

**Technical Article (added by publisher)****Cu K-edge XANES: Polymer, organic, inorganic spectra and experimental considerations**

Mikael Larsson<sup>1,2</sup>, Johan B. Lindén<sup>2,3</sup>, Simarpreet Kaur<sup>2</sup>, Brock Le Cert<sup>2</sup>, Ivan Kempson<sup>a),2</sup>

<sup>1</sup>University College London, School of Energy and Resources, 220 Victoria Square, Adelaide, SA 5000, Australia

<sup>2</sup>Future Industries Institute, University of South Australia, Mawson Lakes, SA, 5095, Australia

<sup>3</sup>Research Institutes of Sweden, Division of Bioscience and Materials, Chemistry, Materials and Surfaces, Brinellgatan 4, 50462, Borås, Sweden

In pursuit of design and characterization of Cu adsorbing in thin films, we present data from a large variety of Cu-K edge X-ray Absorption Near Edge Spectroscopy spectra obtained from organic and inorganic standards. Additionally, we have explored the impact of beam damage inducing redox alterations. Polymer nanoparticles were tested against films to produce higher concentration samples while maintaining high surface area to bulk effects. Spectra from nanoparticles were highly comparable to thin films of ~8 nm thickness, implying comparable contributions by surface effects on copper association. Finally, we observed no impact on Cu XANES spectra from vitrification with DMSO to produce amorphous frozen, hydrated samples. The spectra should act as a valuable resource in assisting the design of experiments and identification of copper associations.

Key words: Cu-K edge XANES; thin films; nanoparticles; polymers; anti biofouling.

**I. INTRODUCTION**

Copper is a metal of relevance for a wide range of applications, from medicine to roofing (Dalecki *et al.*, 2017, Delangle & Mintz, 2012, Hedberg *et al.*, 2015). In many cases, it is important to understand the chemical environment around the copper atoms, and the oxidation state in materials and during processes. Of interest to the authors is the use of copper in marine anti-biofouling. Conventional marine antifouling coatings are loaded with copper that is slowly released and due to the inherent biocidal activity of copper prevents fouling of the surface by marine organisms (Lifset *et al.*, 2012, Chen *et al.*, 2013). Unfortunately, the released copper accumulates in marinas and harbours with detrimental effects on the environment (Kiaune & Singhasemanon, 2011).

We are researching an alternative approach to use copper-binding polymer ligands to capture copper present in the sea and release it upon a trigger, creating a cyclic flux of copper across the coating-water interface to prevent fouling, without any net addition of copper to the environment (Lindén *et al.*, 2014, Lindén *et al.*, 2015). We demonstrated that nano-thin coatings of glutaraldehyde (GA)-crosslinked polyethyleneimine (PEI) can effectively and selectively

a) To whom correspondence should be addressed. Electronic mail: Ivan.Kempson@unisa.edu.au

accumulate copper from real and artificial seawater and that the copper binding is effective even when polysaccharides adsorb on the surface (Kaur *et al.*, 2017).

In the development of these coatings, information on the oxidation state of copper in the coatings has been important and synchrotron X-ray Absorption Near Edge Spectroscopy (XANES) has been used to investigate this (Kaur *et al.*, 2017, Lindén *et al.*, 2015). The polymer systems and marine applications are highly complex and as such it is difficult to predict how Cu can be associated. Consequently, we have pursued collection of a broad range of XANES spectra to consider how the local Cu binding environment may be described and to reveal spectral features identifying subtle differences between conditions. The investigations of nanometer-thin coatings and a wide range of standards using XANES have provided us with a valuable source of experimental data from copper association in thin-coatings/films, polymers, organic ligands, inorganic species. Here we present many of these spectra as a resource for research into Cu association studies with XANES.

## II. EXPERIMENTAL

### A. Chemicals

Copper(II) oxide (CuO), copper(I) oxide (Cu<sub>2</sub>O), EDTA tetrapotassium salt, ethylenediamine, ammonia solution, copper(II) nitrate trihydrate (Cu(NO<sub>3</sub>)<sub>2</sub>•3H<sub>2</sub>O), copper(I) chloride (CuCl), copper(II) chloride (CuCl<sub>2</sub>), branched polyethyleneimine, PEI, (50 wt% in H<sub>2</sub>O, Mn ~ 60,000 and MW ~ 750,000), glutaraldehyde, GA, (25 wt% in H<sub>2</sub>O), dimethyl sulfoxide (DMSO) and microcrystalline cellulose, MCC, (powder, 20 μm) were purchased from Sigma-Aldrich. Copper(II) sulphate pentahydrate (CuSO<sub>4</sub>•5H<sub>2</sub>O) was purchased from Chem-Supply Pty Ltd. Sea Salts from Sigma-Aldrich were used to prepare artificial seawater at 38 g/l. Used water was from a Milli-Q® Advantage A10® water purification system and had a resistivity of 18.2 MΩ cm<sup>-1</sup>.

### B. Sample Preparation

Nano-thin coatings of GA-PEI were prepared on 20 μm thick 1x1 cm<sup>2</sup> fused silica Corning 7980 of UV grade substrates (Valley Design Corp.), using previously described spin-coating protocol (Lindén *et al.*, 2014).

Nanoparticles of crosslinked GA-PEI were prepared as follows: A solution of 1 wt.% PEI in Milli-Q (MQ) water was mixed with an equal volume of GA (1.7, 0.86, 0.43, or 0.21 wt.%) in MQ water. The GA concentrations corresponding with 2:1, 1:1, 1:2 and 1:4 ratios between crosslinking functionalities of the GA and primary and secondary nitrogen of the PEI. The mixtures were treated in an ultrasonication bath (Soniclean, Australia) for 2 h, followed by stirring with a magnetic rod for 1 day. The dispersions were dialyzed in 12 kDa MWCO tubing (Sigma Aldrich) for 2 days in 5 l of MQ water, replacing the water two times per day. Finally, a fraction of each solution was dried and the concentration of nanoparticles calculated from the dry weight. The nanoparticles were characterized with dynamic light scattering, zeta potential analysis, AFM, UV-Vis and FTIR spectroscopy. The copper binding of the particles was investigated in MQ water and artificial seawater using UV-Vis spectroscopy and XPS, where for XPS films of the nanoparticles were prepared on silicon wafers using a spin-coating protocol (Lindén *et al.*, 2014). Fully crosslinked nanoparticles were used in the analyses.

- a) To whom correspondence should be addressed. Electronic mail: Ivan.Kempson@unisa.edu.au

For ligands and nanoparticles in solution, copper(II) sulfate and ligands were mixed in MQ water so that the molar excess of ligand to copper was 20X and 10X for ligands with one or more coordinating groups, respectively. For polymers, the repeating unit was considered as one ligand. This ensured that a large majority of the copper was complexed to ligands. Coatings were loaded with copper by submersion in MQ water or artificial seawater containing 200 ppb copper, from copper(II) sulfate. Submersion time was chosen to achieve close-to equilibrium saturation (Lindén *et al.*, 2015). Following the submersion excess water was removed by gently touching the edge against a Kimtech wipe.

### C. X-ray Absorption Near Edge Spectroscopy (XANES)

XANES was conducted at the X-ray Absorption Beamline at the Australian Synchrotron. Samples were enclosed in a perspex mount and sealed with Kapton foil. Powder samples were prepared by mixing the analyte with microcrystalline cellulose (MCC), Sigma Aldrich) using an agate pestle and mortar to a copper concentration of about 500 ppm. Copper(I) complexes were extracted from the storage containers, mixed with MCC and sealed for analysis inside a glove box under argon atmosphere. Liquid samples with copper concentration of about 70–1000 ppm were prepared by injecting the solution through the Kapton foil using a syringe and subsequently sealing the hole. Film samples were attached to the perspex mount using Kapton foil. All samples were plunge frozen in liquid nitrogen before being introduced to the analysis chamber and analysed at < 10K. No DMSO was used in solutions other than those explicitly stated. XANES was performed over the Cu-K edge (8,979 eV) with the pre-edge region acquired in 10 eV steps before using 0.25 eV steps from 8,975 to 9,012.5 eV followed by 0.06 steps in K out to 6 K. Data across detector elements were averaged using the ‘Average’ software package and further post-processed with Athena (Ravel & Newville, 2005).

## III. RESULTS AND DISCUSSION

### A. Inorganic Cu Standards

XANES spectra of Cu inorganic standards are shown in Figure 1. Some samples have been compared in a solid and solution phase. These spectra demonstrate differences in the spectral features based on the solid versus solution form and is consistent with, although not as pronounced as, data for Cu(I) species (Etschmann *et al.*, 2011, Brugger *et al.*, 2007).

### B. Organic Associations

A variety of samples, or solutions containing different organic molecules and Cu, were analyzed for Cu association. This included constituents used in the formation of the polymeric thin films. The samples denoted as being additive (i.e. “+”) were samples of  $\text{Cu(II)SO}_4$  in solution and with the potential organic ligand subsequently added. Based on the position of the absorption edge, all samples appear to be representative of Cu(II) species. It is worthwhile to note that addition of the organic compounds to solutions generated different Cu absorption spectra, indicating the usefulness in Cu XAS distinguishing subtly different organic ligands in solution phase.

### C. Using DMSO for Vitrification

- a) To whom correspondence should be addressed. Electronic mail: Ivan.Kempson@unisa.edu.au

Ice crystals can scatter the incident analyzing X-rays and produce severely detrimental artefacts in spectra when incident on detectors. To avoid this issue, vitrification of liquid samples is preferable to freeze the sample in an amorphous, rather than crystalline, state. This is assisted by incorporation of DMSO into the sample. We tested inclusion of 40% by weight DMSO into test solutions to gauge any influence on the copper XANES as shown in Figure 3. For these three samples, there was no observable effect on XANES spectra with the inclusion of 40% DMSO. It should be noted however that these are all Cu(II) complexes and similar observations may not extend to Cu(I) complexes. The nanoparticle sample is described in more detail in Section III E.

#### D. Beam Damage Tests

Incident X-rays used for XANES can be detrimental to samples, resulting in poorly understood and unpredictable redox chemistry. In the case of arsenic for example, reduction from As(V) to As(III) can occur on the order of seconds (Kempson *et al.*, 2009, Kempson & Henry, 2010). Out of concern for possible beam alteration of the copper species we ran consecutive scans for some samples and monitored any spectral evolution as a function of beam exposure. Examples shown in Figure 4 represent a series of 3 minute scans. Figure 4a shows a reduction in Cu in an almost linear fashion for copper (II) chloride while no alteration was observed for copper sulfate pentahydrate (Figure 4b). The spectra for copper nitrate trihydrate shown in Figure 4c exhibited a steadily increasing pre-edge feature between each scan. The white line intensity however initially decreased and subsequently varied with either increases or decreases in relative absorption. Some of this is likely to be explained by subtle differences in spectra normalization. However it does not appear an adequate explanation due to the spectra reproducibility and consistent trends observed in the evolution of other spectral features. It may thus elude to a more complex balancing of redox chemistry. Overall, very minimal changes between scans were observed.

#### E. Surface Area Effects. Films Versus Nanoparticles

In our research, we are interested in glutaraldehyde (GA) cross-linked thin films on the order of ~8 nm thick. The quantity of material in these films is extremely low, making characterization very challenging. We aimed to compare spectra from nanoparticles to thin films that could provide similar surface to bulk properties. Nanoparticles could be analysed in much greater quantities for purposes of improving characterization. Nanoparticles of GA-PEI with varying degree of crosslinking were prepared to: (i) act as a model system to achieve good signal to noise for copper in the XAS experiments, due to the possibility to achieve higher total copper concentration over the beam path compared to the nano-thin coatings, and (ii) elucidate if there were differences in the chemical state of copper located at the surface or in the bulk of GA-PEI samples.

The nanoparticles were prepared by mixing GA and PEI at ratios between the molar crosslinking functionality of GA and primary and secondary nitrogen of PEI = 1:4, 1:2, 1:1, and 2:1. Conventional and diffusion NMR confirmed that an initial crosslinking reaction occurred within minutes where GA reacted with the available crosslinking groups of PEI. Visual observation and UV-Vis spectroscopy revealed that subsequently a slower reaction occurred in the material, causing samples to shift from colorless to increasingly red over a couple of days, indicating a significant change in the molecular structure. Calculations based on the mass of the

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product and UV-Vis spectroscopy revealed that the nanoparticles were fully crosslinked at a 1:1 ratio between GA crosslinking functionality and primary and secondary nitrogen of PEI (results not shown).

The diameter of the fully crosslinked nanoparticles was determined to about 10–20 nm by Atomic Force Microscopy (AFM) and Dynamic Light Scattering DLS (Figure 5). This means that the radial diffusion distance for copper to fully saturate the particles in solution was 5–10 nm, comparable to the nano-thin films previously investigated (Lindén *et al.*, 2014, Lindén *et al.*, 2015). All references to nanoparticles in the XAS experiments in this work refer to the fully crosslinked particles, designed to chemically resemble the nano-thin GA-PEI coatings.

The fully crosslinked nanoparticles presented a zeta potential of about +26 mV at pH 7 and it decreased to about +7 mV at pH 11, a similar behaviour as reported for nano-thin GA-PEI coatings (Lindén *et al.*, 2016). More importantly, as for nano-thin GA-PEI coatings (Lindén *et al.*, 2014, Lindén *et al.*, 2015), the nanoparticles were highly effective and selective in binding copper. This was concluded from X-ray Photoelectron Spectroscopy (XPS) analysis of nanoparticles on silicon wafer squares after submersion overnight in MQ water containing 200 ppb copper, or artificial seawater containing 200 ppb of copper and 45 other elements (from certificated standard solution), although the N:Cu ratio for the nanoparticles was determined to be about 20, compared with about 12 reported for the nano-thin coatings. The difference may be from the spin-coated nanoparticle films being thicker, so that the copper concentration in solution was significantly decreased during the uptake, and that erosion of the non-crosslinked nanoparticle films revealed underlying sample with less bound copper. Nonetheless, it was concluded that the nanoparticles could bind copper to at least a 1:20 Cu:N ratio from a concentration as low as 200 ppb, both in MQ water and in artificial seawater with competing ions.

XANES spectra acquired from the fully cross-linked nanoparticles are compared to a thin and thick film in Figure 6. Spectra from the nanoparticles is highly comparable to the thin, ~8nm thick, film. There are some subtle differences when compared to the thick film which was estimated to be at least ten times thicker than the thin film. This is presumably demonstrating the spectra to be a composition of Cu associated with bulk structure and Cu associated at the water-polymer interface. The thick sample would then be dominated by the bulk structure. Note that some small glitches are present in the spectra. These do not detract from the overall spectral curves but do demonstrate an improved signal to noise ratio for the nanoparticles compared to the films. In comparing the spectra of the films, nanoparticles with varying degrees of crosslinking and the standards, it can be concluded that the ligands involved in the cross-linking of the polymer do not detract from the Cu forming ligands and therefore the capacity of the material to accumulate Cu out of solution.

#### IV. CONCLUSION

The results from analysis of different copper salts and solutions containing organic molecules and copper provides a source of knowledge that should prove valuable for researchers interested in the oxidation state and chemical complexation of copper. The use of a nanoparticle model system was verified to represent nano-thin coatings with a thickness similar to the radius of the nanoparticles. It was further shown that addition of 40% DMSO as a vitrification agent did not significantly alter the XANES spectra for any of the investigated samples, a finding that is highly

- a) To whom correspondence should be addressed. Electronic mail: Ivan.Kempson@unisa.edu.au

important for cryo-analysis of hydrated samples, where ice crystals that form without the use of a vitrification agent prevents analysis due to scattering.

### ACKNOWLEDGEMENTS

This research was undertaken on the X-ray Absorption Spectroscopy beamline at the Australian Synchrotron, Victoria, Australia. We sincerely thank Dr Peter Kappen and Dr Chris Glover for assistance and advice in those measurements. We further acknowledge that work was performed at the South Australian node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nano and micro-fabrication facilities for Australia's researchers. We thank Nathan H Williamson at UniSA for his assistance with NMR measurements (data not shown) and Professor Magnus Nydén at UCL for valuable discussions.

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**Figure 1.** Cu-K edge XANES of a variety of inorganic compounds in solid (S) or solution (L) forms. All samples were analysed at a temperature <10K.

**Figure 2.** Cu-K edge XANES spectra of Cu in association with a variety of organic ligands or in their presence in solution to test for association. All samples were analysed at a temperature <10K.

**Figure 3.** Inclusion of 40% DMSO by weight into aqueous samples had no apparent effect on XANES spectra for copper sulfate or copper associated to polymer in solution or in nanoparticles form. All samples were analysed at a temperature <10K.

**Figure 4.** Testing of beam induced alteration XANES spectra of Cu in solution. Arrows indicate evolution in features with consecutive scans. All samples were analysed at a temperature <10K.

**Figure 5.** Size characterization of GA-PEI nanoparticles. Bar diagram shows the average peak hydrodynamic diameter of PEI and nanoparticles prepared using different crosslinker functionality to primary and secondary nitrogen ratio; Error bars indicate 1 SD (n=3). The AFM micrograph shows fully crosslinked nanoparticles in the dry state on a silicon wafer.

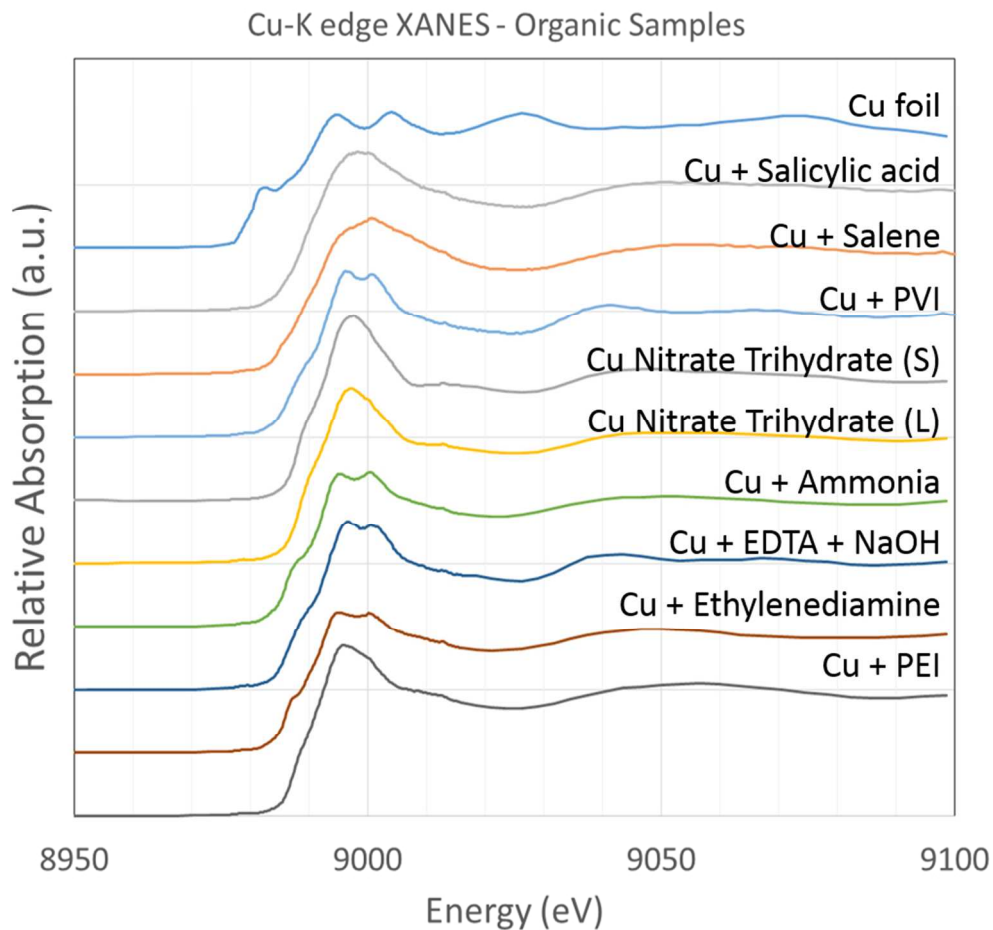
a) To whom correspondence should be addressed. Electronic mail: Ivan.Kempson@unisa.edu.au

**Figure 6.** Cu-K edge XANES shows Cu associated with nanoparticles is highly comparable to the thin film and is an alternative model system for improving signal intensities in characterization techniques. All samples were analysed at a temperature  $<10\text{K}$ .

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a) To whom correspondence should be addressed. Electronic mail: [Ivan.Kempson@unisa.edu.au](mailto:Ivan.Kempson@unisa.edu.au)

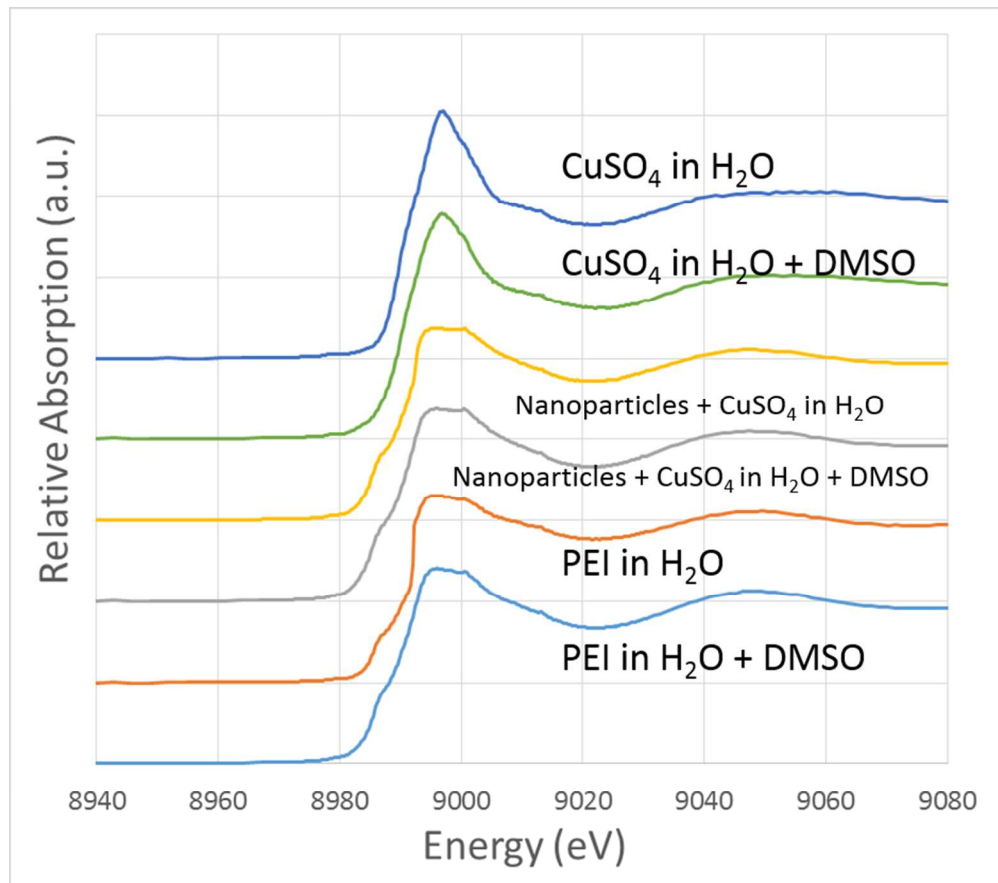




Cu-K edge XANES spectra of Cu in association with a variety of organic ligands or in their presence in solution to test for association. All samples were analysed at a temperature <10K.

174x164mm (150 x 150 DPI)





Inclusion of 40% DMSO by weight into aqueous samples had no apparent effect on XANES spectra for copper sulfate or copper associated to polymer in solution or in nanoparticles form. All samples were analysed at a temperature <10K.

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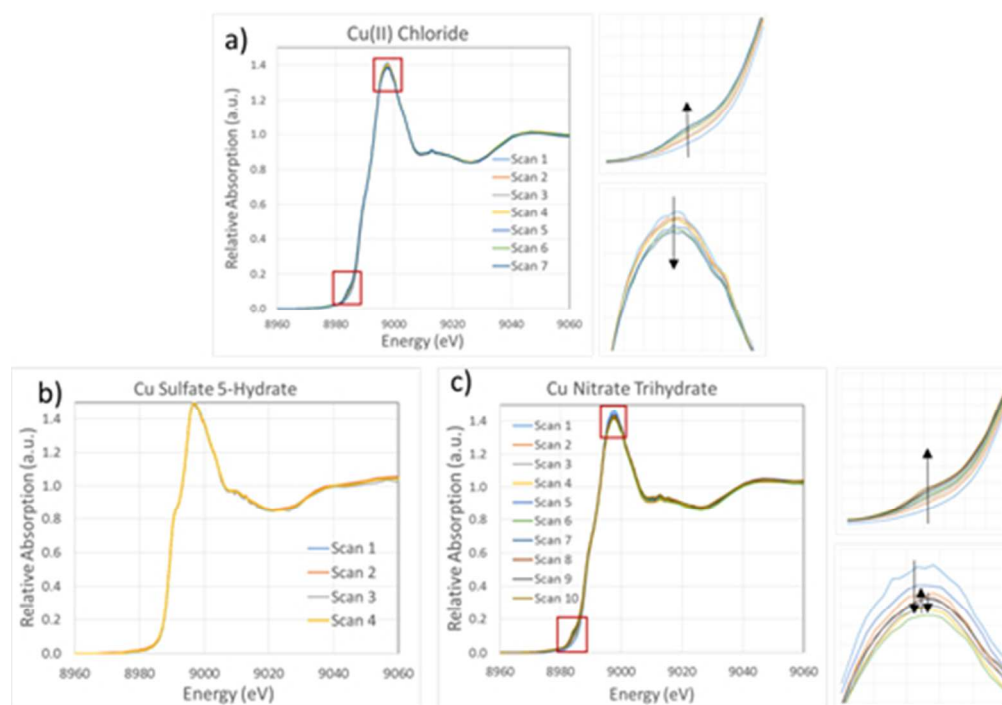


Figure 4. Testing of beam induced alteration XANES spectra of Cu. Arrows indicate evolution in features with consecutive scans. All samples were analysed at a temperature <10K.

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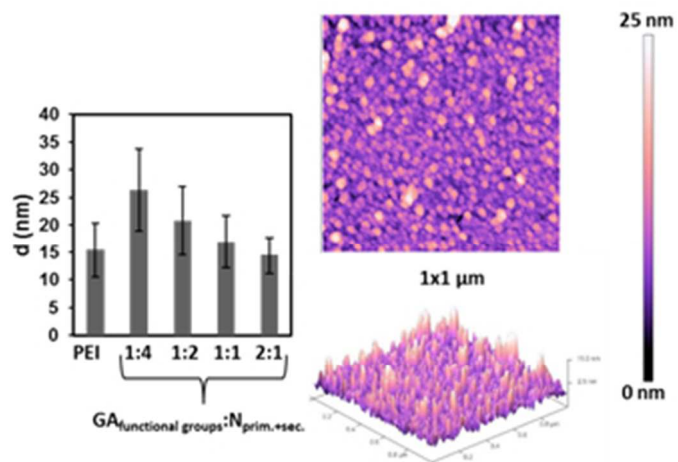


Figure 5. Size characterization of GA-PEI nanoparticles. Bar diagram shows the average peak hydrodynamic diameter of PEI and nanoparticles prepared using different crosslinker functionality to primary and secondary nitrogen ratio; Error bars indicate 1 SD (n=3). The AFM micrograph shows fully crosslinked nanoparticles in the dry state on a silicon wafer.

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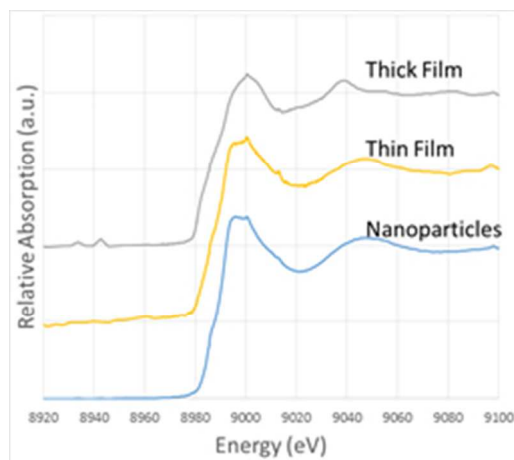


Figure 6. Cu-K edge XANES shows Cu associated with nanoparticles is highly comparable to the thin film and is an alternative model system for improving signal intensities in characterization techniques. All samples were analysed at a temperature <10K.

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