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Colloids and Surfaces A: Physicochem. Eng. Aspects 302 (2007) 121–125
COLLOIDS AND SURFACES A
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Direct observation of macromolecular structures of humic acid by AFM and SEM

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Received 1 December 2006; received in revised form 31 January 2007; accepted 2 February 2007
Available online 11 February 2007

Abstract

Humic substances (HSs) can be easily adsorbed on natural solid surface and thereby affect the physicochemical behavior of metal ions in the natural environment. The influence of HSs is mainly attributed to the macromolecular structures and shapes of solution soluble and surface adsorbed HSs. Herein, to our knowledge, we firstly use both AFM and SEM to image and to compare the macromolecular structures of humic acid on the basal-plane surface of mica and silicon. Under our experimental conditions, spherical colloids are formed at low pH and high salt concentrations, while network or linear structures are formed at high pH and low salt concentrations. The shapes of HA are heterogeneous under different natural environmental conditions. The knowledge of macromolecular structures and shapes of HA in the natural environment is significant for the evaluation of metal ions' behavior in HA–metal ion–mineral ternary systems.

Keywords: Humic acid; Macromolecular structure; SEM; AFM

1. Introduction

Humic substances (HSs) are the major components of aquatic organic colloids and ubiquitous in natural groundwater. Humic substances, of which humic acids (HAs, insoluble at acidic pH) and fulvic acids (FAs, water soluble at acidic to alkaline pH) are the major fractions, presence various chemically reactive functional groups, including carboxyls, phenolic and alcoholic hydrocyls, with pH-dependent properties [1,2]. HSs have marked influence on the species of cation ions, and thereby can control/affect the biological availability, physicochemical properties, and environmental sorption/desorption or macro- and micro-nutrients, toxic metal ions, and xenobiotic organic cations [1,3–6]. When HS was adsorbed to mineral surfaces, HS may bind metal ions and hence immobilize trace metal ions, radionuclides, and non-ionic organic pollutions [7], and they may alter the charge properties of clay mineral surfaces [8]. Effect of humic substances on metal ions' sorption to oxides and minerals has been studied extensively [9–15]. The results indicated that the influence of HS on sorption was different for the different types and sources of humic substances.

The influence of HSs on metal ions' sorption to solid surface is mainly dominated by the surface structures of HSs. However, the direct observations on the macromolecular structures and shapes of HSs are still scarce, only few literatures are available for the macromolecular structures [16–18]. Both the primary chemical structures and the conformational structures of humic substances are still a matter of debate. Aqueous humic substances exist as soluble at low concentration and form colloids or precipitates when they react with cation ions. These changes can alter the macromolecular structures of HSs, and thereby affect the physicochemical properties of HSs [17]. Different research works indicate that HSs exhibit different structures at different conditions. HSs form coils in acidic and strong electrolyte solutions, and elongated structures is formed in dilute alkaline solutions [17]. HSs behave as rigid spherocolloids or closed/compacted network at high HSs concentration, low pH or high neutral electrolyte concentrations but as flexible linear colloids at low HS concentration (except at very low pH or high electrolyte concentrations), and high pH value [19]. Humic sub-

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doi:10.1016/j.colsurfa.2007.02.014

stances can form linear structure as pH is increased, electrolyte concentration is decreased.

Sorption/complexation of metal ions to humic substances generally depends on pH values [20,21], and other foreign cation ions [22]. However, sorption of methylmercury does not show a strong pH dependence with the pH range of 5–9 [23]. The results of humic substances measured by photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM) indicate that the average size increases with increasing ionic strength [16]. The characterization of the size, shape, conformation, structure and composition of HSs is crucial to understand the physicochemical reactions and to evaluate their role in the natural environment [16]. HSs can be characterized by fluorescence spectroscopy [24,25], AFM [17,18] and TEM [16]. The macromolecular structures of different HSs are quite different and sometimes they are inconsistent with each other under very similar conditions [16–19]. Of all the methods mentioned above, AFM is for the most part a non-destructive technique, and it allows for extremely high-resolution (molecular-scale) image of surfaces under environmental relevant conditions [19]. It is a valuable tool for the study of colloidal morphologies, agglomeration, and adsorption of HS on solid surfaces.

To directly determine the macromolecular structures of HS aggregates, we herein used AFM and SEM to image HA sample naturally dried on mica and silicon surfaces. The objectives of this work are: (1) to image the physical shapes and macromolecular structures of HA on the basal-plane surface of mica and silicon using AFM and SEM; (2) to study the influence of pH and ionic strength on the macromolecular structures of HA; and (3) to discuss the parameters which affect the agglomeration and/or loosely self-association of HA.

2. Experimental section

2.1. Sample preparation

HA was dissolved in NaOH solution (pH 9.0) to get the concentration of HA 100 mg/L. Then the mother HA solution was used to prepare the concentration of HA (50 mg/L) at different pH values.

It is well known that the sample for SEM analysis must be carried out under high vacuum conditions. Thereby, the first step was to cast one drop of HA solution on the silicon substrate (the substrate was successively cleaned with deionized water, ethanol and acetone for about 30 min, respectively, by ultrasonic wave cleaner, and dried in nitrogen (N_2) ambient) and let it naturally dry in the ambient conditions at room temperature for 24 h. Then the sample was treated with metal spraying (platinum (Pt)) in the vacuum tank whose vacuum degree was below 0.05.

2.2. AFM and SEM measurements

The SEM measurements (HITACHI S-4300, Hitachi, Japan) were performed under vacuum conditions (about 10^{-3} to 10^{-4} Pa), and scanned directly to achieve the SEM image. The AFM measurements (Nanoscope IIIa SPM, Digital Instruments, Santa Barbara, USA) were performed in tapping mode under ambient conditions. The mica samples with adsorbed HA were scanned and height histograms were derived from the maximum heights of individual particles measured in line profiles. A hard silicon cantilever tip was used with a spring constant of 40 N/m.

3. Results and discussion

3.1. SEM study

The SEM images of the as-prepared HA samples in 0.01 M $NaNO_3$ solution at pH 5.0 and 11.0 were shown in Figs. 1 and 2, respectively. The typical compacted phase of HA at low pH and high electrolyte concentrations as rigid spherical colloids in the natural environment was found in Fig. 1. Maurice and Namjesnik-Dejanovic [19] used tapping-mode AFM to observe the peat fulvic acid in 0.01 M $CaCl_2$ at pH ~5.5 and found that FA molecules aggregated into ring-shaped structures, and not all of the rings were fully closed. Stumm and Morgan [26] predicted that HS molecule in solution would have a coiled configuration and a small hydrodynamic radius at low pH and high ionic

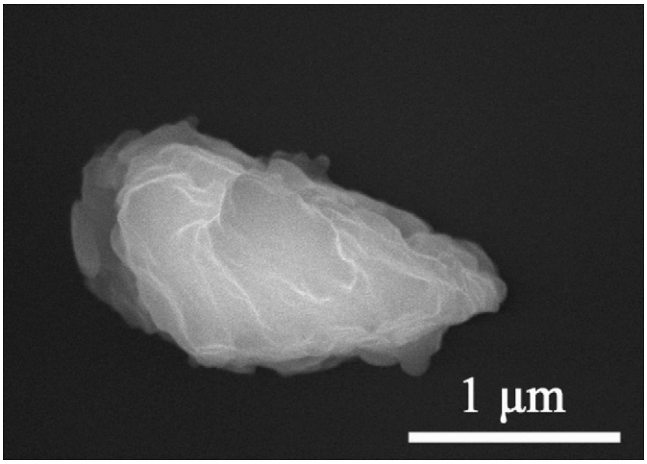

Fig. 1. The SEM image of the as-prepared HA sample in 0.01 M $NaNO_3$ solution at pH 5.0.

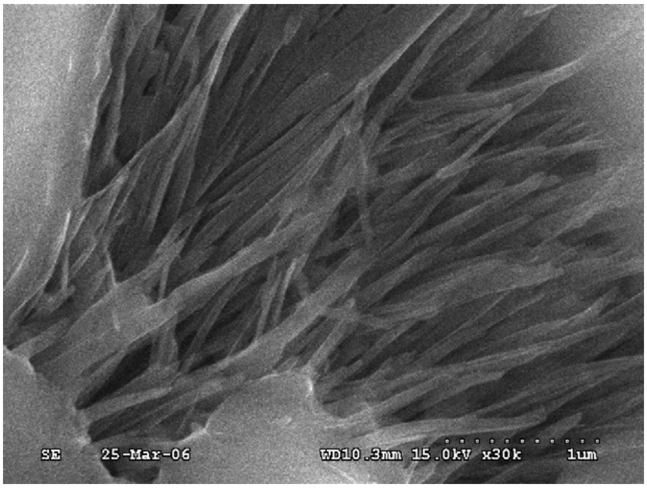

Fig. 2. The SEM image of the as-prepared HA sample in 0.01 M $NaNO_3$ solution at pH 11.0.