Supporting Information

Resolving early stages of homogeneous iron(III) oxyhydroxide formation from iron(III) nitrate solutions at pH 3 using time-resolved SAXS

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1. THE INVERSE FOURIER TRANSFORM METHOD FOR SAXS DATA ANALYSIS

As an alternative to the UM approach, we also attempted to fit data using the Inverse Fourier Transform (IFT) method assuming a polydisperse distribution of globular objects.\(^1\) However it was not possible obtain reasonable fits using this approach: at shorter times fitting was problematic at high \(q\) values because of the low value of the exponent of the power law region (i.e. significantly lower than 4), which required the addition of a large mass of very small spheres; at longer times fitting was problematic at high \(q\) where additions of a small masses of very large particles was required to achieve fits. Hence, for shorter times the resulting size distributions were highly non-unique and unrealistic, while at longer times distinct peaks at sizes that matched well with \(R_g\) resulted. Ultimately, while the two approaches gave reasonably consistent results at longer times, the IFT analysis did not add much additional information to that obtained from the UM approach and was not directly comparable due to the different assumptions behind each modeling approach. A similar conclusion has previously been drawn in a study of soot nanoparticle aggregation in which both model approaches were used.\(^2\) Therefore, the IFT method was not used further for data analysis and interpretation in this work.
2. DYNAMIC LIGHT SCATTERING EXPERIMENTS

Dynamic light scattering (DLS) measurements were conducted on solutions containing the same final composition as those used for SAXS measurements using a similar experimental configuration to that shown in Fig. 2 except that a static micromixer chip (Dolomite Microfluidics) was used rather than the static T-mixer and the mixed solution was directed into a quartz cuvette rather than a capillary. Immediately after collection of ~2 mL of solution (which took ~5 s), the cuvette was placed inside a Malvern Zetasizer ZS and data acquired every 15 s for 30 min (with 3 runs of 5 s per acquisition), with signal attenuation configured to achieve counts in the optimal range for photon correlation analysis.

Ageing solutions/suspensions of Fe oxyhydroxide at pH 3 absorb and scatter visible light with increasing magnitude as ageing continues. However the absorbance of the solution/suspension at the laser wavelength used (633 nm) was measured by spectrophotometry to be negligibly small up to 30 min (and in fact for some time beyond this). This was also supported by visual observations, in which no color was detectable in the experimental solutions/suspensions at the beginning or conclusion of DLS experiments. Therefore the color of solutions was not expected to pose any problems for DLS analysis. This was also confirmed by the fact that correlograms obtained did not display any aberrations that would be expected if sample color were a problem; the correlation function intercept remained constant at ~0.8 and the decay of the correlation function to zero was smooth. The measurements met the Malvern quality criteria.

DLS measurements exhibited no change in count rate over 30 min (data not shown), implying that sedimentation was unlikely to have occurred. Despite attempts to use several different modeling procedures, further useful information on particle sizes and size distributions could not be reliably obtained from DLS due to the confounding influence of polydispersity and because information about primary particles within aggregated structures cannot be resolved by this technique.
3. TRANSMISSION ELECTRON MICROSCOPY

In order to provide a simple qualitative test of the reasonableness of the interpretation of the SAXS data with regard to the size and dimensionality of particles determined using the UM fit, we employed transmission electron microscopy (TEM) to observe FeOx particles 10 s and 1000 s after initiating FeOx formation. Reagent solutions were prepared identically to those used in the SAXS experiments. Polymerization was initiated by rapidly pipetting 5 mL of PIPES buffer solution into 5 mL of acidified Fe solution contained within a 30 mL polypropylene tube that was continuously and rapidly stirred by a Teflon-coated stir bar on a magnetic stirrer plate. After 10 s of stirring, 5 mL of 5 g.L⁻¹ chitosan (previously purified as described by Sipos et al.³) was added to the mixture, which was stirred for another 5 s. (Chitosan was added to inhibit particle aggregation during subsequent sample drying³ and thereby maintain particles in a dispersed form for TEM.) The mixture was aspirated into a 20 mL plastic syringe, then snap frozen by rapid injection into a 50 mL polypropylene centrifuge tube containing ~30 mL of liquid nitrogen (LN2). The centrifuge tube was also partially submerged in LN2 to maintain the sample in frozen form after the procedure was complete. Subsequently, excess LN2 was decanted from the tube, which was then capped and stored at -85°C until further processing. The final pH in the solutions was verified to be 3.00 ± 0.05 by measuring the pH in several replicate samples prepared in exactly the same way. The entire process was then repeated, except that chitosan was not added to the mixture until 1000 s after initiation of polymerization.

The 50 mL centrifuge tubes were subsequently freeze dried for ~18 h, by which time a dry honeycomb-like material remained in the tubes. The dry material was ground to a powder in LN2 using an agate mortar and pestle. Acid-washed pure silica was also added to assist with grinding. The powder was suspended in ethanol and a small drop of the suspension deposited on to a holey carbon grid, which was then placed on a copper support and mounted inside a JEOL JEM-2100 LaB₆ Transmission Electron Microscope. Images of samples were obtained at up to 250,000x magnification. Electron Dispersive Spectroscopy (EDS) was also conducted to verify that the particles examined were indeed FeOx.

Although we did not perform sufficient TEM measurements to form a statistically rigorous conclusion, TEM images confirmed the formation of FeOx particles with size and dimensions of the same order of magnitude as...
values obtained from the UM fit to the SAXS data (Figure S1). This provides basic confirmation of the reasonableness of the UM parameters. However, any further interpretation of the TEM data must be considered with caution, as the method of sample preparation is significantly different to that used in the SAXS experiments for two main reasons:

1. FeOx formation was initiated by conventional mixing using a magnetic stirrer, rather than the rapid mixing apparatus used during SAXS experiments. The SAXS data indicate that nucleation is essentially complete within 1 s at the Fe concentration and pH used, while magnetic stirring can typically achieve mixing times of at best around 1 s under optimal conditions. Therefore, the procedure used could potentially have favored formation of larger particles through localized high concentrations of Fe during mixing. Despite this possibility, it seems unlikely that this occurred to such an extent as to invalidate the TEM study. First, the SAXS results strongly suggest that FeOx formation proceeds initially through formation of polycations and small colloids that undergo restructuring on a relatively rapid timescale (seconds to minutes). Second, particles observed during the TEM study were not substantially larger than those expected from the SAXS experiment, suggesting that the TEM results were reasonable. These factors therefore suggest that the size of FeOx particles was not significantly influenced by the different mixing techniques used in the TEM and SAXS studies. Nonetheless, it is possible that other properties of the particles may have been affected by the different techniques used.

2. Samples were frozen and subsequently freeze-dried prior to TEM analysis. To inhibit aggregation of particles during the process, chitosan was added immediately prior to the freezing step. This treatment was previously shown to inhibit FeOx particle aggregation even at relatively high pH where aggregation is otherwise typically rapid, by formation of a non-specifically bound polysaccharide coating layer around FeOx particles. As samples were frozen rapidly (within a few seconds) by immersion in LN2, it is unlikely that substantial changes in particle size would have occurred during this process, given that primary particle restructuring and aggregation to secondary particles were observed to occur on timescales of at least several seconds during SAXS experiments. Furthermore, the consistency between the size of
FeOx particles observed during TEM measurements and SAXS experiments again supports the notion that any particle aggregation during the procedure was negligible.

On this basis, we therefore believe that the size of the FeOx primary particles and aggregates observed in the TEM study was not significantly modified by the sample preparation procedure. However, it is entirely possible that other properties of the FeOx particles may have been influenced by the relatively slow mixing, freezing and freeze drying steps; therefore no attempt has been made to extract any additional information from the TEM images.
Figure S1. TEM images of FeOx freeze-dried (A) 10 s and (B) 1000 s after the initiation of FeOx formation by neutralization of an acidified Fe(NO₃)₃ solution.
4. REFERENCES

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