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Scanning electron and atomic force microscopy investigation of extracellular polymeric substances, hematite and EPS-hematite colloids and aggregates

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Natural colloids are involved in a multitude of biogeochemical and physicochemical processes in aqueous systems. However, the chemical composition, mineralogical diversity and morphological variability of natural colloids are the reasons for the difficulty to understand their formation, stability and mechanisms of interaction with other solutes.
In this study we explore the effects of different amount of extracellular polymeric substances (EPS) of Bacillus subtilis on the aggregation and stability of hematite colloids.
The hematite colloids were synthesized using Schwertmann and Cornell method [1], where ferric nitrite solution slowly drops into the boiling water. Bacillus subtilis EPS was obtained using Omoike and Chorover method [2], where EPS was precipitated from the supernatant solution by using three volumes of cold ethanol. Then the mixture was centrifuged and dialyzed to remove ethanol and residual media components and stored at -20C. Synthetic hematite was mixed with different amounts of EPS resulting in solutions with EPS/hematite ratios of 1:5, 1:2, 1:0.5 and 1:0.2. Droplets of the colloidal suspension were put on silicon wafer and subject to air drying. The wafers were then analyzed by Scanning Electron Microscopy (SEM) with energy-dispersive Xray spectroscopy and Atomic Force Microscopy (AFM). A control sample with pure synthetic hematite colloid was also prepared and analyzed.
Pure hematite colloids form homogenic distribution of relatively small aggregates of 40 to 100 nm size. Theses aggregates loosely connect to each other creating skeletal or fisher-net like structures.
The smallest amount of EPS results in coagulation of hematite in very large (up to 80 μm) islands/aggregates of tightly packed hematite nanoparticles. Adding EPS decreases the size of islands to the point where again only 40 to 100 nm size aggregates are visible, but they are strictly separated in comparison to the pure hematite colloid.
Although separation of hematite aggregates with higher EPS content can be attributed to the steric forces of organic matter, the mechanism behind creation very large islands of hematite with small amount of EPS is very interesting and do not have clear explanation at the moment.

[1] Schwertmann & Cornell (2000) “Iron Oxides in the Laboratory”, Wiley-VCH.
[2] Omoike & Chorover (2006) Geochimica et Cosmochimica Acta 70, 827-838.