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Towards a better understanding of the aggregation mechanisms of iron (hydr)oxide nanoparticles interacting with extracellular polymeric substances: role of pH and electrolyte solution

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Abstract

Extracellular polymeric substances (EPS) are ubiquitous in the soil and water environment and interact strongly with mineral surfaces. However, these interactions and their impacts on the behavior and fate of minerals remain poorly understood. Here, for a better understanding of the colloidal stability of minerals in the environment, we investigated the aggregation of goethite (α-FeOOH) nanoparticles (NPs) in the presence of EPS from Bacillus subtilis under different environmental conditions (pH, ionic strength and ionic valence). Results showed that the aggregation processes of goethite NPs are determined by the solution chemistry, and the colloidal stability of goethite NPs is strongly influenced by the addition of EPS. In the absence of ionic strength, the addition of EPS promotes the aggregation of goethite NPs only when the pH (pH=6) is less than the point of zero charge for the goethite nanoparticles ($pH_{pzc} \approx 8$). In the presence of ionic strength, the aggregation rate of goethite NPs increases with increasing concentration of NaCl, $NaNO_3$ and Na_2SO_4 solutions, and after the addition of EPS solution, the critical coagulation concentrations (CCC) of goethite NPs are increased from 43.0, 56.7 and 0.39 mM to 168.0, 304.9 and 126.2 mM in the three electrolyte solutions, indicating that the addition of EPS inhibits the aggregation of goethite NPs. While in Na_3PO_4 solution, when the concentration of Na_3PO_4 solution ranged from 0 to 1 mM, the aggregation rate of goethite NPs increases first, followed by a decrease, and with the concentration of Na_3PO_4 solution exceeding 1 mM, the aggregation rate of goethite NPs increases again, due to the charge screening by sodium counter ions. This study provides a fundamental understanding of the behavior of goethite NPs in natural soil and water environments.

Keywords: aggregation; EPS; goethite; surface charge; electrolyte concentration

1. Introduction

In the natural environment, microorganisms do not typically live as dispersed single cells, but assemble at interfaces to form microbial aggregates such as biofilms (Davey et al., 2000). For the majority of biofilms, the microorganisms account for about 10 % of the dry mass, whereas the matrix can account for up to 90 % of the dry mass, with the matrix comprised predominantly of complex high molecular weight extracellular polymeric substances (EPS) produced by the growth and metabolism of the microorganisms (Flemming and Wingender, 2010). EPS are mainly composed of polysaccharides, proteins, nucleic acids and lipids (Cao et al., 2011). As the main component of the biofilm, EPS can protect microorganisms against chemicals (e.g. heavy metals, hydrocarbons, biocides, antibiotics, etc.) and mechanical challenges present in the environment (Peterson et al., 2015). Once released into soils or aquatic environments, EPS can be adsorbed on the surfaces of inorganic colloids. Previous studies have shown that interactions between EPS and inorganic colloids can affect a broad variety of geochemical processes, such as microbial attachment and biofilm formation (Ma et al., 2017; Whitchurch et al., 2002; Zhao et al., 2014), particle aggregation and deposition (Lin et al., 2016a; Chowdhury et al., 2012), mineral dissolution (Bundeleva et al., 2014), bioleaching (Sand et al., 2006), biomineralization (Bontognali et al., 2008) and the sequestration of toxic substances (Fang et al., 2014; Liu et al., 2017).

Several studies concerning the effect of EPS on the stability of inorganic colloids, have demonstrated that the change in the stability of inorganic colloids can obviously alter their environmental behavior. For example, Zhang et al. (2015) have shown that the aggregation of polymeric luminescent nanomaterials based on dyes could induce their emission properties. Sheng et al. (2016) reported that the aggregation of hematite NPs can reduce the adsorption of metal Cu (II). The effect of EPS on the stability of manufactured nanoparticles (NPs) and natural iron (hydr)oxide colloids have also been investigated by several authors (Khan et al., 2011; Miao et al., 2016; Xu et al., 2016). For instance, exopolysaccharides extracted from the bacterium Bacillus pumilus are shown to play an important role in the stability of silver NPs in water (Khan et al., 2011), and flocculent sludge-derived EPS are found to enhance the stability of CuO NPs (Miao et al., 2016). It has also been reported that the addition of EPS extracted from bloom-forming cyanobacteria that are ubiquitously present in eutrophic waters can greatly decrease the hydrodynamic diameters of Al_2O_3 colloidal particles, and the electrolyte cations can induce the aggregation of colloidal particles (Xu et al., 2016). In our previous research, the effect of EPS extracted from Bacillus subtilis on the stability of TiO_2 NPs was investigated, and EPS constituents and environmental conditions (ionic strength, ionic valence and solution pH) were found to influence the NP stability significantly (Lin et al., 2016a; Lin et al., 2017).

In the past two decades, many studies have been performed on the effects of organic substances on the stability of natural iron (hydr)oxide colloids (e.g., Kretzschmar et al., 1994; Stemig et al., 2014; Vindedahl et al., 2016; Xu et al., 2015), because of their ubiquity and play critical roles in element cycling as well as pollutant fate and transport (e.g., Amstaetter et al., 2010; Elsner et al., 2004; Klupinski et al., 2003; Lead et al., 2006; Pecher et al., 2002; Wigginton et al., 2007). In the soil environment, iron (hydr)oxides tend to encounter EPS from microbial communities before direct interaction with bacterial cells. However, the stability and reactivity of natural iron (hydr)oxide colloids were usually investigated in the presence of natural organic matter (e.g., humic acid, fulvic acid), rather than EPS in the aforementioned studies. In this study, for a better understanding of the colloidal stability of minerals in the environments, we investigated the effect of EPS on iron (hydr)oxide colloid stability under the conditions of various pHs, ionic strengths, and different electrolyte anions. Goethite, a prevalent crystalline iron (hydr)oxide mineral in soil, was selected as a model iron (hydr)oxide in this study.

2. Materials and methodology

2.1. Synthesis and characterization of goethite

Goethite was synthesized by the simultaneous addition of a 0.15 M $Fe(NO_3)_3$ solution to a neutralizing 2.5 M KOH solution in a high-density polyethylene bottle, to give a final pH near 12.0, followed by aging for 24 h in a 60 ℃ oven according to the method of Atkinson et al. (1967). Briefly, X-ray diffraction (XRD) analysis was used to identify the synthetic goethite and the results are shown in Fig. S1. The diffraction data well matched the corresponding goethite standard XRD data (JCPDS 00-29-0713). Atomic force microscopy (AFM) and scanning electron microscopy (SEM, ZEISS MERLIN Compact, Germany) showed that the goethite NPs are 333.52 ± 10.47 nm long and 81.79 ± 5.36 nm wide, with needle-shaped crystals (Fig. S2). The specific surface area of the goethite NPs was determined by N_2 BET adsorption at

96.44 m^2 g^{-1}. X-ray photoelectron spectroscopy (XPS) measurements of goethite NPs were carried out using a VG Scientific ESCALAB 250 spectrometer, with Al Ka X-ray (1486.6 eV) as the light source.

2.2. EPS extraction and purification

EPS used in this study were extracted from Bacillus subtilis, which was cultivated in Luria broth at 28 ℃ in an aerobic condition to the early stationary (24 h) growth phase. The details on the EPS extraction methods are shown in the Supporting Information. Stock solutions of EPS at 2 g/L were prepared in deionized water and stored at 4 ℃. The concentrations of EPS solution were maintained at 0 - 20 mg/L during goethite NPs aggregation experiments at different pH values, and 1 mg/L EPS was used in the aggregation experiments with different electrolyte solutions.

2.3. Goethite aggregation experiments

The hydrodynamic size of the goethite NPs in solution and the aggregation kinetics of goethite NP suspension were investigated using time-resolved dynamic light scattering (TR-DLS) (Zetasizer Nano ZEN 3600, Malvern, UK). During measurement, a 633 nm He-Ne laser beam passed through the particle suspension, and the scattered light was detected and collected by a photo-detector at a fixed scattering angle of 173°. In these experiments, the aggregation of goethite NPs at different pH values was measured using 10 mg/L goethite NPs and 0-20 mg/L EPS solution, and the variation of the goethite stability with different electrolyte solutions was investigated using 10 mg/L goethite NPs and 1 mg/L EPS solution. NaCl, $NaNO_3$, Na_2SO_4 and Na_3PO_4 solutions were used to provide the ionic strength, with their concentration at 0 - 1500 mM, 0 - 1000 mM, 0 - 250 mM and 0 - 120 mM, respectively. For all experiments, 0.6 mL of a goethite NP suspension was pipetted into the DLS cuvette, followed by the addition of an EPS stock solution and the electrolyte solution to bring the final suspension volume to 1.2 mL. After mixing for 1 s, the measurement was started and each reaction was measured continuously for 60 min.

For the aggregation measurement at different pH values, the aggregation rate (k), was calculated for the time period from the initial aggregation (t_0) to the time when the goethite diameter (D_h) exceeded 1.5 D_h, which can be expressed as:

$$k \propto \frac{1}{N_0}\left(\frac{\mathrm{d}D_h(t)}{\mathrm{d}t}\right)_{t\to 0} \qquad (1)$$

For the experiments with different electrolyte solutions, the stability of the suspensions was evaluated by examining the variation of the attachment efficiency (α), with increasing electrolyte concentration. α is defined as the ratio of the aggregation rate in the reaction-limited regime (k) to that in the diffusion-limited regime (k_{fast}) (ionic strength above the critical coagulation concentration (CCC)) as follows (Elimelech et al., 1995):

$$\alpha = \frac{k}{k_{\mathrm{fast}}} = \frac{\frac{1}{N_0}\left(\frac{\mathrm{d}D_h(t)}{\mathrm{d}t}\right)_{t\to 0}}{\frac{1}{(N_0)_{\mathrm{fast}}}\left(\frac{\mathrm{d}D_h(t)}{\mathrm{d}t}\right)_{t\to 0,\mathrm{fast}}} \qquad (2)$$

The subscript “fast” refers to the stage where aggregation is a diffusion-limited process. Because the initial concentration of the goethite NP suspension is held constant, this equation can be simplified by eliminating N_0. The value of $(dDh(t)/dt)_{t\to 0,fast}$ can be obtained from the average values of the aggregation rate constant in the diffusion-limited regime when the concentration of NaCl, $NaNO_3$, Na_2SO_4 and Na_3PO_4 is higher than the CCC.

2.4. Zeta potential measurements

The zeta potential of goethite NPs at 25 ℃ were determined using a Zetasizer Nano ZS Instrument (ZEN 3600, Malvern, UK). The zeta potential of goethite NPs in the presence of EPS (0-20 mg/L) was measured at pH 4.0, 8.0 and 10.0 in deionized water. The zeta potential of the goethite NPs was also measured in both the absence and presence of EPS in NaCl, $NaNO_3$, Na_2SO_4 and Na_3PO_4 solutions. The samples used for zeta potential measurements were prepared in a similar manner to those for the aggregation experiments. For each condition, triplicate measurements were performed, with more than ten runs for each measurement.

2.5. FTIR and XPS spectroscopy measurements

The adsorption experiments of EPS on goethite colloids were performed in a 50 mL centrifuge tubes, in which 20 mL of goethite suspension (5 g/L) mixed with 20 mL 2 g/L EPS solution to reach a final EPS concentration of 1 g/L with Na_3PO_4 concentrations of 50 mM. The mixture was gently shaken at 25 °C for 2 h and centrifuged at 12,000 × g for 30 min. After freeze-drying, FTIR spectra of goethite, goethite-Na_3PO_4, and goethite-EPS-Na_3PO_4 complexes were obtained on a spectrometer (IFS 66 v/s, Bruker, Karlsruhe, Germany) equipped with a MCT-MIR liquid nitrogen-cooled detector and OPUS 5.5 processing software. All spectra were collected at pH 5.5, with 256 scans over the 800-4000 cm^{-1} range at a resolution of 4 cm^{-1}. The KBr pressed disc technique were used by mixing sample with KBr powder (around 1: 100) and using a press at the pressure of 10 tonnes. XPS spectra of goethite, goethite-Na_3PO_4, and goethite-EPS-Na_3PO_4 complexes were carried out using a VG Scientific ESCALAB 250 spectrometer, using Al Ka X-ray (1486.6 eV) as the light source.

3. Results and discussion

3.1. Zeta potential of goethite and EPS

Zeta potential measurements for pure goethite and pure EPS are shown in Fig.1. For goethite, the zeta potential is relatively constant at +41.0 ± 3.50 mV between pH 3.0 to 6.0, followed by a decrease, switching from positive to negative values at the Point of Zero Charge (PZC; pH 8.3) and finishing at -23.6 ± 1.3 mV at pH 9.5, which is consistent with the data reported in previous studies (Day et al., 1994). For EPS, the zeta potential is negative throughout the pH regime, with a steady decreasing from -16.6 ± 0.3 mV at pH 3.0 down to -21.5 ± 1.3 mV at pH 9.5.

3.2. Goethite nanoparticle aggregation as a function of EPS concentration at different pH values

For determining the aggregation rates of goethite NPs under different EPS concentrations at different pH values, we measured the goethite size evolution with time. If the suspension of goethite NPs remains stable with time, the aggregation rate (k) will be zero, and if the suspension is not stable with time, the k value will be positive. The aggregation kinetic experiments as a function of EPS concentration were performed at pH = 4 ($pH < pH_{PZC,\ goethite}$), 8 ($pH = pH_{PZC,\ goethite}$) and 10 ($pH > pH_{PZC,\ goethite}$), and the aggregation rates and the aggregation profiles are shown in Fig. 2 and

Fig. S3, respectively. When pH = 4.0 (< $pH_{PZC,\ goethite}$), the goethite NPs suspension is stable in ultrapure water, but three different behaviors are observed with the addition of various concentrations of EPS solution: (a) with EPS below 0.04 mg/L, the aggregation rate of goethite NPs is approximately zero; (b) with EPS between 0.04 mg/L and 0.41 mg/L, the aggregation rate of goethite NPs increases sharply and reaches a maximum at 0.41 mg/L; (c) with EPS beyond 0.41 mg/L, the aggregation rate of goethite NPs decreases rapidly back to zero. When pH = 8.0 (= $pH_{PZC,\ goethite}$), the aggregation rate of goethite NPs is at a maximum in ultrapure water, then drastically decreases to approximately zero with the addition of 0.01 mg/L EPS and remains approximately constant with increasing EPS concentration. When pH = 10.0 (> $pH_{PZC,\ goethite}$), the addition of EPS shows no discernible effect on the aggregation rate of goethite NPs.

Fig. 3, 4 and 5 show the corresponding values of zeta potentials and z-average diameters of goethite NPs as a function of EPS concentration at different pHs, the samples equilibrated for 30 min before the determination of the values. At pH = 4.0 (Fig. 3), in the absence of EPS, the zeta potential and z-average diameter values of goethite NPs are 38.6 ± 0.26 mV and 325.2 ± 22.3 nm, respectively. Three distinct behaviors are also identifiable in the zeta potential and z-average diameter values after the addition of different concentrations of EPS solution and they can be used to explain the aggregation behavior: (a) goethite NPs remain positively charged at low EPS concentration (≤ 0.1 mg/L), with the zeta-potential value greater than +20 mV and the z-average diameter value smaller than ~600 nm, suggesting that electrostatic repulsion between the NPs likely inhibits the aggregation and the suspension is relatively stable; (b) then, by increasing the EPS concentrations (from 0.15 to 1.5 mg/L), goethite NPs are less positively charged, with the zeta potential value decreased from > +20 mV to -10 mV, and the z-average diameter of goethite NPs increased to a maximum (~1200 nm) at 0.41 mg/L, implying that the reduced electrostatic repulsion between the NPs likely favors the rapid aggregation to very large aggregates at zero zeta potential, which subsequently experiences an increased repulsion again due to the increasing negative charge of NPs with increasing EPS concentration; (c) by further increasing the EPS concentration (> 1.5 mg/L), the zeta potential and z-average diameter values are approximately -20 mV and 200 nm, respectively, and the electrostatic repulsion between the NPs likely inhibits the aggregation of goethite NPs, and the suspension is relatively stable again.

When pH = 8.0, approximating the PZC (Fig. 4), the zeta potential of the goethite NPs is close to zero, with the minimal electrostatic repulsion between the NPs and the maximal z-average diameter at ~1900 nm. The addition of EPS solution decreases the zeta potential, leading to an increase of the electrostatic repulsion between the now negative NPs, and an obvious decrease of the z-average diameter of goethite NPs to ~400 nm.

When pH = 10.0 (Fig. 5), the zeta potential of the goethite NPs is negatively charged at < -30 mV, and the addition of EPS has little effect on the zeta potential of the NPs, resulting in considerable electrostatic repulsion between the NPs at all concentrations of EPS, and a z-average diameter at near ~200 nm regardless of the absence or presence of EPS.

3.3. Goethite nanoparticle stability as a function of electrolyte solution in the absence and presence of EPS

Ionic strength is also a crucial factor influencing the NP stability. The effect of various anions on the stability of goethite NPs was investigated using sodium chloride (NaCl), sodium nitrate ($NaNO_3$), sodium sulfate (Na_2SO_4) and sodium phosphate (Na_3PO_4) as background electrolytes.

The attachment efficiencies (α) of goethite NPs as a function of electrolyte (NaCl, $NaNO_3$ and Na_2SO_4) solutions at pH 5.5 are presented in Fig. 6a, b, and c, and representative aggregation profiles are shown in Fig. S4a, b, and c, respectively. At pH 5.5, the goethite NPs are positively charged and experience considerable electrostatic repulsion, leading to the formation of a stable suspension. The aggregation behavior of the goethite NPs in the three electrolyte solutions can be described by the classic Derjaguin, Landau, Verwey and Overbeek (DLVO) theory. As shown in the data for the goethite NP suspensions without the addition of EPS, when the concentration of NaCl < 40 mM, $NaNO_3$ < 50 mM and Na_2SO_4 < 0.3 mM, the degree of charge screening of the positive goethite NPs by the negative electrolyte anions increases with increasing electrolyte concentration, leading to an increase in attachment efficiency and aggregation kinetics. This is known as the reaction-limited regime ($\alpha < 1$). When the concentration of NaCl > 40 mM, $NaNO_3$ > 50 mM and Na_2SO_4 > 0.3 mM, the complete charge screening of the goethite NPs by the electrolyte anions eliminates the energy barrier between them, enabling the NPs to undergo diffusion-limited aggregation (α = 1). The minimum electrolyte concentration in the diffusion-limited regime is defined as the critical coagulation concentration (CCC) (Chen and Elimelech, 2006). The CCC for goethite NPs is 43.0 mM and 56.7 mM in NaCl and $NaNO_3$ solution, respectively, which is much higher than the CCC of 0.39 mM in Na_2SO_4 solution. These CCC values obtained in the present study are consistent with those obtained by Xu et al. (2015), who reported that the CCC values for goethite NPs are 54.7, 62.6 and 0.2 mM in NaCl, $NaNO_3$ and Na_2SO_4 solution, respectively. According to the CCC values, divalent anions have much higher aggregation ability than monovalent anions for goethite NPs, which can be explained by the zeta potential data in Fig. 7a, b, and c. The zeta potential value of goethite NPs is approximately +35 mV in the absence of the electrolyte solutions, then decreases slowly with increasing concentration of NaCl and $NaNO_3$ solution from 0 to 200 mM until reaching an equilibrium, but decreases rapidly with increasing concentration of Na_2SO_4 solution from 0 to 0.5 mM. This indicates that the divalent sulphate counter ions in the Na_2SO_4 solution are more effective in charge screening of the goethite NP than the monovalent chloride and nitrate counter ions in the NaCl and $NaNO_3$ solutions, leading to the reduction of the electrostatic repulsive force between goethite NPs, and the occurrence of the CCC at a much lower Na_2SO_4 concentration.

The aggregation rates and representative aggregation profiles of goethite NPs in Na_3PO_4 solution are shown in Fig. 6d, Fig. S4d and Fig. S5, respectively. According to the data for the goethite NP suspensions without the addition of EPS in Fig. 6d, the aggregation rate does not reach its maximum even after the addition of 120 mM

Na_3PO_4 solution, because this system does not reach a diffusion-limited regime. As shown in equation (2), the attachment efficiency (α) of goethite NPs is defined as the ratio of the aggregation rate in the reaction-limited regime (k) to that in the diffusion-limited regime (k_{fast}), which means that the attachment efficiency or the CCC cannot be determined for the Na_3PO_4 system. However, the aggregation behavior in the Na_3PO_4 system can be explained in light of the particular behavior of phosphate with iron (hydr)oxides and the zeta potential data for goethite NPs in Na_3PO_4 solution in Fig. 7d. Previous studies (Arai and Sparks, 2007; Xu et a., 2017) have shown that phosphates are mainly specifically adsorbed on (hydr)oxides through ligand exchange to form inner-sphere complexes. This specific adsorption has a pronounced effect on the goethite NP surface charge. Specifically, an increase in Na_3PO_4 concentration from 0 to 1 mM leads to a notable fall in the zeta potential of goethite NPs with the surface charge changing from positive to negative. When the zeta potential value approximates zero, the NPs aggregate immediately and the aggregation rate reaches its maximum. As the zeta potential becomes increasingly negative, the electrostatic repulsion between the goethite NPs leads to a fall in the aggregation rate at 1 mM Na_3PO_4 solution. When the concentration of Na_3PO_4 solution increases above 1 mM, the NP surfaces are increasingly negatively charged, which favors a screening of the negative charges by the electrolyte sodium counter ions, leading to a reduction in the net negativity of the zeta potential, and an increase in the aggregation rate. The results in this study demonstrate that the strong specific adsorption of phosphates can significantly affect the charge properties of goethite NPs, and the charge neutralization induced by specific adsorption can cause obvious variation in NP aggregation.

The effect of EPS on the stability of goethite NPs was explored by examining the aggregation profiles of goethite NPs in the presence of EPS (1 mg/L) as a function of NaCl, $NaNO_3$, Na_2SO_4 and Na_3PO_4 concentration (Fig. S6a, b, c and d) as well as the attachment efficiencies or aggregation rates of goethite NPs (Fig. 6a, b, c and d). In NaCl, $NaNO_3$ and Na_2SO_4 solution, the addition of EPS results in much lower α values compared within the EPS-free solution, indicating that EPS significantly hinders the aggregation of goethite NPs. The CCC values of goethite NPs in the presence of 1 mg/L EPS are 168 mM, 304.9 mM and 126.2 mM in NaCl, $NaNO_3$ and Na_2SO_4 solutions, respectively. A most probable explanation for the reduced attachment efficiency is that the steric repulsion between the goethite NPs from the adsorption of EPS molecules onto the NPs, which greatly enhances the stability of the systems. According to the corresponding zeta potential values, the surface charges of goethite NPs are instantly reversed upon the addition of negatively charged EPS, and with increasing concentration of electrolyte solution, the positively charged sodium counter ions can promote the aggregation of goethite NPs by shielding the electrostatic repulsion between them, implying that electrostatic repulsion also plays an important role in this reaction process (Fig. 7a, b, and c).

In contrast, the addition of EPS does not significantly alter the aggregation rates of goethite NPs in Na_3PO_4 solution except at the highest concentration (above 100 mM), probably due to the reason that, with both phosphate and EPS adsorbed onto the goethite NPs, some of the electrostatic repulsion experienced by the NPs can only be overcome at a very high concentration of sodium counter ions. In this reaction regime, the zeta potential is closest to zero, thus promoting NP aggregation (Fig. 7d). The interaction mechanisms between EPS and goethite in Na_3PO_4 solution were also investigated by XPS and FTIR spectroscopy. FTIR spectra of goethite, goethite-Na_3PO_4 and goethite-EPS-Na_3PO_4 are shown in Fig. S7. The FTIR spectrum of unreacted goethite shows only one broad absorbance band at 1650 cm^{-1}, which is attributed to the overtones of OH vibrations (Cao et al., 2011). The main absorption bands of adsorbed EPS at 1662 cm^{-1}, 1552 cm^{-1} and 1410 cm^{-1} are assigned to C=O of amides associated with proteins (amide I), N-H and C-N in CO-NH- of proteins (amide II), and C-O of COO^- groups, respectively (Fang et al., 2012; Lin et al., 2016b; Omoike et al., 2004). For goethite-Na_3PO_4 and goethite-EPS-Na_3PO_4 complexes, the emerging band at 1049/1052 cm^{-1} is due to the adsorption of phosphate on the goethite surface (Wang et al., 2017). Similar results can also be obtained from the XPS spectra (Fig. S8). Table S1 presents the elemental composition of goethite, goethite-Na_3PO_4 and goethite-EPS-Na_3PO_4, obtained by integrating the C1s, O1s, N1s and P2p peaks from the wide scan spectrum. Compared to the unreacted goethite, the emergence of the phosphorus peak at 132.7 eV and 133.8 eV suggests the adsorption of HPO_4^{2-} and PO_4^{3-} on the goethite surface in goethite-Na_3PO_4 and goethite-EPS-Na_3PO_4 complexes (Xie et al., 2013).

4. Conclusions

Our results indicate that the addition of EPS can promote the aggregation of goethite NPs only when the NP surface is positively charged in the absence of IS. Electrolyte solutions (NaCl, $NaNO_3$ and Na_2SO_4) can promote the aggregation of goethite NPs through the counter ion screening effect by reducing the electrostatic repulsion between NPs, and this effect can be weakened by the addition of EPS. At a low Na_3PO_4 solution concentration, the specific adsorption of phosphate first neutralizes the goethite NP surface charge and promotes the aggregation of NPs, then the aggregation is inhibited due to the increasingly negative charge on the NP surface, and at a high Na_3PO_4 solution concentration, the aggregation rate of goethite NPs can be increased again owing to enhanced charge screening by sodium counter ions. Overall, at circumneutral pH and in most natural electrolyte solutions, EPS inhibits the aggregation of goethite NPs and thus enhances their stability. This study facilitates the understanding of the behaviors of goethite NPs in the environment.

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