Cryogenic electron microscopy for native state analysis of soft- and nano-materials

Dario Luis Fernandez Ainaga[1], Teresa Roncal-Herrero[1], Martha Ilett[1], Stuart Micklethwaite[1], Zabeada Aslam[1], Eleena Naveed[1], James Hitchcock[1], Cheng Cheng[1], Benjamin Lobel[1], Olivier Cayre[1], Dr Nicole Hondow[1]
[1]University of Leeds, Leeds, UK

Background
Nanomaterials can potentially be used in a range of areas, including in consumer goods through to medical applications. In many areas, the nanomaterials are used while dispersed in a liquid, or contain hard-soft interfaces, which, while increasing their applicability to their end-use, can complicate characterisation and determination of structure-property relationships.

Electron microscopy, while ideally suited to the nanoscale imaging and analysis of these materials, can encounter limitations due to the vacuum requirements, which exclude many in situ or native state studies. In addition, necessary sample preparation can often lead to artefacts. A viable alternative is electron microscopy conducted on frozen hydrated samples, where the nanomaterials are 'captured' in the native state and any electron beam induced damage products are immobilised.

This work aims to develop representative native state analysis of dispersed nanoparticles in soft materials using cryogenic electron microscopy approaches, with application shown here to a commercial sunblock sample containing metal oxide nanoparticles and Pickering emulsions (water-oil mixtures) designed to contain an active ingredient.

Methods
Results from two soft materials containing dispersed nanoparticles will be detailed. The first sample is a Pickering emulsion, comprised of oil in water droplets stabilised by ~5 nm platinum nanoparticles. The approaches are further developed by examining a commercial product, a sunscreen containing active ingredients of 4.5% of TiO_2 and 6.5% of ZnO nanoparticles.

All samples were prepared for cryo-TEM using an FEI Mark IV Vitrobot©. A 3.5 µl drop of suspension was loaded onto a lacey carbon-coated copper TEM grid (EM resolutions) before being blotted and then rapidly plunge frozen in liquid ethane. Transfer into the microscope was done using a Gatan-914 cryo TEM holder, and the temperature was maintained below −165 °C during analysis. Comparison was made to a static liquid cell (LC) commercially sold as a K-kit and supplied by Bio-Matek. S/TEM analysis was carried out using an FEI Titan3 Themis G2 equipped with a monochromator operating at 300 kV and fitted with 4 EDX silicon drift detectors and a Gatan One-View CMOS camera. The probe current was kept below 100 pA for all cryo and LC experiments. Samples were prepared for cryo-SEM using a Quorum Technologies PP3010 Cryo-SEM preparation system and examined in an FEI Helios G4 CX Dual beam FIB-SEM with a beam voltage of 1–10 kV and beam current 100 pA, while elemental mapping via an Oxford instruments EDX spectroscopy system was conducted at 15 kV.

Results
We have previously shown the advantage of cryogenic-EM approaches to the analysis of dispersed nanoparticles, including those in complex biological cell culture [1,2]. In this work we will show the advantages of using a cryogenic approach for more complex soft materials systems incorporating nanoparticles, with extension to the use of cryo-STEM-tomography and cryo-FIB-SEM.

Cryo preparation, transfer and analysis is essential for Pickering emulsions as the integrity of the sample is maintained as drying and the microscope vacuum results in bursting of the droplets. Undertaking higher magnification imaging with careful consideration of total electron fluence it is possible to examine the distribution of the nanoparticles using cryo-STEM [3], and we will show that utilising cryo-HAADF STEM over a ±60° tilt range permits 3D visualisation of the sample structure. This results in the confirmation of both the position of the stabilising nanoparticles and the overall droplet shape in 3D space. Using a combination of cryo-EDX and -EELS the elements in both the nanoparticles and oil droplets are confirmed. Cryo-STEM and associated spectroscopies are also used to analyse the commercial sunscreen, with comparison to alternative in situ electron microscopy techniques – static liquid cell STEM and cryo-SEM [4]. While cryo-STEM does allow for higher resolution analysis, in this case both the concentration of dispersed particles and viscosity of the product causes complications with sample preparation. In analysis of a diluted product, both nanoparticle types are identified, something which was not possible in the static liquid cell due to electron beam artefacts causing dissolution of one nanoparticle type. Cryo-SEM was used to analyse the pure product without dilution but biased the characterisation to the larger fraction of nanoparticles and agglomerates.

Conclusions

Cryo electron microscopy offers route to the representative native state analysis of dispersed nanoparticles in soft materials. Complex systems, such as the soft hybrid inorganic-organic Pickering emulsions can be analysed by a combination of STEM-analytical techniques to provide nanoscale 3D information. Commercial products, with numerous components and required to be used at a set concentration can be more complicated, however with a combination of different in situ EM techniques an accurate, native state characterisation can be achieved.

Keywords:

cryo; STEM; cryo-SEM; nanomaterials

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