AFM study on the adsorption and aggregation behavior of dissolved humic substances on mica

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Abstract Humic substances constitute the major organic fractions of soils, sediments and natural waters, and play a dominant role in the binding, mobilization, transport and ultimate fate of organic contaminants in subsurface systems. In this paper, two humic acid samples, Guanting and Tianjin, with different origin and chemical compositions have been investigated with AFM imaging for their adsorption and aggregation behaviors on mica. While the Tianjin humic sample is found to form small spheres with 250 to 330 nm in diameter at lower concentrations, irregular loop-chain assemblies of hundreds of nanometer in diameter with the chain width of about 40 nm are dominant for the Guanting humic sample, which may attribute to the more polar aliphatic fractions in the chemical composition in the latter. The heterogeneous and polydisperse nature of humic substances with multiple structural features, such as sponge-like structures, perforated sheets, aggregate of spheres, branches and chain-like assemblies etc., is apparent at higher concentrations for both humic samples, showing morphologically new evidence for the dominant view of the humic dual-mode sorption model. With naphthalene introduced, the assemblies of Guanting humic substances clearly become more compact with significantly narrowed branches and less porous the perforated sheet-like structures. It is indicative of that smaller nanometer scale rings present along the perforated assemblies could potentially represent hydrophobic domains, which may facilitate the adsorption and aggregation of naphthalene onto the natural particle surfaces and therefore lead to an important role of dissolved humic substances in the sorption of environmental pollutants.

Keywords: atomic force microscopy (AFM), dissolved humic substances, morphology, aggregation, adsorption.

Humic substances (HS) are a category of naturally occurring, biogenic, heterogeneous organic materials found in or extracted from soils, sediments, and natural waters that can generally be characterized as being yellow-to-black in color, of highly variable relative molecular masses, and refractory^[1,2]. Derived from a

variety of organic precursors (plant biopolymers such as lignin etc.), plant residues and animal debris via both transformation and synthesis processes^[3] under the profound geochemical alteration, including the microbiological and chemical degradation, sedimentation and diagenesis as well, humic substances com-

prise highly complex composites of partially or completely degraded biomolecules, carbohydrates, lipids, and proteins etc. However, they cannot be classified into any of the normal, easily defined categories of discrete materials such as proteins, polysaccharides, and polynucleotides. As the major organic constituents of soils, sediments and waters, humic substances are ubiquitous in all terrestrial and aquatic environments. They can form molecular aggregates, dispersed in different colloidal states in solution or deposited on mineral surfaces^[4,5], and therefore, strongly influence a wide range of environmentally important processes. For example, they may modify the chemical speciation of trace metals, stabilize inorganic colloids via adsorption^[6,7], and significantly impact the fate and transport of hydrophobic organic pollutants in subsurface systems^[8–16]. A better understanding of their morphology and the underlying adsorption and aggregation processes is therefore of fundamental importance.

Humic macromolecules have been described as flexible linear chains with a polyelectrolytic character^[17] or as random coils that could form fairly compact spheres^[18] for the theoretical conceptual model. Nevertheless, a precise description of their size, shape and structure remains difficult because of the complex, heterogeneous and polydisperse nature of the humic substances. In this context, a lot of physical instruments and techniques have been exploited for the structural characterization of humic substances, including diffusion coefficient measurement^[18,19], luminescence spectroscopy (pyrene fluorescence probe etc.)^[20], Size-Exclusion Chromatography (SEC)^[3], Cross Polarization Magic Angle Spinning carbon-13 Nuclear Magnetic Resonance (CPMAS ¹³C-NMR)^[21–23], Infrared (IR) analysis, and microscopic observation techniques like Scanning Electron Microscopy (SEM)^[21,24] and Transmission Electron Microscopy (TEM) etc. For example, Stevenson and Schnitzer^[25] performed TEM analysis on dried HS samples and showed that depending on the HS concentration their structures change from spheroids with dimensions in the nanometer range at low concentrations to chainlike structure or flattened linear fibers and to perforated sheet-like assemblies at higher HA concentrations. More recently, atomic force microscopy (AFM) has been used for observation on the morphology of humic

substances. Compared with other microscopies (SEM and TEM), AFM offers more flexibility in sample preparation (no need for dehydration, embedding, and coating etc.), three-dimensional images and in some cases better image resolution^[26], opening up an excellent opportunity for probing the structure and sorption behavior of natural particles and the associated humic substances. Heil and Sposito^[27] provided differences in AFM images of surface topography between the Handford soil colloids and the specimen illite particles to show visual evidence of organic coatings on the particles in the flocculation process of illitic soil colloids. Recently, Gerin and Dufrêne^[28] developed a procedure for probing the native surface structure of natural soil particles and characterized the properties of the adsorbed organic matter by combining AFM imaging and X-ray photoelectron spectrometry (XPS) analysis. A few reports have also described the use of AFM to characterize the structure of humic substances on mica after isolation from water streams and soils^[29–36]. Maurice *et al.*^[29,30], for example, have imaged aggregate structures of humic substances in aqueous solutions, and reported small spheres (10–50 nm) and “sponge-like ring structures”. Wilkinson *et al.*^[31,32] performed both transmission electron and atomic force microscopy imaging on the freshwater colloidal organic matters and investigated the effects of solution pH and ionic strength on the size and conformation of two different HS on mica. Plaschke *et al.*^[33–35] performed *in situ* Fluid-AFM imaging on the sorbed humic acid colloids and investigated the influence of different pH conditions and Eu(III) ions on the agglomeration of humic acid. Liu *et al.*^[36] compared the thickness of adsorption layers obtained by AFM imaging for the two different sample preparation methods of freezing and air-drying. Liu *et al.*^[37] investigated the effect of substrate with different characteristics on the composition and amount of extracellular polymer substances (EPS) in activated sludge and provided SEM and AFM images of the EPS morphology. In this study, AFM is employed to further investigation of the aggregate structures of humic substances on mica in order to reveal the possible mechanism of humic substances in modifying the sorption behavior of hydrophobic organic contaminants onto the natural particles. Two humic acid sam-

ples of Guanting and Tianjin with different origin and chemical composition have been investigated and naphthalene is introduced into the Guanting sample solution to show the structural changes during their interfacial adsorption processes.

1 Experimental

1.1 Materials and instrumentation

Ultrapure deionized water (Resistivity: 18 MΩ/cm) was used through all the experiments; chemical reagents used were all of analytical grade; nitrogen gas was of analytical pure.

(i) Humic substances and their characteristics.

Two humic acid (HA) samples were investigated for this study. One was a commercially available HA manufactured in Tianjin. The other was extracted from the sediment collected from the Guanting Reservoir, one of the major natural potable water resources near Beijing. It was operated according to the extraction and purification procedure given by the International Humic Substances Society (IHSS)^[38], which is shown in the following.

Appropriate portions of sediment were immersed in 0.1 mol/L NaOH, a nitrogen flow was introduced into the solution for 1 h. After purging the air with nitrogen the vessel was sealed and placed on a shaker for 12 h. The suspension was separated by centrifugation and the supernatant was collected. The residual sediment was treated twice as described in the above. The combined supernatants were then adjusted to pH = 2 using 6 mol/L HCl, and the solution was left overnight in order for the dissolved humic acids to release. After centrifugation, the supernatant was removed, and the

obtained humic acid substances were rinsed with distilled water for 3 times followed by air-drying at ambient conditions.

The basic physicochemical properties of the two HA samples used are given in Tables 1 and 2^[39]. The content of total organic carbon (TOC) and dissolved organic carbon (DOC) were determined on an Apollo-9000 TOC analyzer (Tekman-Dohrmann Co., U.S.A.). Potassium biphthalate (dried for 2 h before use) was used for the preparation of carbon standard solution. Elementary analysis was made on Vario-EL (Elementar Co., Germany) using the high temperature combustion method. Cation exchange capacity (CEC) was determined by BaCl₂ method as described in the literature^[40]. CPMAS ¹³C NMR and ¹H NMR (D₂O as solvent) spectra were made on Avance-DPV 300 MHz (Bruker Co., Germany. Measuring conditions: Probe, 4 mm in diameter; spinning rate, 13 kHz; contact time, 2 ms; delay, 1 s; scanning width, 27 kHz at δ = 368; Line broadening, 100 Hz) and Avance-DPX 500 MHz (Bruker Co., Germany. Measuring conditions: Probe, 5 mm in diameter; delay, 1 s; scanning width, 8012 Hz at δ = 16; line broadening, 3.00 Hz, Temperature, 295 K), respectively.

(ii) Naphthalene stock solution. Aqueous solution of naphthalene was prepared by dissolving excess amount of solid naphthalene (with a purity of 99%, purchased from Beijing, China) in a solution of 0.005 mol/L CaCl₂ as a background electrolyte and 100 mg/L NaN₃ to prevent bacterial growth. The mixture was subsequently treated by ultrasonic for 1 h, shaken overnight on a reciprocating shaker, and then filtered using GF/F filter paper (Whatman Co., England) to remove any insoluble solute. The filtrate was finally

Table 1 The physicochemical properties of the two HA samples of Guanting and Tianjin

Humic acid sample	Elemental analytical results (g/g) (%)				Analyzed by CPMAS ¹³ C NMR					TOC (%)	DOC ^{a)} (%)	CEC (cmol (+)/kg)
	C	H	O	N	aliphatic C (δ0–100) (%)	aromatic C (δ100–165) (%)	COOH (δ165–185) (%)	carbonyl (δ185–220) (%)	hydrophobic index			
Guanting	24.01	3.96	25.20	2.97	55.9	21.8	15.0	5.3	0.72	28.76	2.78	9.36
Tianjin	62.53	4.80	32.34	Null	41.1	41.1	2.5	12.4	0.92	64.77	0.87	3.22

a) Measured in the presence of 0.005 mol/L CaCl₂ as the ion strength medium.

Table 2 ¹H NMR results for the dissolved fraction of the two HA samples of Guanting and Tianjin

Humic acid samples	Aliphatic C (δ0–3.0) (%)	Aromatic C (δ6.5–8.5) (%)	Polarity (δ3.0–6.5) (%)	Hydrophobic index
Guanting	24.6	null	75.4	0.33
Tianjin	15.4	0.5	84.1	0.19

transferred into a brown flask and tightly sealed with Parafilm immediately. The naphthalene stock solution was preserved in a refrigerator at 4°C.

Freshly cleaved mica surface was used as substrate in all experiments. Natural muscovite slides with dimension of 2 cm × 3 cm and 0.18–0.20 mm in thickness were first cut into approximately 10 mm × 10 mm square pieces using a sharp, clean, single-edged razor blade. The mica pieces were then subjected to a through cleaning procedure which includes rinsing with distilled water to remove the small residual particulates created during the cutting process followed by a rinse with 95% ethanol and finally with absolute alcohol. The mica sheets were allowed to dry in air at ambient conditions in a clean room, and placed in a Petri dish for stock.

The atomic force microscope used was a NanoScope IIIa Multimode Scanning Probe Microscopy Instrument (Digital Instruments, Santa Barbara, CA) operating in air at room temperature with 50%–60% humidity. The images presented in this paper contain 256×256 data points (pixel resolution), acquired by using the retrace signal in the contact mode. The standard V-shaped silicon nitride (Si₃N₄) cantilevers (with integral tips, Model OTR8-35) of different stiffness and tip sharpness were used for imaging. Their nominal dimensions were 100- and 200- μm in length and 0.8 μm in thickness and possessed a spring constant ranging from 0.06 to 0.58 N/m. The scanning speed and the loop gain factors were varied during the imaging process. Line scan rates were typically set at about 2.5 Hz with other scan parameters such as Scan size, Set point, Integral gain, Proportional gain etc. varied and optimized. In order to avoid tip-related artifacts, imaging was performed with a minimal force and image features were reproduced before being accepted as representative. The prepared samples were investigated by AFM within a few hours after preparation. For each experimental condition, two mica sheets were prepared and at least three images were obtained for each mica sheet sample. Images were taken with online filtering and subsequently by flattening to remove the background slope. Using NanoScope Image analysis software, section analysis of images was conducted to measure the size of particles and their aggregates.

1.2 Experimental methods

Adequate amounts of HA samples were added to a mixture containing 0.005 mol/L CaCl₂ and 100 mg/L NaN₃, and the dispersion mixture was fully stirred overnight with a suspended magnetic stirrer while 0.1 mol/L NaOH was added drop-wise to maintain the alkalinity of the solution at pH = 9. After dissolution, the solution was left undisturbed for 24 h and the supernatant was filtered through the Millipore membrane. The solution was stored in a refrigerator at 4°C.

Two HA solutions with the concentration of 10 and 100 mg/L respectively (with 0.005 mol/L CaCl₂ and 100 mg/L NaN₃ of presence, pH = 8–9) were made from dilution of the above stock solutions. Aliquots of the HA solution were then transferred into a 50 mL conical flask for the AFM sample preparation experiments. Mica was selected as the model surface to simulate the adsorption process of the dissolved humic substance on clay minerals. Before use of fresh basal-plane, surfaces were exposed by cleaving several upper layers with a scotch tape and then introduced into the HA solution. After stirring for 60 h on a shaker bed the mica was removed from the solution and gently rinsed by immersion into the deionized water (30 s) in order to remove any non-adsorbed humic acid and loosely attached substances. The mica was then allowed to dry in an enclosed Petri dish under ambient conditions and mounted on magnetized stainless steel stub by means of double-sticky tape for AFM observation.

For studying the adsorption of HA in the presence of naphthalene, in parallel with the HA control blank, a saturated solution of naphthalene was introduced into the HA dispersions before the mica sheet was immersed into the solution and operated in the same manner as described above for the adsorption and the AFM observation.

2 Results and discussion

Figs. 1 and 2 give a comparison of the morphological features of Guanting and Tianjin humic samples at the nano-scale level under different concentrations. It is shown that irregular loop-chain assemblies of hundreds of nanometer in diameter with doped particulates are dominant for the Guanting sample at 10 mg/L,

Fig. 1. AFM images showing the structural difference between Guanting and Tianjin humic samples at lower concentration (10 mg/L). (a) and (b) Topography of Guanting sample and its 3-D close-up of the irregular loop-chain assemblies with the chain width of c.a. 40 nm; (c) and (d) topography of Tianjin sample and its 3-D close-up of the discrete spheroids with the diameter of 250–330 nm.

the chain width of which is about 40 nm with a height of 0.5–1.0 nm, while the Tianjin sample, however, was found to form more compact assembly structures of small discrete spheres with 250 to 330 nm in diameter and 8.6 nm in height. At higher concentrations (30 or 100 mg/L, Fig. 2), both HA samples are shown to have multiple assembly structures, in which sponge-like structures, perforated sheets, aggregates of spheres, etc. are apparent. Compared with the compact structure of Tianjin humic sample, the heterogeneous and polydisperse features of Guanting humic substances are more evident, showing both loose and compact assemblies with a great variety, including sponge-like structures, branches, gel-like clumps etc. (Fig. 3). These morphological differences may attrib-

ute to their different origin, composition and physico-chemical nature.

As shown in Tables 1 and 2, a major difference is shown for their composition and the physicochemical properties. The Guanting sample gives a TOC content of 28.76% with the DOC value of 2.78%, while the Tianjin humic substance, however, shows a higher TOC of 64.77% but much lower content of DOC (0.87%). CPMAS ^{13}C NMR and ^1H NMR spectra provide further evidence on the functional group and structural composition of the two HS samples. The Guanting sample presents large N content (2.97%) and high O/C ratio (0.79) with H/C ratio of 1.98, and the CPMAS ^{13}C NMR spectra show a high aliphatic and carboxylic carbon content (55.9% and 15.0, respec-

Fig. 2. AFM images of Guanting ((a) and (c)) and Tianjin ((b) and (d)) humic samples at higher concentrations ((a) and (b), 30 mg/L; (c) and (d), 100 mg/L). (a) and (b) Perforated sheet-like structures; (b) and (d) aggregated spheroids and globular clusters.

tively) with the relatively low aromatic and carbonyl carbon contents (21.8% and 5.3%, respectively), indicating that it is rich in polar groups and aliphatic compounds. The Tianjin humic substance, however, exhibits a high content of C (62.53%) with its H/C ratio (0.92) close to 1 and the O/C ratio of 0.39, and the CPMA¹³C NMR spectra indicate a nearly equivalent amount of both aliphatic and aromatic carbon (41.1%), very low carboxylic carbon (2.5%) with the high carbonyl carbon of 12.4%, implying a higher content of aromatic compounds and high content of low polar groups in the sample.

Since aliphatic and polar organic fractions constitute the majority of Guanting humic sample, these components have amphiphilic groups as in the molecular structure of surfactants, which may form micelle-like aggregates in solution and aggregate into various assembly structures at mineral surfaces. Fig. 3 presents morphologically more evidence on the heterogeneous and polydisperse features of Guanting

humic sample, especially when higher HA concentrations are used. These results are in accordance with the above discussion about its structure and composition.

As shown in Fig. 4, Guanting humic sample was further examined for its morphological changes on mica under the coadsorption of naphthalene in order to investigate the interaction mechanism of humic substances in the sorption of naphthalene onto the natural particles. It is obvious that under the coadsorption of naphthalene the assemblies of humic substances clearly become more compact than those of the controlled sample blank without naphthalene, the chain width changing from 500–650 nm to 300–350 nm and the perforated sheet-like structure reducing from 1.5 μ m to 500 nm in width. Moreover, gel-like clumps were further observed for the naphthalene-incorporated sample. The aggregated branches become significantly narrowed down and the perforated sheet-like structures become less porous, implying that naphthalene molecules may form associates with the humic

Fig. 3. Polydispersity of Guanting humic sample on mica at 100 mg/L. (a) Gel-like clumps and micro flocs; (b) loosely assembled structures and sponge-like domains; (c) and (d) aggregated globular clusters and their close-up image, showing the tightly assembled structure of 50 nm in diameter and 8–15 nm in thickness; (e) and (f) branched chain-like structures and their close-up image, indicating the compact ribbon assembly or bundle of flattened filaments.

substances, reinforce connections between the HA molecules, and therefore cause the assembly structures to be more compact. It is indicative of that smaller nanometer-scale rings present along the circumstance in the perforated sheet-like assembly structures could potentially represent hydrophobic domains, which may alter the assembly structure via the hydrophobic interaction and therefore lead to an important role of

dissolved humic substances in the sorption of environmental pollutants.

Despite much discrepancy in their complex molecular structures in the literature, humic substances have been substantiated to consist of separate hydrophobic and hydrophilic parts and form self-associating aggregates in solution by intermolecular hydrophobic interactions^[20,41]. As amphiphilic polyelectrolytes,

Fig. 4. Changes in the morphology of Guanting humic sample with the coadsorption of naphthalene (naphthalene, 30 mg/L, right images of (b), (d) and (f); humic concentration, 30 mg/L), showing that the aggregates of humic substances become more compact when naphthalene is introduced.

humic substances can be dissolved or precipitated in aqueous media, can accumulate at interfaces, can form self-assemblages, can exist in different colloidal states. Their morphology may not be permanently unchanged. Whether they take the form as flexible linear chains or

as random coils in their molecular conformation, depend on their solution conditions, including the concentration of humic substances, solution pH, ion strength or salinity, polyvalent metal ions^[16,34,35] and temperature as well. Hydrophobic organic contami-

nants, such as naphthalene etc., have strong affinity toward the hydrophobic groups of the humic substances, and their adsorption onto the natural particles acts mainly on the organic layers of the natural particles. Therefore, earlier investigations^[10,11,42–45] showed that sorption of hydrophobic organic contaminants (HOCs) by soils and sediments is dominated by partitioning into relatively homogeneous, lipophilic, gel-like humic matrices, which can be recognized as a partition or dissolution process and has the linear adsorption features. A number of more recent studies^[12–15,46–55], however, have indicated that such sorption often manifests substantial deviations from a linear partition model, provided that very low concentrations of HOCs are studied. In that case, a presumed adsorption process is often observed, as evidenced by the nonlinear, slow, hysteretic and competitive sorption behaviors. The overall sorption process of HOCs is distinctively shown to be biphasic, generally involves a fast initial uptake followed by a very slow sustained rate that may require periods of weeks or even months or years to reach apparent equilibrium^[56]. It has been further hypothesized that sorption of HOCs by soils and sediments is highly dependent on the structure and chemical composition of associated humic substances at the microscopic scale, which may comprise two distinctly different types of domains: a soft, highly amorphous and swollen domain and a hard, relatively condensed and tightly knit domain. While the concept of two principal types of SOM domains appears quite reasonable as a basis for reconciling the non-partitioning-like behaviors exhibited by most soils and sediments, further experimental evidence on the morphology and structure of the two different domains is preferred. We have here investigated the sorption and aggregation behavior of dissolved humic substances on mica and presented direct evidence on the heterogeneity and polydispersivity of humic substances at interface. Furthermore, the comparative study on the sorption behavior of dissolved humic substances under the coadsorption of naphthalene indicates that dissolved humic substances may present the ring circumstances along the small pores inside the perforated sheet-like assemblies as the hydrophobic domains to form associates with HOCs, which makes the interaction between the HA molecules much

stronger, and therefore causes the assembly structures to be more compact.

3 Conclusion

A major difference in the morphology has been identified for the two humic samples of Guanting and Tianjin. The Guanting HA was found to form irregular loop-chain assemblies of hundreds of nanometer in diameter, while the Tianjin HA showed small discrete spheres. Compared with the compact assembly of the Tianjin HA, the Guanting HA showed both loose and tight assemblages with a great variety at higher concentrations, which can be attributed to the more polar aliphatic fractions in the chemical composition in the latter.

With the coadsorption of naphthalene, assemblies of Guanting HA clearly become more compact, showing significantly narrowed branches and the perforated sheet-like structures being less porous. It is indicative of that naphthalene molecules may take up the smaller nanometer-scale rings present along the perforated assemblies as the hydrophobic domains to form associates with humic substances and cause the assembly to become more compact, which may facilitate the binding and transport of naphthalene onto the natural particles.

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