# Spectroscopic studies of ternary interactions in an öocyst-surfactant-hematite system

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## Abstract

Prior studies have indicated that the subsurface transport of *Cryptosporidium parvum* is diminished in sediments containing iron oxides, but the molecular mechanisms are poorly known, as are the impacts thereon of natural organic matter (NOM). Using in-situ attenuated total reflectance (ATR)-FTIR spectroscopy, we examined the molecular mechanisms of viable *Cryptosporidium parvum* öocyst adhesion at the hematite ($\alpha$-$Fe_2O_3$)-water interface over a wide range in solution chemistry. The anionic surfactant sodium dodecylsulfate (SDS) was used as a surrogate for NOM to examine the impacts of surfactant-type components on öocyst adhesion mechanisms. Results indicate that, in the absence of SDS, öocyst surface carboxylate groups form inner-sphere complexes with hematite Fe metal centres at low pH and outer-sphere complexes at high pH. Such direct chemical bonding is likely one mechanism whereby Fe oxides diminish öocyst transport. The presence of SDS significantly diminishes Fe-carboxylate complexation in the öocyst-SDS-hematite ternary system. Results suggest that the sulfate groups ($OSO_3^-$) of SDS compete effectively for $\alpha$-$Fe_2O_3$ surface sites, and this is likely the primary mechanism for decreasing Fe-carboxylate complexation. Sorptive competition with NOM may, therefore, increase the mobility of *C. parvum* öocysts in soils.

## Key Words

ATR-FTIR spectroscopy, öocyst adhesion, hematite, SDS, Fe-carboxylate complexes

## Introduction

*Cryptosporidium parvum* is a water-borne protozoan pathogen that is responsible for the gastrointestinal disease Cryptosporidiosis, which is potentially lethal for immuno-compromised individuals (Casemore *et al.* 1997). Öocysts, the encysted, environmental form of the obligate pathogen, exhibit a complex mixture of surface biomacromolecules consisting primarily of amide, carboxylate, phosphate, and polysaccharide functionalities (Gao and Chorover 2009). Ionizable functional groups, such as carboxylate and phosphate groups, may serve as reactive sites for direct bonding to mineral surfaces. In a previous spectroscopic study, we found that formation of inner-sphere versus outer-sphere complexes between öocyst surface carboxyls and hematite surface hydroxyls was dependent on solution chemistry (Gao *et al.* 2009). Such molecular-scale chemical bonding is likely to retard öocyst transport in Fe-rich tropical soils.

Ionic surfactants are ubiquitous in the environment and may play important roles in mediating the fate and transport of pathogenic cells in the subsurface. For example, anionic surfactants can decrease or even reverse the positive surface charge on metal oxides by forming monolayer or bilayer surface patches (Fuerstenau and Colic 1999; Bai *et al.* 2004). This could diminish öocyst adhesion due to an increase in electrostatic repulsive force. However, it is also possible that surfactant coatings could enhance öocyst adhesion to mineral surfaces because of an increase in sorbent hydrophobicity. Furthermore, ionic surfactant compounds can form surface complexes at mineral surfaces (Bai *et al.* 2004; Gao and Chorover 2010), competing for the reactive surface sites with öocyst surface biomolecules. Despite the prevalence of naturally produced and synthetic surfactants in waters contaminated with *C. parvum* öocysts, their influence on öocyst fate and transport remains poorly understood. The main objective of this study was to use SDS as a model compound to examine the effect of surfactant-type NOM components on öocyst adhesion mechanisms using in-situ ATR-FTIR spectroscopy, and to explore the potential effect of anionic surfactant on pathogen transport in the subsurface. Specifically, we compared the ATR-FTIR spectra of öocysts at the aqueous solution – hematite interface in the presence and absence of SDS. By close examination of spectral changes, the molecular interaction mechanisms were determined.

## ATR-FTIR spectra of Öocysts suspension

ATR-FTIR spectrum of viable öocyst suspension on the ZnSe internal reflection element (IRE) exhibits IR bands corresponding to amides (1635, 1542, and 1337-1313/cm for amide I, amide II, and amide III, respectively), carboxylate ($COO^-$ at 1400/cm), phosphate (1237/cm), and polysaccharide functional groups (C-O-C, C-C, 1150-950/cm) (Figure 1a). The spectrum on the same IRE coated with nano-particulate $\alpha$-$Fe_2O_3$ (Figure 1b) is distinctly different from the spectrum for the uncoated IRE. It exhibits much stronger asymmetric and symmetric $COO^-$ stretching bands, suggesting the formation of Fe-carboxylate complexes during öocyst adhesion. In addition, the separation in wavenumber ($\Delta\nu$) of $\nu_{as}$ and $\nu_a$ $COO^-$ stretching bands (e.g., $\Delta\nu > 200$/cm for monodentate, 180 - 150/cm for binuclear bidentate, and < 100/cm for mononuclear bidentate) (Deacon and Phillips 1980) allows the complexation mode (inner- versus outer-sphere) to be assigned (Gao *et al.* 2009).

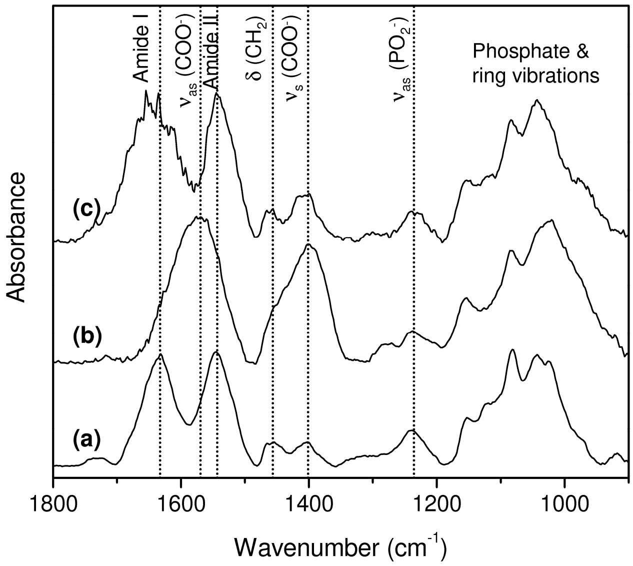

**Figure 1. ATR-FTIR spectra of viable öocyst suspension in 10 mM NaCl solution at pH 6 on (a) ZnSe IRE alone, (b) α-$Fe_2O_3$ surface in the absence of SDS, and (c) α-$Fe_2O_3$ surface in the presence of 0.01 mM SDS.**

When SDS is introduced to the ternary system (Figure 1c), the intensity of the asymmetric stretching of $COO^-$ is significantly reduced to the extent that it is masked by the amide group vibrations. The intensity of the symmetric stretching band is substantially reduced as well. Such intensity decreases in carboxylate stretching modes clearly suggests that the presence of SDS significantly diminishes Fe-carboxylate complexation at the hematite surface.

## Kinetics of Öocyst-SDS-Hematite ternary interaction

Figure 2 presents the result of experiments of öocyst adhesion to hematite in the absence (Figure 2a-e) and presence (Figure 2f-j) of SDS as a function of interaction time (proceeding from bottom to top) and pH (left to right) in 10 mM NaCl solution. We focus on the spectral region of $\nu_s(COO^-)$ (1500 - 1200/cm) because it is subject to minimum influence from the bending vibrations of $H_2O$ and exhibits most distinct changes responding to reaction time. As discussed above, when SDS is absent, the symmetric stretching band increases progressively with interaction time for all pH values due to the formation of the Fe-carboxylate complexes (Figure 2 a-e). Two symmetric stretching bands were observed at low pH (3 and 4.5) at 1370 and 1400/cm, respectively, whereas only one stretching band at 1400/cm was observed at higher pH. On the basis of $\Delta\nu$ of $\nu_{as}$ and $\nu_a$ $COO^-$, öocysts are bound to hematite via monodentate (Figure 3a) and binuclear bidentate (Figure 3b) complexes. The former predominates at low pH, whereas the latter becomes increasingly prevalent with increasing pH.

In contrast, we observe the opposite trend when SDS is introduced to the ternary system. As shown in Figure 2f-j, the symmetric stretching band decreases with interaction time for all pH values, suggesting that the extent of Fe-carboxylate complexation is diminished gradually in the ternary system. In addition, similar to the spectra in the absence of SDS, the symmetric stretching also contains two bands at 1418 and 1400/cm in the pH range of 3 - 7.5, indicating the presence of two inner-sphere complexes. However, one of the bands shifted from ~ 1370/cm in the absence of SDS to 1418/cm in the presence of 0.01 mM SDS, suggesting that SDS also changed the complexation mode between hematite and öocyst surface carboxylate groups.

The peak position of the asymmetric $COO^-$ stretching was determined at 1577/cm by peak deconvolution. Thus, based on the $\Delta\nu$ between $\nu_{as}$ and $\nu_s$ of $COO^-$, both peaks at 1418/cm ($\Delta\nu$ = 159/cm) and 1400/cm ($\Delta\nu$ = 177/cm) could be potentially attributed to binuclear bidentate complexes. In addition, at pH 3, the band at 1400/cm decreases more quickly relative to the band at 1418/cm, indicating the complexation mode at

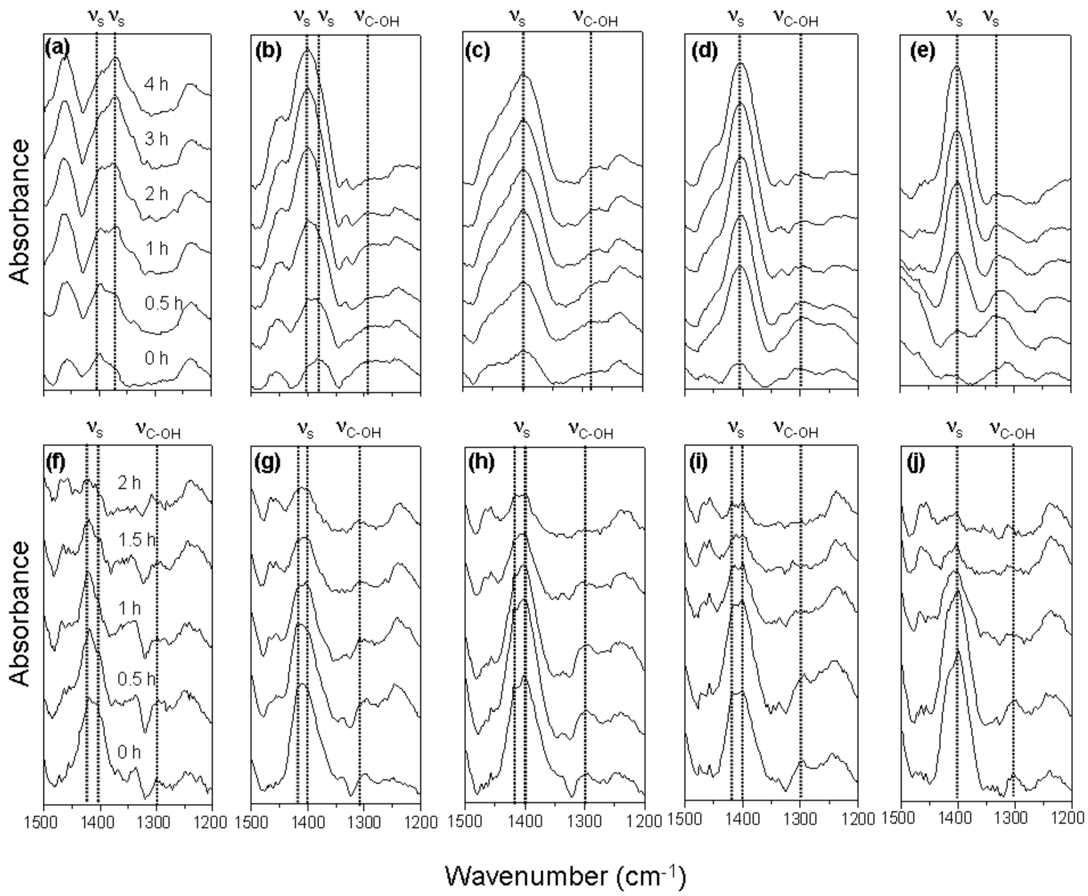

**Figure 2. ATR-FTIR spectra of viable öocyst suspension on α-$Fe_2O_3$ surface in the absence of SDS in 10 mM NaCl solution as a function of reaction time in the region of $\nu_s(COO^-)$ (1500 - 1200/cm) at (a) pH 3, (b) pH 4.5, (c) pH 6, (d) pH 7.5, (e) pH 9, and in the presence of 0.01 mM SDS at (f) pH 3, (g) pH 4.5, (h) pH 6, (i) pH 7.5, (j) pH 9.**

1418/cm is relatively more stable at this pH (Figure 2f). Because the band at 1400/cm occurs in both systems (absence or presence of SDS), we propose that the presence of SDS has shifted the other band (at 1370/cm in the absence of SDS) to 1418/cm in the öocyst-SDS-hematite ternary system, which could be interpreted as a change from monodentate to binuclear bidentate coordination. However, since this assignment derives from symmetry considerations, the band at 1418/cm is also possibly due to carboxylate binding to one Fe(III) (monodentate) and H-bond formation with a hydroxyl group, which possesses similar symmetry to binuclear bidentate coordination. The hydroxyl groups in the ternary system may derive from both the hematite surface and sulfate groups of SDS. The absence of this band in the binary öocysts-hematite system, however, suggests that hematite surface hydroxyls do not form H-bonds with the cell surface carboxylate groups. Hence, we attribute the observed shift to the formation of H-bonds between the sulfate groups of SDS and the carboxylate groups of the monodentate complex, forming a ternary, carboxylate-SDS-mineral complex (Figure 3c). This interpretation is consistent with the change of the dominant monodentate complex in the absence of SDS at pH 3 to the dominant carboxylate-SDS-hematite ternary complex in the ternary system after the introduction of SDS.

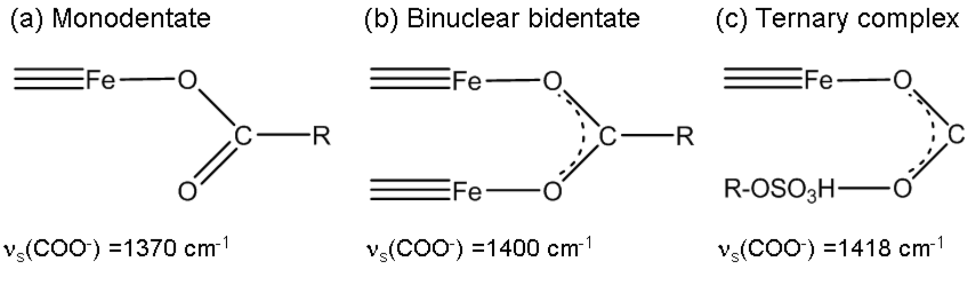

**Figure 3. Schematic illustration of the surface complexation structures formed between öocyst surface carboxylate groups and hematite based on the Δν of $\nu_{as}$ and $\nu_s$ $COO^-$. The peak position associated with each complex was labelled in the figure.**

With increasing pH, the spectra exhibit a similar trend with the symmetric $COO^-$ stretching decreasing with reaction time (Figure 2g-j). In the pH range of 4.5 - 7.5, the spectra still contain two bands at 1418 and 1400/cm, corresponding to the carboxylate-SDS-hematite ternary complex and binuclear bidentate complex, respectively. However, the contribution from each complexation mode is noticeably changed. The binuclear bidentate complex represented by the band at 1400/cm becomes relatively stronger with increasing pH. There are two possible explanations for this observation. First, we found that high pH favors the binuclear bidentate complex in NaCl background electrolyte (no SDS) (Gao *et al.* 2009). Another possibility is that gradual deprotonation of sulfate and carboxylate groups with increasing pH hinders the formation of H-bond between them, and thereby reduces the contribution from the ternary complex. When pH increases to 9, the band at 1400/cm becomes the only dominant band.

## Molecular mechanism of SDS effect on Öocyst adhesion

We speculate that the competition between the $OSO_3^-$ group of SDS and the carboxylate groups in the microbe-mineral interfaces is a primary mechanism for the decrease of the asymmetric and symmetric $COO^-$ stretching in the spectra of the ternary system. If the substitution of the sulfonate groups for the carboxylate groups occurs in the ternary system, the spectra should exhibit an increase of $\nu_{as}(OSO_3^-)$ with reaction time associated with the decrease of $\nu_s(COO^-)$ due to the formation of Fe-sulfate complexation, which was confirmed by the ATR-FTIR data (data now shown).

## Conclusions

A molecular understanding of the effect of SDS on öocyst adhesion mechanism to hematite was achieved using in-situ ATR-FTIR spectroscopy. Our results suggest that small concentration of surfactants can have profound effect on mechanisms of öocyst adhesion and, potentially, transport in the environment. The results indicate that the Fe-carboxylate surface complexation formed between öocyst surface biomolecules and hematite was significantly diminished in the öocysts-SDS-hematite ternary system. The primary mechanism causing decreased Fe-carboxylate complexation in the presence of SDS is likely due to sulfate substitution by forming Fe-sulfate complexes at hematite surface. In addition, such substitution is favored at higher pH, implying that weakly bounded öocysts by outer-sphere complexation can be easily displaced by ionic surfactants. Biosurfactants produced by soil microorganisms are structurally comparable to SDS, and thereby may have similar effect on öocyst transport. The results of this study provide critical information of the molecular bonding mechanisms for predicting the fate and transport of pathogenic cells in the subsurface prevalent with surfactant-type compounds.

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