Progress on Cryogenic Analytical STEM of Nanomaterials

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The potential for the physicochemical properties of engineered nanomaterials to be exploited in numerous product areas including medicine, catalysis, energy and the environment is beginning to be fulfilled. While many techniques measure nanoparticles in the pristine state, characterisation of the same particles under actual application conditions, i.e. dispersed at low concentrations in environmental or physiological media, is far more challenging. Conventional TEM, with samples prepared simply by drop casting enables imaging and analysis of individual nanoparticles but, because of the drying process, does not capture the particle agglomeration in the dispersion or the surface chemistry when hydrated [1]. Yet these characteristics are vital to understand for successful exploitation of nanoparticles and can be addressed if the nanoparticle suspension can be captured by rapidly freezing and transferring into the microscope frozen to avoid drying and re-dispersion.

To capture nanoparticle suspensions we have implemented cryogenic sample preparation and transfer for our physical sciences-based electron microscope, a Titan Themis[3] G2 TEM. This microscope is optimized for analytical work, with a SuperX 4 EDX detector arrangement, Gatan Quantum spectrometer and a monochromator. While this microscope is often used for conventional imaging and analysis, we can also utilize a FEI Vitrobot for sample preparation and a Gatan 914 TEM cryo-holder to examine frozen samples. On the microscope we have focused on using scanning transmission electron microscopy (STEM), and through control of the probe current (10 – 280 pA) and imaging or spectroscopy conditions we can collect data prior to devitrification (i.e. melting) of the amorphous ice. We have used energy dispersive X-ray (EDX) spectroscopy to identify and spatially map different elements and electron energy loss spectroscopy (EELS) for elemental and oxidation state analysis. This setup has permitted the native state or in situ analysis of a range of nanomaterials in aqueous suspensions, with applications in areas such as medicine, personal products and environmental science.

We have undertaken low dose electron microscopy of nanoparticles suspended in vitreous ice, with our initial work being focused on a model nanoparticle mixture containing iron oxide, zinc oxide and ceria nanoparticles [2]. In this study we compared the conditions of cryo-TEM to cryo-STEM, finding that electron beam induced damage to the vitreous ice occurs at far higher total electron fluences in STEM mode. This permits the opportunity for spatially resolved cryo-STEM-EDX and cryo-STEM-EELS, with individual nanoparticle identification (Figure 1). EELS investigations could be undertaken in a way which either permitted mapping or higher resolution information, such as oxidation state, to be investigated [1]. We have since applied these principles to an iron oxide-based material, undertaking monochromated cryo-STEM-EELS to investigate iron oxidation state [3]. Figure 1(h) shows the cryo-EELS collected from an agglomerate of ceria nanoparticles encased in amorphous ice, and when compared to standard spectra for Ce(III) and Ce(IV), it can be clearly seen that in the suspension the Ce(IV) oxidation state is retained. Furthermore, we have shown that artefacts formed by drying nanoparticles in complex suspensions such as cell culture media used for nanomedicine and nanotoxicology studies can be avoided using a cryogenic sample preparation and imaging approach [4].

Our goal is to extend the capability of near native state imaging and analysis of nanoparticle systems by TEM. Using the cryogenic approaches developed here, we will detail further investigations on identifying and analysing surface coatings on suspended nanoparticles such as protein coronas and complex 3D nanoparticle based systems in the frozen hydrated state.

References:

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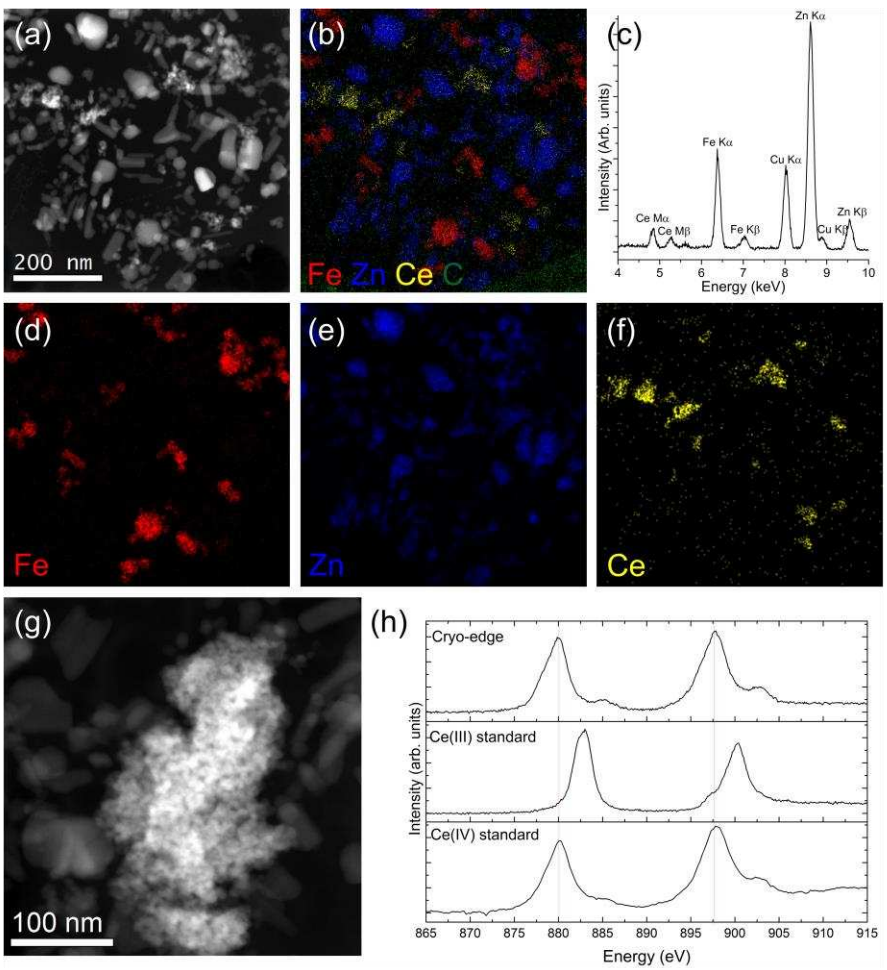

Figure 1. Cryo-analytical STEM of a model nanoparticle mixture. (a) Cryo-HAADF-STEM image with corresponding EDX maps and spectrum (b – f). (g) Cryo-HAADF STEM image with (h) EELS spectrum from the area compared to standard spectra (non-cryo) confirming the agglomerated CeO_2 nanoparticles in suspension retain a Ce(IV) oxidation state.