

Article

### Subscriber access provided by UNIV MASSACHUSETTS AMHERST

# Colloidal Behavior of Aluminum Oxide Nanoparticles As Affected by pH and Natural Organic Matter

Saikat Ghosh, Hamid Mashayekhi, Bo Pan, Prasanta Bhowmik, and Baoshan Xing Langmuir, 2008, 24 (21), 12385-12391 • DOI: 10.1021/la802015f • Publication Date (Web): 30 September 2008 Downloaded from http://pubs.acs.org on November 21, 2008



### **More About This Article**

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML



## Colloidal Behavior of Aluminum Oxide Nanoparticles As Affected by pH and Natural Organic Matter

Saikat Ghosh, Hamid Mashayekhi, Bo Pan, Prasanta Bhowmik, and Baoshan Xing\*

Department of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, Massachusetts 01003

Received June 26, 2008. Revised Manuscript Received August 18, 2008

The colloidal behavior of aluminum oxide nanoparticles (NPs) was investigated as a function of pH and in the presence of two structurally different humic acids (HAs), Aldrich HA (AHA) and the seventh HA fraction extracted from Amherst peat soil (HA7). Dynamic light scattering (DLS) and atomic force microscopy (AFM) were employed to determine the colloidal behavior of the NPs. Influence of pH and HAs on the surface charges of the NPs was determined. 2-Potential data clearly showed that the surface charge of the NPs decreased with increasing pH and reached the point of zero charge (ZPC) at pH 7.9. Surface charge of the NPs also decreased with the addition of HAs. The NPs tend to aggregate as the pH of the suspension approaches ZPC, where van der Waals attraction forces dominate over electrostatic repulsion. However, the NP colloidal suspension was stable in the pHs far from ZPC. Colloidal stability was strongly enhanced in the presence of HAs at the pH of ZPC or above it, but in acidic conditions NPs showed strong aggregation in the presence of HAs. AFM imaging revealed the presence of long-chain fractions in HA7, which entangled with the NPs to form large aggregates. The association of HA with the NP surface can be assumed to follow a two-step process, possibly the polar fractions of the HA7 sorbed on the NP surface followed by entanglement with the long-chain fractions. Thus, our study demonstrated that the hydrophobic nature of the HA molecules strongly influenced the aggregation of colloidal NPs, possibly through their conformational behavior in a particular solution condition. Therefore, various organic matter samples will result in different colloidal behavior of NPs, subsequently their environmental fate and transport.

#### Introduction

Nanoparticles (NPs) are generally defined as particles having a diameter less than 100 nm; however, there is no well-accepted definition.<sup>1</sup> Production and use of engineered nanoscale materials has increased enormously in the recent years. Nanoscale objects can now be manipulated and modified, providing great potential for the development of new technologies and products.

In spite of their great applications, recent studies have demonstrated potential problems if nanomaterials are released into the environment, especially considering their toxicity to microorganisms, plants, and animals.<sup>2</sup> Colloidal behavior of the NPs is one of the major factors that greatly influences their environmental fate, transport, and toxicity.

Colloidal behavior of engineered NPs in an aquatic environment depends on several factors, such as pH, ionic strengths, and the presence of natural organic matter (NOM) commonly found in the natural environment. At lower ionic strength, pH often plays a dominant role in the aggregation of inorganic colloids. The aggregation and disaggregation of inorganic colloids mainly arise from the pH-induced surface charge as well as organic matter present. Colloidal interaction primarily follows the classical DLVO model, but the steric factor may also play a significant role in the colloidal stability.<sup>3</sup>

NOM comprises a heterogeneous mixture of different functional moieties derived from geochemical and microbial processes. Therefore, the physicochemical characteristics of NOM fractions may strongly alter the stability of inorganic colloids.<sup>4</sup> Humic substances, especially humic and fulvic fractions, along with polysaccharides predominantly constitute NOM fractions present in natural aquatic bodies.<sup>4</sup> Numerous studies have shown the stabilizing impact of humic and fulvic acids upon their sorption on inorganic colloids,<sup>5</sup> predominantly through repulsive interactions due to the net negative surface charge development of the inorganic colloids. However, polysaccharide fractions can destabilize stable colloidal suspensions.<sup>6</sup> Polysaccharides present in the environment vary notably on their polarity, possibly due to variation in their molecular weight. Thus, the aggregation mechanisms of NPs may be significantly different in the presence of different polysaccharides. Chen et al.<sup>4</sup> showed that charge neutralization as well as gel-bridging of alginate polymers, in the presence of divalent cations such as Ca<sup>2+</sup>, strongly enhanced the aggregation rate of hematite NPs. Ferretti et al.<sup>7</sup> showed experimentally and with simulation studies significant aggregation of hematite particles by schizophyllan, a large, hydrophobic extracellular polysaccharide at a particular hematite/schizophyllan ratio. Several other researchers have observed the destabilization function of long-chain polymers when added to a stable colloidal suspension.8,9

Therefore, NOM is known to play a crucial role in the colloidal behavior of inorganic colloids, but the specifics behind these processes are not fully understood. Furthermore, it can be presumed that the physicochemical properties of different organic matter fractions may impart significant changes on the colloidal

<sup>\*</sup> Corresponding author. Phone: 413-545-5212. Fax: 413-545-3958. E-mail: bx@pssci.umass.edu.

<sup>(1)</sup> Guzman, K. A. D.; Finnegan, M. P.; Banfield, J. F. *Environ. Sci. Technol.* **2006**, *40*(24), 7688–7693.

<sup>(2)</sup> Oberdorster, E. Environ. Health Perspect. 2004, 112(10), 1058–1062.

<sup>(3)</sup> Chen, K. L.; Elimelech, M. J. Colloid Interface Sci. 2007, 309, 126–134.

<sup>(4)</sup> Chen, K. L.; Mylon, S. E.; Elimelech, M. Environ. Sci. Technol. 2006, 40, 1516–1523.

<sup>(5)</sup> Tipping, E.; Higgins, D. C. Colloids Surf. 1982, 5(2), 85-92.

<sup>(6)</sup> Wilkinson, K. J.; Joz-Roland, A.; Buffle, J. Limnol. Oceanogr. 1997, 42(8), 1714–1724.

<sup>(7)</sup> Ferretti, R.; Stoll, S.; Zhang, J. W.; Buffle, J. J. Colloid Interface Sci. 2003, 266(2), 328–338.

<sup>(8)</sup> Guyot, A.; Audebert, R.; Botet, R.; Cabane, B.; Lafuma, F.; Jullien, R.; Pefferkorn, E.; Pichot, C.; Revillon, A.; Varoqui, R. J. Chim. Phys. Phys.-Chim. Biol **1990**, 87((11-12)), 1859–1899.

<sup>(9)</sup> Lafuma, F.; Wong, K.; Cabane, B. J. Colloid Interface Sci. 1991, 143, 9-21.

 
 Table 1. <sup>13</sup>C NMR Integration and Relative Elemental Composition Data for AHA and HA7

	NMR integration				elemental composition (%)			
	0-50 ppm <sup>a</sup>	50-109 ppm <sup>b</sup>	109–163 ppm <sup>c</sup>	163-220 ppm <sup>d</sup>	С	Н	0	N
AHA	33.4	6.0	45.1	15.6	60.0	4.5	34.5	1.0
HA7	22.5	28.9	26.5	24.9	56.6	6.2	33.5	3.6
<sup>a</sup> Aliphatic C. <sup>b</sup> Carbohydrate C. <sup>c</sup> Aromatic C. <sup>d</sup> Carboxylic C.								

stability of mineral particles. In this study, we used sequentially extracted HAs with different structures to investigate the effect of HA structure on NP colloidal stability. It is known that with increasing number of sequential HA extractions from a soil, the polarity of the HA fractions decreases, along with a significant increase in HAs aliphaticity and molecular weight.<sup>10–12</sup> Therefore, we hypothesize that the charge and conformational difference of HAs may profoundly alter the colloidal stability of mineral NPs. Recent reports revealed that aluminum oxide NP (Al<sub>2</sub>O<sub>3</sub> NP) is one of the major engineered nanosized materials available in the US market.<sup>13</sup> Considering the wide range of industrial applications of Al<sub>2</sub>O<sub>3</sub> NPs, their release into the environment and water would be inevitable.

Thus, the objective of our study was to determine the colloidal behavior of Al<sub>2</sub>O<sub>3</sub> NPs as a function of pH and in the presence of structurally different HAs. We used dynamic light scattering (DLS) and  $\zeta$ -potential measurements to follow the changes of particle size and surface charge and their subsequent effect on the colloidal stability. Atomic force microscopy (AFM) was used to visualize aggregate structure and the attachment of HAs to the NP surface. To the best of our knowledge, this is the first study on how structurally different HAs change the colloidal behavior of Al<sub>2</sub>O<sub>3</sub> NPs.

#### **Materials and Methods**

**Preparation of Natural Organic Matter Solution.** Two structurally different HAs were used in this study. AHA was obtained from Aldrich Chemicals (Milwaukee, WI), while HA7 was obtained after seven sequential extractions of Amherst peat soil. Detailed extraction and purification procedures of AHA and HA7 were presented in our previous works.<sup>10,14</sup> Both AHA and HA7 were subjected to elemental composition determination and <sup>13</sup>C NMR analysis, which are displayed in Table 1. It is clear that AHA and HA7 have similar carbon content; however, HA7 has a more pronounced polysaccharide (carbohydrate) fraction compared to AHA.

**Preparation of Colloidal Suspension of Al<sub>2</sub>O<sub>3</sub> NP for Dynamic Light Scattering (DLS) Measurements.** The Al<sub>2</sub>O<sub>3</sub> NP used in our experiment was obtained from Hongchen Material Science & Technology Co. The surface area of the material was 230 m<sup>2</sup>/g and the particle size was 60 nm. A total of 100 mg of Al<sub>2</sub>O<sub>3</sub> NP was suspended in 1 L of 0.002 M NaCl solution. The pH of suspension was adjusted to 11, to minimize the interparticle attraction. The suspension was then sonicated for 1 h using an ultrasonic bath (Fisher Scientific) to break down any aggregate structure, and the suspension was allowed to settle. The colloidal suspension of NP was stored for subsequent experiments. The final concentration of the NPs in the suspension was 40 mg/L, determined turbidimetrically at a wavelength of 500 nm.

 $\zeta$ -Potential.  $\zeta$ -Potential was measured with a Zetasizer (Malvern Instruments) with a folded capillary cell and laser beam at 632 nm. The temperature of the samples was maintained at  $25 \pm 0.1$  °C during the experiment. The instrument was calibrated by measuring the  $\zeta$  of a standard latex sample, having  $\zeta = -55 \pm 5 \text{ mV}$ .<sup>15</sup> Charge variation of the colloidal materials under various solution conditions as well as with the addition of HAs was measured with DLS using laser Doppler electrophoresis.16 To determine the ZPC (zero point of charge) of NP suspension, 10 mL of the suspension was transferred into a 20 mL glass vial, where the pH was adjusted for the entire pH range of 4-11. To determine the  $\zeta$  of NP in the presence of AHA and HA7, desired amounts of either of the HAs were added to the NP suspension to attain 5 or 20 mg/L HA concentrations at each pH. Then the pH of the mixture was adjusted again and allowed to equilibrate. The  $\zeta$  of two different HAs was also examined separately in the entire pH range of 4-11. The concentration of both AHA and HA7 was 50 mg/L for the entire pH range of 4-11 in these experiments.

**Particle Size Determination.** Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer with a laser beam at 632 nm.  $Al_2O_3$  NP suspension was collected in a 20 mL glass vial from the stock followed by pH adjustment. Each sample was transferred to a green Malvern Zetasizer cell for the size measurement. The effect of both AHA and HA7 on the aggregation of the  $Al_2O_3$  NP was measured by adding different amounts of both AHA and HA7 into the NP suspension to keep the final concentration of 5 and 10 mg/L AHA and 5 mg/L HA7. Maximum peak intensity was plotted to obtain the particle size distribution.

The size distribution of pure AHA and HA7 was also determined with the same technique at a pH range of 4-11. The concentration of both AHA and HA7 was kept at 100 mg/L, and 0.002 M NaCl was used as background electrolyte. A higher concentration of HA was used for the size measurement to get better signals resulting from higher scattering intensity.

Atomic Force Microscopy Studies. Atomic force microscopy (AFM) has been widely used to determine the size of colloidal mineral particles<sup>17</sup> and conformational changes of NOM.<sup>15</sup> Freshly cleaved mica surface was vertically placed in the colloidal suspensions of Al<sub>2</sub>O<sub>3</sub> NP at pH 3, 7, and 11 for 20-30 min to allow adsorption of the NPs on the mica surface. The mica samples were then placed momentarily for a few times into deionized water for the removal of weakly sorbed particles followed by air drying in a dust-free environment. A similar sample preparation was used for the Al2O3-NP-AHA 5 mg/L and Al<sub>2</sub>O<sub>3</sub>-NP-HA7 5 mg/L suspensions, but the sorption time was 10-15 min, since sorption of Al<sub>2</sub>O<sub>3</sub>-NP-HA complexes on mica surface was significantly higher compared to pure Al<sub>2</sub>O<sub>3</sub> NP. The samples were then examined with a Dimension 3100 AFM instrument (Digital Instruments). Tapping mode AFM (TMAFM) measurements were carried out with a silicon cantilever auto-tuned between 0 and 300 kHz frequency region.15 Both height and phase images were recorded. Phase-contrast imaging provides additional information about the material properties, including stiffness of the material.<sup>18</sup> Hard material causes a larger phase shift and, therefore, appears brighter in color, whereas relatively soft material causes smaller phase shifts due to higher tip-sample interaction, thus appearing darker in color. All the images obtained from AFM imaging were subjected to section analysis using a Nanoscope VI (Digital Instruments, Plainview, NY).

#### **Results and Discussion**

**Influence of HA on the Surface Charge of Colloidal Al<sub>2</sub>O<sub>3</sub> NP.** The influence of pH and the effect of addition of different

 <sup>(10)</sup> Kang, S. H.; Xing, B. S. Environ. Sci. Technol. 2005, 39(1), 134–140.
 (11) Heymann, K.; Mashayekhi, H.; Xing, B. S. Spectrosc. Lett. 2005, 38(3), 293–302.

<sup>(12)</sup> Khalaf, M.; Kohl, S. D.; Klumpp, E.; Rice, J. A.; Tombacz, E. Environ. Sci. Technol. 2003, 37(13), 2855–2860.

<sup>(13)</sup> Abraham, T. http://www.ceramicindustry.com, retrieved on September 5, 2000.

<sup>(14)</sup> Pan, B.; Xing, B. S.; Liu, W. X.; Tao, S.; Lin, X. M.; Zhang, X. M.; Zhang, Y. X.; Xiao, Y.; Dai, H. C.; Yuan, H. S. *Environ. Pollut.* **2006**, *143*(1), 24–33.

<sup>(15)</sup> Pan, B.; Ghosh, S.; Xing, B. S. Environ. Sci. Technol. 2008, 42(5), 1594–1599.

 <sup>(16)</sup> Csáki, F. K.; Csempesz, F. Colloids Surf., A 2001, 190(1-2), 3-8.
 (17) Plaschke, M.; Romer, J.; Kim, J. I. Environ. Sci. Technol. 2002, 36(21), 4483-4488.

<sup>(18)</sup> Namjesnik-Dejanovic, K.; Maurice, P. A. Geochim. Cosmochim. Acta 2001, 65(7), 1047–1057.



**Figure 1.** The effect of pH and HA's on the  $\zeta$ -potential of the Al<sub>2</sub>O<sub>3</sub> NP. The  $\zeta$ -potential of Al<sub>2</sub>O<sub>3</sub> NP decreased in the presence of HAs as well as with increasing pH.  $\blacklozenge$  represents the change in  $\zeta$ -potential of the pure NP system, while  $\Delta$  and  $\blacktriangle$  represent the variation in  $\zeta$ -potential of AHA and HA7, respectively, as a function of pH. Influence of addition of 5 mg/L ( $\Box$ ) and 20 mg/L ( $\Delta$ ) AHA showed that the NP surface charge decreased with increasing AHA concentration. Similar phenomenon was observed with the addition of 5 mg/L ( $\blacksquare$ ) and 20 mg/L ( $\triangle$ ) of HA7. In all the measurements, 0.002 M NaCl was used as a background electrolyte. The  $\zeta$ -potential of pure particles decreased with increasing pH, reaching the ZPC at pH 7.9.

amounts of AHA and HA7 on the  $\zeta$  of the pure Al<sub>2</sub>O<sub>3</sub> NP are presented in Figure 1. The pH significantly altered the  $\zeta$  of pure Al<sub>2</sub>O<sub>3</sub> NP. The  $\zeta$  of Al<sub>2</sub>O<sub>3</sub> NP decreased with increasing pH of the suspension and reached ZPC at pH 7.9. This value was lower than the ZPC of the bulk alumina particles pH =  $8.7 \pm 0.1$ , in the presence of KNO<sub>3</sub> as a background electrolyte.<sup>19</sup> This phenomenon is in accordance with the result obtained for TiO<sub>2</sub> NP,<sup>1</sup> which showed that small nanocrystalline TiO<sub>2</sub> NP had lower ZPC than the larger TiO<sub>2</sub> NP, possibly due to a higher charge density.

The Al<sub>2</sub>O<sub>3</sub> NP had positive surface charges in acidic pH range but the addition of AHA or HA7 lowered the  $\zeta$  of Al<sub>2</sub>O<sub>3</sub> NP at that corresponding pH. For example, pure Al<sub>2</sub>O<sub>3</sub> NP had a  $\zeta$  of 35 mV at pH ~4, but with the addition of 5 mg/L of AHA, the  $\zeta$  decreased to 29 mV, whereas addition of 20 mg/L of AHA lowered the  $\zeta$  to 18 mV (Figure 1). The  $\zeta$  of Al<sub>2</sub>O<sub>3</sub> NP also decreased with the addition of HA7 at pH 4. Addition of 5 mg/L HA7 lowered the  $\zeta$  to 18 mV (Figure 1). Illés and Tombácz<sup>20</sup> observed a significant decrease in  $\zeta$  of magnetite NPs from 40 mV to -40 mV with the addition of 2 mg/L humic acid (HA). Amal et al.<sup>21</sup> also observed a significant decrease in  $\zeta$  of fulvic acid (FA). In addition, Tombácz et al.<sup>22</sup> showed that organic anions increased the negative charge density adjacent to the mineral particle surface and could cause a shift in the position of the shear plane further



**Figure 2.** Time-dependent aggregation of the pure Al<sub>2</sub>O<sub>3</sub> NP in various pHs (A) and pH-dependent aggregation of Al<sub>2</sub>O<sub>3</sub> NP in the presence and absence of HAs (B). AFM height image of Al<sub>2</sub>O<sub>3</sub>-NP-AHA 20 mg/L suspension at pH 8 reflected minimal aggregation of the NPs (C). Al<sub>2</sub>O<sub>3</sub> NPs formed stable colloidal suspension in pHs far from ZPC. The hydrodynamic diameter of the Al<sub>2</sub>O<sub>3</sub> NP remained relatively unchanged with time at the pHs of 3, 4, 5, 6, and 11, whereas the particle size increased with time at pH 7, near the ZPC. The pH-dependent aggregation of the Al<sub>2</sub>O<sub>3</sub> NP in the absence of HAs showed that the maximum aggregate size was produced at ZPC or pHs close to ZPC ( $\blacklozenge$ ). However, the presence of 20 mg/L of both AHA ( $\bigcirc$ ) and HA7 ( $\blacktriangle$ ) strongly decreased the aggregate size, when added at ZPC or above (B). Aggregation of the Al<sub>2</sub>O<sub>3</sub> NP in acidic pH range was very strong in the presence of AHA and HA7, which produced very large aggregates followed by fast sedimentation indicated by hypothetical dashed lines (B).

away from the surface. This phenomenon could lead to a decrease in the  $\zeta$  of the mineral surface. Addition of 20 mg/L of either HAs into Al<sub>2</sub>O<sub>3</sub> NP suspension significantly decreased the  $\zeta$ value at the ZPC. In this study, addition of 20 mg/L AHA and HA7 at pH 8 lowered  $\zeta$  to -33.3 and -33.7 mV, respectively. Therefore, both AHA and HA7 could enhance the colloidal stability of Al<sub>2</sub>O<sub>3</sub> NP at pH = ZPC or above. This significant decrease in  $\zeta$  of Al<sub>2</sub>O<sub>3</sub> NP after addition of 20 mg/L of HAs might have arisen from the dissociation of the readily ionizable functional moieties present in both HAs (Table 1).

Influence of pH on Aggregation of  $Al_2O_3$  NP. Timedependent aggregation behavior of  $Al_2O_3$  NP was studied at different pHs using the DLS technique (Figure 2). The DLS measurements clearly indicated that the hydrodynamic size of  $Al_2O_3$  NP aggregates did not increase with time in the pH range from 3 to 6 and at pH 11, i.e., pHs far from the ZPC. The stability of colloidal  $Al_2O_3$  NP in these pH ranges is in line with the classical DLVO model of colloidal stability.<sup>1,20,23</sup> However, we did observe size variation even at these pHs, which may be due

<sup>(19)</sup> Tombacz, E.; Dobos, A.; Szekeres, M.; Narres, H. D.; Klumpp, E.; Dekany, I. *Colloid Polym. Sci.* **2000**, 278(4), 337–345.

 <sup>(20)</sup> Illes, E.; Tombacz, E. J. Colloid Interface Sci. 2006, 295(1), 115–123.
 (21) Amal, R.; Raper, J. A.; Waite, T. D. J. Colloid Interface Sci. 1992, 151(1), 244–257.

<sup>(22)</sup> Tombacz, E.; Filipcsei, G.; Szekeres, M.; Gingl, Z. Colloids Surf., A 1999, 151(1), 233-244.



**Figure 3.** AFM height images of pure  $Al_2O_3$  NP at pH 3, 7, and 11 after sorption on freshly cleaved mica surface in the presence of 0.002 M NaCl. AFM height images of the pure  $Al_2O_3$  NP at pH 3 showed that the majority of the particles were not aggregated. AFM height images of pure  $Al_2O_3$  NP at pH 7 reflected much higher size distribution of NPs due to a higher charge-induced aggregation at pHs close to the ZPC. AFM height images of the pure  $Al_2O_3$  NP at pH 11 showed no aggregation behavior of the NPs due to strong electrostatic repulsive interaction operating between the NPs at pHs above ZPC.

to the polydispersity of the Al<sub>2</sub>O<sub>3</sub> NPs. The colloidal stabilization of Al<sub>2</sub>O<sub>3</sub> NP in the pH range from 3 to 6 and at pH 11 is in correlation with the higher  $\zeta$  (absolute value) of pure Al<sub>2</sub>O<sub>3</sub> NP at those corresponding pHs.

Aggregation was clearly observed at pH 7 (closer to the ZPC) even at the very low ionic strength maintained in our experiments. At pH 7, the London–van der Waals attractive force may be prevailing over the electrostatic repulsive force, leading to aggregation. Thus, Al<sub>2</sub>O<sub>3</sub> NPs tends to aggregate at any pH close to ZPC, leading to colloidal instability.

The pH-dependent aggregation of Al<sub>2</sub>O<sub>3</sub> NP was also studied in the presence of both AHA and HA7 (Figure 2B). The hydrodynamic size of Al<sub>2</sub>O<sub>3</sub> NP did not alter notably at highly acidic or alkaline conditions. However, the particles tend to form aggregates as the pH of the suspension was in the vicinity of ZPC. The maximum aggregate size was observed at ZPC. Another study revealed that TiO2 NP also had similar aggregation behavior over a varied range of surface potential.<sup>1</sup> The size measured in an aggregating system may not be authentic, but the presence of larger aggregates can clearly be assessed.<sup>20</sup> To further demonstrate the aggregation phenomenon observed from the DLS measurements, AFM examinations of Al<sub>2</sub>O<sub>3</sub> NP were carried out at three different pHs, i.e., 3, 7, and 11 (Figure 3A-C). AFM images clearly showed almost similar size distribution of Al<sub>2</sub>O<sub>3</sub> NP at pH 3 and 11; however, few aggregate structures of Al<sub>2</sub>O<sub>3</sub> NPs were observed at pH 3, which was absent at pH 11. This could be due to higher sorption of the positively charged Al<sub>2</sub>O<sub>3</sub> NPs on the negatively charged mica surface followed by aggregation. The increased particle size (e.g., >200 nm) was observed at pH 7, confirming the aggregation of the NPs at pHs close to the ZPC.

Addition of 20 mg/L of either AHA or HA7 to colloidal suspension of Al<sub>2</sub>O<sub>3</sub> NP imparted both stabilizing and destabilizing influence, depending on the pH. Al<sub>2</sub>O<sub>3</sub> NP had a very unstable colloidal suspension in the vicinity of ZPC. However, addition of either AHA or HA7 at the ZPC or above it strongly increased the colloidal stability. The particle size distribution (Figure 2B) showed a sharp decrease in the aggregate size of Al<sub>2</sub>O<sub>3</sub> NP upon HA addition, which is due to the reduction of  $\zeta$ . This reduction of  $\zeta$  has presumably arisen from the ionization of the polar functional moieties of HAs adsorbed on the Al<sub>2</sub>O<sub>3</sub> NPs surface. The electrostatic repulsive force operating between the interacting HA-coated Al<sub>2</sub>O<sub>3</sub> NP contributed to the colloidal stability. Kretzschmar et al.23 also reported a decrease in coagulation behavior of the kaolinite particles after addition of a small amount of HA at the ZPC. The stabilization by the HA molecules may be purely electrostatic in nature. AFM height image (Figure 2C)





**Figure 4.** Time-dependent aggregation of Al<sub>2</sub>O<sub>3</sub> NPs as a function of AHA and HA7 concentrations at pH 4 with 0.002 M NaCl as background electrolyte. In the presence of 5 mg/L AHA ( $\blacklozenge$ ), the suspension of NPs was stable during the experiment. With increasing concentration of AHA to 10 mg/L ( $\blacklozenge$ ), the aggregation rate increased from surface charge neutralization of NPs. A significantly higher aggregation rate was observed with the addition of 5 mg/L HA7 ( $\blacksquare$ ), which pointed out that the physicochemical behavior of the two structurally different HAs prominently contributed to the colloidal interaction of Al<sub>2</sub>O<sub>3</sub> NPs.

of the Al<sub>2</sub>O<sub>3</sub>-NP-AHA 20 mg/L at pH 8 showed individual NPs referring colloidal stability. The average height of the adsorbed NPs on the mica surface reached up to 5 nm.

A completely different phenomenon of  $Al_2O_3$  NP colloidal stability was observed when 20 mg/L of either AHA or HA7 was added at acidic conditions.  $Al_2O_3$  NP strongly aggregated in acidic conditions, thereby precipitating out and being removed from the suspension. Therefore, it was virtually impossible to measure the size distribution of very large aggregates by DLS. Therefore, a separate experiment was conducted to study the time-dependent aggregation of the NPs at pH 4 in the presence of AHA and HA7.

Aggregation of Al<sub>2</sub>O<sub>3</sub> NP in the Presence of AHA and HA7 under Acidic Conditions. It is quite clear in Figure 4 that the rate of Al<sub>2</sub>O<sub>3</sub> NP aggregation was much higher with increasing concentration of AHA. Al<sub>2</sub>O<sub>3</sub> NP suspension was stable at 5 mg/L AHA during the experimental time period. However, the aggregation rate increased prominently with 10 mg/L AHA. Surface charge neutralization of Al<sub>2</sub>O<sub>3</sub> NP possibly influenced the pronounced aggregation. Tiller and O'Melia<sup>24</sup> reported that at low ionic strength anionic polyelectrolytes changed the coagulation process of the positively charged particles by altering the net surface charge. A significantly higher aggregation rate of Al<sub>2</sub>O<sub>3</sub> NP was observed with 5 mg/L HA7. Comparison of the AFM topography images (Figure 5) of Al<sub>2</sub>O<sub>3</sub>-NP–AHA and Al<sub>2</sub>O<sub>3</sub>-NP–HA7 suspensions revealed a higher degree of

(24) Tiller, C. L.; Omelia, C. R. Colloids Surf., A 1993, 73, 89-102.



**Figure 5.** Comparison between the AFM height images of  $Al_2O_3$  NP in the presence of AHA and HA7. AFM height image of  $Al_2O_3$ -NP-5 mg/L AHA suspension showed much smaller size distribution of the NPs compared to the AFM height image of  $Al_2O_3$ -NP-5 mg/L HA7 suspension. We observed very little aggregation of NPs with AHA, where the majority of the NPs were weakly attached. However, the aggregation of NPs is predominant with HA7, which is in line with the DLS data.

aggregation in the latter. Therefore, the difference in physicochemical properties between AHA and HA7 dominantly affected aggregation rate.

AFM height and phase-contrast images (Figure 6) of Al<sub>2</sub>O<sub>3</sub>-NP-HA7 suspensions further revealed that small spherical Al<sub>2</sub>O<sub>3</sub> NPs were entangled within the helical fibrillar structure of HA7. AFM images also demonstrated that single Al2O3 NP was entrapped by several fibrils of HA7 molecules. At pH 4, due to minimal electrostatic repulsion, several long-chain fibrils could come close together, forming a gel-like network, possibly due to strong H-bonding along with hydrophobic interaction between the long chains, where NPs were entrapped. Buffle et al.<sup>25</sup> proposed a pearl-necklace simulation model to explain the strong aggregation of inorganic colloid particles when interacted with large semiflexible biopolymeric materials ubiquitous in aquatic environment. AFM images in this work further revealed that these long-chain fractions could act as a bridge between different Al<sub>2</sub>O<sub>3</sub> NP, which promoted aggregation. Chen et al.<sup>4</sup> observed with TEM measurements that hematite NPs were embedded within a gel-like aggregate produced by alginate, a long-chain, highly charged polar polysaccharide in the presence of divalent Ca<sup>2+</sup> ions. In another study, highly charged alginate chains took up a more coiled conformation in solution rather than the rodlike structures.<sup>25</sup> However, the possible mechanism of aggregate gel formation is entirely different, as observed in our experiment. In the presence of  $Ca^{2+}$ , the electrostatic repulsive barrier of a single polymeric chain of the alginate polymer is effectively screened, which facilitates the aggregation of alginate polymer chains. Moreover, Ca<sup>2+</sup> can act as a bridging ligand between carboxylate moieties present in the alginate chains; thereby favoring intermolecular association to form a gel-like network.



Langmuir, Vol. 24, No. 21, 2008 12389

**Figure 6.** AFM height (A) and phase (B) images of Al<sub>2</sub>O<sub>3</sub>-NP-HA7 suspension at pH 4. Both images clearly demonstrated the presence of long-chain polysaccharides. It is also evident that NPs are physically entrapped within the gel network consisting of long-chain fractions, hence producing large aggregates leading to colloidal instability. The phase image showed the presence of organic matter fractions at the edges of the NP surface (appeared darker), while the basal plane is lighter in color. The line profile plot showed a negative shift in phase. The height (C) and phase imaging (D) of the pure NPs did not show any dark color at the edges of NP surface, as well as showing no decline in phase angle, which indicated the absence of HAs.

On the contrary, in our study the ionic strength was maintained at a very low level using Na<sup>+</sup> instead of Ca<sup>2+</sup>, which could not effectively screen the surface charge. Thereby, gel-like network formation is entirely dependent on the hydrophobicity and through H-bonding between HA7 molecular chains. Long chain coiled fractions of HA7 might have arisen from the polysaccharide molecules behaving like semiflexible biopolymer, though we do not have any direct structural data except for the <sup>13</sup>C NMR results (Table 1). A TMAFM study of hyaluronan, a linear polysaccharide, deposited on freshly cleaved mica revealed a weakly helical, coiled conformation.<sup>26</sup> Intramolecular association of both extended and coiled conformation hyaluronan formed a network structure. Therefore, the presence of these very long-chain hydrophobic fractions in HA7 may be the determining factor in increased aggregation of Al<sub>2</sub>O<sub>3</sub> NP compared to that of AHA. Moreover, enhanced aggregation of the colloidal mineral particles was verified in the presence of weakly charged, high molecular weight, rod-like polysaccharides derived from planktons.<sup>25</sup>

Association of HA with the NP surface can be distinguished from the AFM phase-contrast imaging (Figure 6). The phase plot of NP-HA7 suspension clearly illustrated higher tip-sample interaction at the edge of spherical NP surface along with a shift in phase angle. The edge of NP surfaces appeared darker, referring to the presence of softer material on the edge of the particle

<sup>(25)</sup> Buffle, J.; Wilkinson, K. J.; Stoll, S.; Filella, M.; Zhang, J. W. Environ. Sci. Technol. 1998, 32(19), 2887–2899.

<sup>(26)</sup> Cowman, M. K.; Spagnoli, C.; Kudasheva, D.; Li, M.; Dyal, A.; Kanai, S.; Balazsy, E. A. *Biophys. J.* **2005**, *88*(1), 590–602.



**Figure 7.** Schematic representation of the proposed aggregation mechanism of  $Al_2O_3$  NP in the presence of HA7 at pH 4. First, the polar fraction of HA7 molecules likely sorbed on the NP surface, followed by attachment with the long-chain fractions, possibly from the polysaccharides present in HA7. The strong hydrophobic interaction and H-bonding between these long chains at pH 4 strongly facilitated the aggregation. Moreover, the  $Al_2O_3$  NPs attached to the long-chain fractions can also act as a bridging ligand between the individual chains, contributing to the enhanced aggregation of the NPs.



**Figure 8.** AFM height (A) and phase-contrast image (B) of  $Al_2O_3$ -NP-AHA 250 mg/L at pH 11. The dark color on the edge of NPs showed the presence of AHA on the surface. We also observed that the majority of AHA-coated NPs did not show any aggregation, possibly due to strong electrostatic repulsion between the negatively charged functional moieties of HA adsorbed on the NP surface.

surface, whereas the basal plane of particle surface appeared lighter in color.<sup>17</sup> Therefore, phase-contrast images showed a difference in material properties between the harder mineral particles and softer HA fractions. However, the AFM phase image (Figure 6D) of pure particles did not show any dark color on the edge of NPs, which was associated with a positive shift in phase angle. From the phase-contrast image, HA7 associated with the NP surface appeared darker and also showed much higher negative phase shift, whereas the long chains of HA7 appeared with a less negative phase angle shift, thereby reflecting variation in stiffness of different organic matter fractions in HA7. Similarly, the microlayered pattern of polyethylene was revealed using AFM phase imaging, where the bright and dark colored stripes were representing the high- and low-density layers of polyethylene.<sup>27</sup> Therefore, it can be pointed out that HA7 may comprise of both soft and inflexible fractions. We propose that, depending upon the heterogeneity of HA7, the aggregation of Al<sub>2</sub>O<sub>3</sub> NP follows a two-step process (Figure 7). In the first step, possibly the more polar fractions of HA7 molecules sorbed on the NP surface, followed by their entrapment in the weakly diffusible long-chain fractions, most likely through H-bonding. Therefore, these longchain fractions of HA7 loaded with Al2O3 NP can come close together through hydrophobic interaction and form a gel-like aggregate structure to produce large aggregates followed by sedimentation from the colloidal system (step two). Ferritti et al.<sup>7</sup> proposed that entrapment of hematite colloidal particles on schizophyllan (a large extracellular polysaccharide) plays a significant role in aggregation, because hematite particles can



**Figure 9.** The pH-dependent size distribution of AHA and HA7 at pH 4 (A) and pH 11 (B). At pH 4, HA7 had relatively larger size distribution compared to AHA, whereas at pH 11, HA7 showed a bimodal size distribution having one peak at 130 nm and the other at 395 nm. HA7 molecules at pH 4 must have taken a more coiled conformation, therefore showing dominantly one size distribution. The coiled structure of HA7 molecules thereby facilitated the aggregation behavior of  $Al_2O_3$  NP in acidic pH conditions.

act as anchoring points and support internal cohesion of the aggregate.

From the height and phase-contrast imaging (Figure 8), we observed similar phenomenon of AHA sorption on  $Al_2O_3$  NP surface at pH 11. At highly alkaline pH, some fractions of AHA were sorbed on the NP surface, possibly through some specific interaction between HA and the NPs.<sup>28</sup>

The DLS data at pH 4 (Figure 9A) showed relatively higher size distribution of HA7 than AHA, but at pH 11, HA7 had a significant increase in its size distribution compared to AHA (Figure 9B). Therefore, it seems that in acidic solution conditions

<sup>(27)</sup> Magonov, S. N.; Elings, V.; Whangbo, M. H. Surf. Sci. 1997, 375(2-3), L385–L391.

<sup>(28)</sup> Vermeer, A. W. P.; Van Riemsdijk, W. H.; Koopal, L. K. Langmuir **1998**, 14(10), 2810–2819.

#### Colloidal Behavior of Al<sub>2</sub>O<sub>3</sub> Nanoparticles

the long-chain fractions of HA7 molecules may take up a more coiled conformation to gain conformational entropy. However, at pH 11 no intra- or intermolecular attraction exists between the HA molecules, and a strong repulsive force is experienced by polar segments of HA molecules. Thus, HA7 molecules can take up a more open conformation. Peak intensity distribution of AHA at pH 11 was in the size range of 255 nm, whereas HA7 showed a bimodal size distribution having almost 50% of the molecules in the size range of 130 nm and the other 50% in the size range of  $\sim$ 400 nm. Therefore, the coiled conformation of some HA7 fraction along with charge neutralization at pH 4 facilitated strong aggregations of the NPs.

In summary, the colloidal behavior of Al<sub>2</sub>O<sub>3</sub> NPs can be significantly influenced by the solution pH. The physicochemical nature of natural organic matter (NOM) can also dominantly alter the colloidal stability of NPs. In neutral to alkaline conditions, both HAs could stabilize the Al<sub>2</sub>O<sub>3</sub> NP colloidal system. However, in acidic conditions, AHA decreased the colloidal stability, possibly through a charge neutralization mechanism. The presence of long-chain weakly charged fractions of HA7 strongly decreased the colloidal stability of the NP, possibly through entrapment of NPs in a gel-like network. The association of HA to the NP surface can be identified by AFM phase-contrast imaging, because AFM can specifically distinguish the difference in material properties. Therefore, the colloidal stability and mobility of the NPs in the environment is affected not only by environmental factors such as pH and ionic strength but also by the structural properties of NOM. Thus, more thorough research is needed to study the influence of environmental factors including NOM characteristics on the colloidal stability and transport of the engineered NPs in the environment. Again, this work highlights the importance of NOM characteristics in addition to its quantity in altering the colloidal stability of engineered NPs.

Acknowledgment. This project was supported in part by the Massachusetts Agricultural Experiment Station (MA 8532) and Massachusetts Water Resources Research Center (2007MA73B). We express our sincere gratitude to Dr. Kevin D. McCarthy, Dept. of Chemistry (UMass, Amherst), for his guidance in operating the AFM, and Prof. Mark Tuominen, Dept. of Physics (UMass, Amherst), for allowing us to use the AFM.

LA802015F