shape of the $p(r)$ functions ranging between 0 and nearly 3.5 nm distance (D_{max}) (Fig. 2). Monomeric spherical colloidal particles in a monodispersed system indicate a typical Gaussian-like $p(r)$ function with a diameter of D_{max} and with a radius at the peak $p(r)$ value (Kajiwara and Hiiragi, 1996). The FA solute in the solution probably behaves as a spherical colloid with stable dispersion in a kind of "monodispersed" solution system. Observed particles in the Dando and Inogashira FA solutions have D_{max} values of 3.3 and 3.5 nm and R_G values of 1.4 and 1.5 nm, respectively. These estimated sizes are similar in both the Dando and Inogashira FAs, showing that similar behavior of both FAs in the buffer solution results in almost identical particle sizes. Smaller particle sizes of aquatic FAs in a kind of "monodispersed" solution system were calculated by Thurman et al. (1982) using the Guinier plot of SAXS data. The range of their R_G values was between 0.5 and 1.5 nm. Although agglomeration was considered not to be present from the $p(r)$ function in the present analytical conditions (concentration 1 g L^{-1}), an increase in the FA sample concentration could result in a larger particle size due to agglomeration, such as that observed by Pranzas et al., (2003) using SANS (concentration 1 g L^{-1}) and SAXS (concentration 10 g L^{-1}) data from aquatic FAs.

Unlike the FAs, the $p(r)$ functions of HAs indicated relatively complicated shapes (data not shown) and distribution with wide ranges of r reaching D_{max} values of 21 and 29 nm in the Inogashira and Dando HA solutions, respectively (Table 1). The estimated R_G values were 7.3 nm and

Table 1. Size parameters calculated from SAXS data and Einstein's equation using intrinsic viscosity ($[\eta]$) and weight average molecular weight ($\bar{M}_w$) of humic and fulvic acids.

	R_H (nm)	R_G (nm)	D_{max} (nm)	R_G/R_H	D_{max}/R_G	$[\eta]^{\dagger}$	$\bar{M}_w{}^{\ddagger}$
Dando HA	3.16	10.11	28.57	3.20	2.83	8.66	7140
Dando FA	1.21	1.42	3.34	1.17	2.35	10.08	19788
Inogashira HA	2.14	7.33	20.87	3.43	2.85	3.73	2517
Inogashira FA	1.14	1.48	3.52	1.30	2.37	3.84	2896

R_H: Hydrodynamic radii, R_G: Radii of gyration, D_{max}: maximum diameters of humic and fulvic acids estimated from distance distribution functions

† Data from Kawahigashi (2007a).

‡ Data from Fujitake (2007a).

10.1 nm for the Inogashira and Dando HAs, respectively. The R_G values of HAs were similar to previously reported values using soil and aquatic HAs estimated by SAXS, ranging between 1 and 10 nm (Wershaw and Pinckney, 1973; Kawahigashi et al., 1995). The apparent particle dimensions of the Dando HA in solution were larger than that of the Inogashira HA. However, the ratio of D_{max} to R_G (D_{max}/R_G) was very similar in both HAs (approximately 2.8), indicating a similar average particle shape in these solutions. The humic acid particles in the solution are considered to be relatively elongated, rather than spherical colloids. Spherical colloids with a smaller ratio of D_{max}/R_G were probably dominant in the FA solutions.

Estimation of hydrodynamic radii (R_H)

To allow comparison with R_G values, the hydrodynamic radius (R_H) was estimated using Einstein's equation applicable for a hydrated spherical colloid consisting of a flexible polymer in a good solvent (Lovell, 1989). The hydrodynamic radius can be calculated as a Stokes radius of a spherical colloid in a solvent. The size of R_H corresponds to the radius of the solvated particle. The particle size can change with the solution conditions. The Einstein's equation represents the relationship between the intrinsic viscosity $[\eta]$ and the R_H value as

$$[\eta] = 10\pi R_H^3 N_A / 3M$$

where N_A is the Avogadro's number and M is the molecular weight. The weighted average molecular weight ($\overline{M}_w$) is recommended in the equation because of similarity in equations between $\overline{M}_w$ and the viscosity average molecular weight ($\overline{M}_v$) (Kurata et al., 1975, Lovell, 1989). To obtain R_H values, the unknown parameters of $\overline{M}_w$ and $[\eta]$ were taken from published data for the standard humic substances (Fujitake, 2007a; Kawahigashi, 2007a). The values of $\overline{M}_w$ were obtained by a relative determination using the HPSEC analysis calibrated with a set of polystylene sulfonates in a phosphate buffer solution (pH 7.0). This analytical condition was applicable for the determination of the apparent molecular weight of humic substances with high reproducibility and sample recovery (Asakawa et al., 2008). The $[\eta]$ values were determined using Huggins' equation relating concentration dependence of specific viscosities of sample solutions. The R_H values of humic substance samples calculated from Einstein's equation using $\overline{M}_w$ values by the HPSEC analysis fitted well to the R_H values determined by a standard calibration (Kawahigashi et al., 2005).

The calculated R_H value of the Inogashira FA (1.1 nm) was almost the same as that of the Dando FA (1.2 nm) (Table 1), probably due to similarity of $\overline{M}_w$ and $[\eta]$ values in the two FAs. The Dando HA, having larger values of $\overline{M}_w$ and $[\eta]$, had a greater R_H of 3.2 nm than the Inogashira HA (2.1 nm). The order of R_H values was the same as that of R_G values obtained by SAXS measurement.

The relationship between both radii is theoretically represented by the equation: $R_G^2=3/5R_H^2$ (Burchard, 1983). Assuming the humic substance particles to be spherical colloids in order to estimate the radii using Einstein's equation, the calculated R_H value was expected to be larger than R_G determined by the SAXS analysis. However, R_H calculated from Einstein's equation was always smaller than R_G estimated by the SAXS data, even though sample preparations and solution conditions were different between the two analyses. The difference between the R_H and R_G values was especially large in the HAs. Smaller values of $\overline{M}_w$ estimated by the HPSEC technique under very dilute concentration conditions could affect the calculation of R_H and result in the smaller values of R_H than R_G. The sample concentration in the HPSEC analysis should be low enough to avoid interactions between gel materials and humic substances, while the SAXS measurement requires a relatively high concentration to obtain sufficient scattering intensity from humic substances. The different concentration conditions could affect HA particle sizes and shapes.

The ratio of R_G to R_H (R_G/R_H) provides information on solute shape (Van De Sandre and Persoons, 1985). A rigid spherical particle has a relatively low R_G/R_H ratio as compared with a rigid rod particle. Other colloid shapes such as ellipsoidal, disk, flexible and semi-flexible colloid are indicated by ratios between those for rigid sphere and rigid rod colloids. As the ratio is a relative value, it is comparable among colloidal particles under the same solution conditions. Here the R_G/R_H ratios in HA and FA solutions were calculated. The R_G/R_H ratios in the HAs (3.2 and 3.4) are larger than those in the FAs (1.2 and 1.3), indicating that HA particles in the solution are mainly elongate, rather than spherical colloids. Fulvic acid particles in the solution probably behave as spherical colloids, different from the HAs.

Influence of chemical structure on the size and shape of humic substances

Both FAs are characterized by a greater content of acidic functional groups (total acidity 9.08 $\text{cmol}_c\ \text{kg}^{-1}$ C for Dando FA; 10.88 $\text{cmol}_c\ \text{kg}^{-1}$ C for Inogashira FA) as compared with the HAs (5.88 $\text{cmol}_c\ \text{kg}^{-1}$ C for Dando HA; 6.31 $\text{cmol}_c\ \text{kg}^{-1}$ C for Inogashira HA) (Tsutsuki, 2007), while there is a greater aromaticity in the Inogashira HA (59 %) and a larger proportion of saturated aliphatic hydrocarbons in the Dando HA (21.3 %) (Fujitake, 2007b). They are important structural characteristics of the two HAs. These chemical structures can provide higher hydrophobicity in the HAs and higher solubility in the FAs. The HAs may readily form aggregates in solution due to hydrophobic interaction between particles, especially under the conditions of high solution concentration. The Dando HA with long saturated aliphatic hydrocarbons probably tends to form micelle-like aggregates via surface-active properties (Kawahigashi, 2007b). On the other hand, the FA particles can disperse with high stability because of intermolecular electrostatic repulsion between the acidic functional groups.

The elongate shape of HAs was also suggested by the ratio of $D_{\max}$ to R_G under the relatively high concentration of 1000 mg L^{-1}. The HA particles observed by SAXS are probably aggregates consisting of smaller-sized HA particles, as confirmed by the HPSEC analysis at the low concentration of 20 mg L^{-1}. These aggregates could be formed from small HA particles through hydrophobic interaction. In contrast, the FAs with low $D_{\max}/R_G$ and R_G/R_H ratios could be stably dispersed as single particles, indicating no formation of aggregates in the solution due to electrostatic repulsion between FA particles. The difference in size and shape between HAs and FAs can be attributed to their chemical structures contributing to their colloidal behaviors.

In conclusion, the average particle sizes, R_H, in HA solutions estimated using HPSEC under dilute concentration conditions were smaller than R_G values estimated by the $p(r)$ function of SAXS. This suggests that the particle sizes of HAs were variable depending on concentration and probably other solution conditions, such as pH and ionic strength. The particle sizes of HAs were probably influenced by aggregation of small HA particles in the solution through hydrophobic interaction. The aggregation is considered unlikely to be random but to grow in an ellipsoidal manner. However, to confirm the aggregate formation of HA particles, it would be necessary to investigate changes in particle size of HAs with changing concentration using a single analytical method. Changes in colloidal sizes of FAs depending on concentration were not confirmed under the experimental conditions used in this study. The FA particles were probably not easily able to form aggregates due to their stable dispersion in solution as a result of electrostatic repulsion between particles. The differences in colloidal behavior between the HAs and FAs under the solution conditions can be attributed to charge density or hydrophobicity in the FAs and HAs.

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