

# Size and shape of soil humic acids estimated by viscosity and molecular weight

Masayuki Kawahigashi\*, Hiroaki Sumida, Kazuhiko Yamamoto

*College of Bioresource Science, Nihon University, Fujisawa, Kanagawa 252-8510, Japan*

Received 9 April 2004; accepted 20 October 2004

Available online 22 December 2004

## Abstract

Ultrafiltration fractions of three soil humic acids were characterized by viscometry and high performance size-exclusion chromatography (HPSEC) in order to estimate shapes and hydrodynamic sizes. Intrinsic viscosities under given solute/solvent/temperature conditions were obtained by extrapolating the concentration dependence of reduced viscosities to zero concentration. Molecular mass (weight average molecular weight ( $\bar{M}_w$ ) and number average molecular weight ( $\bar{M}_n$ )) and hydrodynamic radius ( $R_H$ ) were determined by HPSEC using pullulan as calibrant. Values of  $\bar{M}_w$  and  $\bar{M}_n$  ranged from 15 to  $118 \times 10^3$  and from 9 to  $50 \times 10^3$  ( $\text{g mol}^{-1}$ ), respectively. Polydispersity, as indicated by  $\bar{M}_w/\bar{M}_n$ , increased with increasing filter size from 1.5 to 2.4. The hydrodynamic radii ( $R_H$ ) ranged between 2.2 and 6.4 nm. For each humic acid,  $\bar{M}_w$  and  $[\eta]$  were related. Mark–Houwink coefficients calculated on the basis of the  $\bar{M}_w - [\eta]$  relationships suggested restricted flexible chains for two of the humic acids and a branched structure for the third humic acid. Those structures probably behave as hydrated sphere colloids in a good solvent. Hydrodynamic radii of fractions calculated from  $[\eta]$  using Einstein's equation, which is applicable to hydrated sphere colloids, ranged from 2.2 to 7.1 nm. These dimensions are fit to the size of nanospaces on and between clay minerals and micropores in soil particle aggregates. On the other hand, the good agreement of  $R_H$  values obtained by applying Einstein's equation with those directly determined by HPSEC suggests that pullulan is a suitable calibrant for estimation of molecular mass and size of humic acids by HPSEC.

© 2004 Elsevier Inc. All rights reserved.

**Keywords:** Viscosity; HPSEC; Weight average molecular weight; Number average molecular weight; Polydispersity; Hydrodynamic radius; Flexible chain; Branched structure; Humic acid; Ultra filtration

## 1. Introduction

Humic substances, either dissolved or attached to minerals, are ubiquitous constituents of terrestrial and aquatic environments. They are chemically active at interfaces between solid, liquid, and gas phases. Humic substances adsorb strongly to minerals, especially hydrous oxides of Al and Fe [1–3] and can be sequestered within nanospaces of the mineral matrix of soils and sediments [4,5]. Microbial metabolism of humic substances depends on the size of the pores wherein they reside [6]. Also, dissolved and mineral-associated humic substances can bind and thus influence the geochemical fate of hydrophobic xenobiotics and

metals [7]. The colloidal properties of humic substances, such as size and shape, are likely crucial for their mobility and sorptive interactions with solids and solutes. However, the size and shape of humic substances have been analyzed less frequently as compared with other properties such as molecular weight.

Viscometry is a suitable method for estimation of sizes and shapes of polymers and/or macromolecules. Previous studies on humic substances have shown that they change their shape depending on pH, ionic strength, and concentration [8–12]. Viscosity analyses can provide information on the viscosity average molecular weight ( $\bar{M}_v$ ) by regarding humic substances as flexible chain polymers [9,10,13,14]. This procedure seems valid because the molecular weights calculated from viscometry data resemble much those obtained by other methods [15]. Nevertheless, conformations

\* Corresponding author. Fax: +81-466-84-3952.

E-mail address: [khigashi@brs.nihon-u.ac.jp](mailto:khigashi@brs.nihon-u.ac.jp) (M. Kawahigashi).

other than flexible chains may occur because of the heterogeneous composition of humic substances depending on origin and formation conditions.

The relationship between viscosity and molecular weight in a series of polymers can provide information on its structure [16]. Visser [17] and Kawahigashi and Fujitake [18] have succeeded in obtaining structural information on humic acids applying the relationship to a series of humic acid-size fractions prepared. The results suggested branched and flexible chain characteristics, respectively.

Based on knowledge of the conformation of a polymer, it is possible to calculate the hydrodynamic size from the intrinsic viscosity by applying an equation appropriate to the shape [19]. In many studies on the dimensions of humic studies, equations were adopted without their shape taken into account [10,13,20]. In this study, structures of soil humic acids were estimated from viscosity and molecular weight by analyzing a series of size fractions. Second, hydrodynamic sizes were calculated, considering these structures.

## 2. Materials and methods

### 2.1. Humic acids

Humic acids were extracted from three different soils (A horizons) in order to obtain samples with different structural compositions. The Minakami sample (MK) was from a Fulvic Andosol under deciduous forest, the Hiratsuka sample (HT) was from a Eutric Fulvisol managed for upland arable land, and the Fujisawa sample (FJ) represented the buried A horizon of a Pachic Melanic Andosol.

The soils were slurried in a 1:1 mixture of 0.2 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and 0.2 M NaOH (10 ml per gram soil) under N<sub>2</sub> atmosphere at room temperature for 24 h. The soil suspensions were centrifuged at 8000 rpm (11,000g) for 15 min, the supernatant was removed, and the settled soil materials were extracted a second time. The two extracts were combined, acidified to pH 1.5 by adding 1 M HCl, and the precipitated humic acids were separated from the supernatants by centrifugation at 8000 rpm for 30 min. The humic acids were further purified by elimination of fulvic acids by redissolution and reprecipitation with 1 M NaOH and 1 M HCl, respectively, and removal of clays by passing through 0.45- $\mu$ m filters (hydrophilic polytetrafluoroethylene, PTFE, membrane) and treatment with 0.3 M HF–0.1 M HCl (pH < 1.5). After dialysis against ultrapure water, the sample was freeze-dried. The humic acid samples were designated according to the respective soil's name.

### 2.2. Ultrafiltration

Subsamples of 500 mg of each humic acid were dissolved in 1 L of 0.1 M NaOH and the pH was adjusted to 7.0. The humic acid concentration of 500 mg L<sup>-1</sup> was

less than the critical concentration for aggregation or micelle formation [21–24]. The humic acid solution was passed through the Remolino ultrafiltration (UF) system (Millipore Co. Ltd., Tokyo, Japan). The retentate on an UF membrane was recovered by rinsing with distilled water. After dialysis under acidic conditions (pH < 1.5) against distilled water, the retentate was freeze-dried. Filtrates were concentrated to >500 mg L<sup>-1</sup> by rotary evaporation and then dialyzed against pure water. Concentrations and pH of the filtrate fraction were adjusted to 500 mg L<sup>-1</sup> and to 7.0, respectively. Then the solution was passed through the UF membrane with the next smaller pore size. Humic acid solutions were successively passed through UF membranes with 100, 30, and 10  $\times$  10<sup>3</sup> g mol<sup>-1</sup> molecular weight cutoff, from a larger to a smaller pore size. The filtrate of the 10  $\times$  10<sup>3</sup> g mol<sup>-1</sup> UF membrane was deionized and freeze-dried. Each retentate was named according to each molecular weight cutoff, 100K, 30K and 10K fraction; the smallest fraction passing the membrane with the smallest pore size was named the 5K fraction.

For analyses of viscosity and for high-performance size-exclusion chromatography (HPSEC), all reagents were superanalytical grade and they were prepared with deionized water followed by reversed osmotic filtration.

### 2.3. HPSEC

A 10-mg subsample of each size fraction was dissolved in 1.0 ml of 0.05 M NaOH and the solution pH was adjusted to 7.0 with 0.01 M NaOH. The solution was brought to 5 ml with deionized water and then adjusted to 10 ml by adding 0.02 M K<sub>2</sub>HPO<sub>4</sub>–0.02 M KH<sub>2</sub>PO<sub>4</sub> buffer solution (pH 7.0) containing 20% of methanol. The solution was diluted to 0.01 g humic acid L<sup>-1</sup> with the HPSEC eluent (0.01 M K<sub>2</sub>HPO<sub>4</sub>–0.01 M KH<sub>2</sub>PO<sub>4</sub> buffer solution (pH 7.0) containing 10% methanol, which gives an ionic strength of 0.026 M). An aliquot of 0.5 ml of the diluted solution was filtered through 0.2- $\mu$ m syringe filters (hydrophilic PTFE) and 100  $\mu$ l of the filtrate was applied to the HPSEC system, comprising an UV detector. Shodex 805HQ (0.75 cm in diameter, 30 cm in height, Showa Denko KK, Tokyo, Japan) being preceded by a guard column was used for separation by size exclusion. The applied flow rate was 1.0 ml min<sup>-1</sup>.

### 2.4. Determination of molecular weights and hydrodynamic sizes

Weight average molecular weights ( $\bar{M}_w$ ), number average molecular weights ( $\bar{M}_n$ ), and hydrodynamic radii ( $R_H$ ) of humic acids were calculated using a calibration curve prepared with pullulan standards (Showa Denko KK) (Fig. 1). The equations of  $\bar{M}_w$  and  $\bar{M}_n$  were expressed by the following equations,

$$\bar{M}_w = \frac{\sum_{i=1}^n (M_i^2 \times N_i)}{\sum_{i=1}^n (M_i \times N_i)},$$

Download English Version:

<https://daneshyari.com/en/article/10378219>

Download Persian Version:

<https://daneshyari.com/article/10378219>

[Daneshyari.com](https://daneshyari.com)