

Application of Wet-Scanning Transmission Electron Microscopy to assess the fate of silver nanoparticles under environmentally relevant conditions

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Introduction

Nanomaterials are a key area of materials science innovation, and silver nanoparticles (AgNP) are one of the most widely utilised nanomaterials, being incorporated into a wide variety of consumer and commercial products, principally because of their anti-bacterial activity, Figure 1.

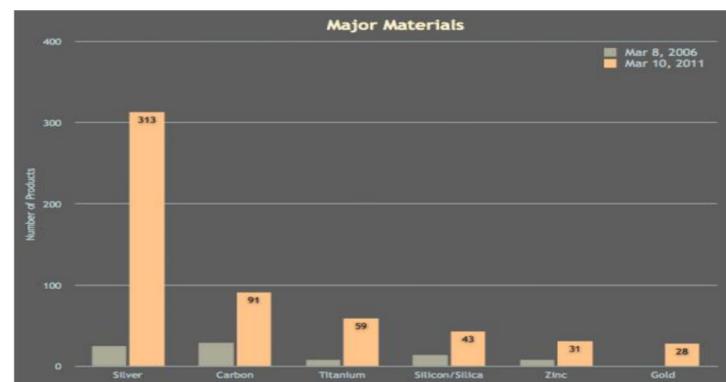


Figure 1: Recorded use of different nanomaterials in consumer products. Source: The Project on Emerging Nanotechnologies

Post-use, AgNP are likely to enter the wastewater stream¹ and may therefore represent a toxicological risk to wastewater treatment processes that depend upon microbial digestion, Figure 2, or to microbial processes in subsequent receiving environments, such as soils, Figure 3.



Figure 2: Activated sludge treatment of wastewater depends upon aerobic digestion by bacteria to reduce the biological oxygen demand of wastewater.

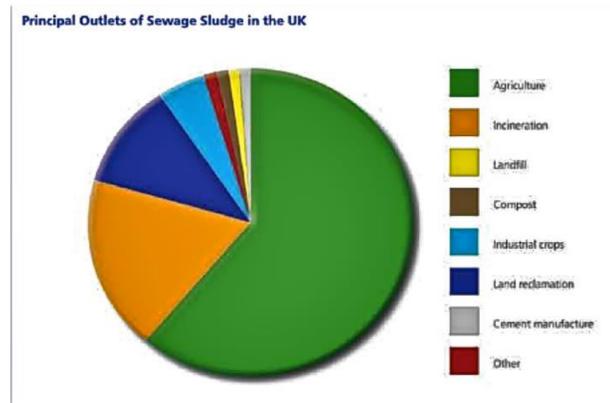


Figure 3: Applications for sewage sludge generated by the wastewater treatment process in the UK. Source: www.water.org.uk

Assessing the post-use, toxicological risk of AgNP requires investigation of the fate of AgNP when exposed to the potentially complex and variable components of wastewater. Whether AgNP interact with components of wastewater in ways that lead to AgNP aggregation, is of particular interest because particle size is likely to influence both the anti-bacterial activity of the AgNP and their partitioning behaviour. It is appropriate to apply a range of techniques to assess the particle size characteristics of AgNP², and these may include microscopy, Dynamic Light Scattering and UV-vis.

The integrity of transmission electron (TEM) and scanning electron microscopy (SEM) in assessing the particle size of AgNP may be compromised, either by the modifications made to samples during their preparation for analysis, such as dry sample preparation, or by the physical conditions in the sample chamber, which can include high vacuum conditions³. In contrast, wet-STEM is a microscopy innovation that allows samples to be analysed under realistic environmental conditions, including in liquids. Wet-STEM may therefore facilitate a more accurate assessment of the fate of AgNP in environmental media.

Method

AgNP with citrate (AgNP-CIT) and polyvinylpyrrolidone (AgNP-PVP) coatings were prepared for use in testing, as citrate and polyvinylpyrrolidone coated AgNP are two of the most common forms of AgNP used in product applications⁴.

Cl⁻ and SO₄²⁻ ions are frequently identified as important free ions in wastewater, and NaCl and MgSO₄ are consequently included in the OECD specification for synthetic influent. Exposure solutions were therefore prepared with NaCl and MgSO₄ at concentrations of 1mM in Millipore water. AgNP-citrate and AgNP-PVP were added to the exposure solutions at concentrations of 0.1mM.

Control and exposure solutions were assessed for AgNP particle size by Dynamic Light Scattering (DLS) using 1ml aliquots and by wet-STEM using 15µl aliquots. Wet-STEM assessment was performed using an FEI Quanta 650 SEM, Figure 4, equipped with a wet-STEM peltier stage, Figure 5.



Figure 4: FEI Quanta 650 SEM

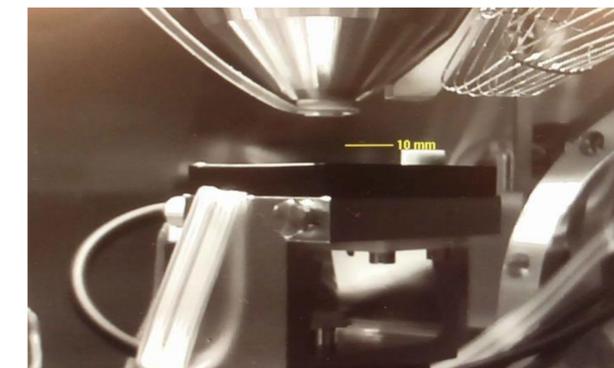


Figure 5: Peltier stage mount that permits analysis of samples in liquid format. Sample chamber requires careful specification of pressure and temperature settings to sustain wet sample conditions.

Wet-STEM images were analysed using SigmaScan software to determine particle size.

Results

Sample chamber parameter optimisation

Correct sample chamber settings are required in order to establish samples in the optimal 'wet' condition, between the extremes of saturated and dry states. Under 'wet' conditions images of the sample can be captured more effectively than under saturated conditions. Figure 6 shows 'wet' conditions to the left of the wet/saturated boundary, and saturated conditions to the right of the boundary; AgNP-citrate particles are present as nano-scale, dispersed particles on both sides of the boundary, but image sharpness is improved under wet conditions and is important for subsequent image analysis, including particle size measurement.

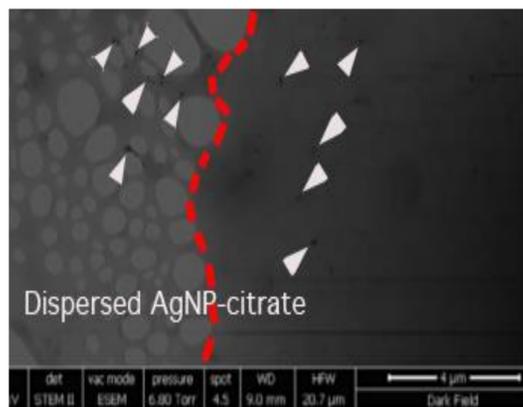


Figure 6: Dark field image of AgNP-citrate under 'wet' (left of red boundary line) and saturated (right of red boundary line) conditions in de-ionised water. In both cases the particles (indicated by white arrows) appear as dispersed particles in the nano-size range.

Establishing samples in the 'wet' condition requires adjustment of chamber pressure and temperature settings in order to achieve optimal humidity and evaporation rate conditions in the sample chamber; the settings used are detailed in Table 1.

Table 1: Wet-STEM equipment settings for stable 'wet' sample conditions and effective image capture of silver nanoparticles

Chamber purge settings	5 cycles at 6.0 – 10.0 Torr
Spot size	4.5
Energy level	30.00kV
Dwell time	5μs (scan) – 20μs (image capture)
Chamber temperature	5° C
Chamber pressure	6.5 Torr

Results

If samples are allowed to transform from 'wet' state to dry state, equivalent to the sample preparation procedure used in TEM microscopy, this promotes aggregate formation. Figure 7 shows an AgNP-citrate aggregation formed when AgNP-citrate in de-ionised water are subject to drying, this contrasts with the true behaviour of AgNP-citrate in de-ionised water, which is to form a stable suspension of dispersed, nano-scale particles, Figure 6.

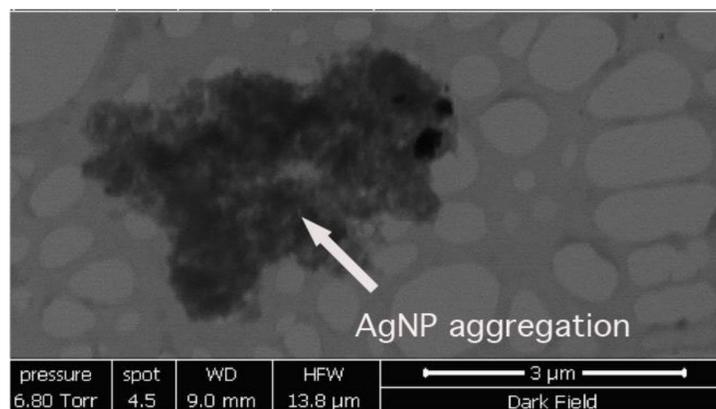


Figure 7: Dark field image of AgNP-citrate aggregation formed in dry sample state.

Use of wet-STEM to assess AgNP under environmentally relevant conditions – static images

AgNP-citrate exhibit moderate particle aggregation under exposure in a 1mM NaCl solution; particle size distribution consists mainly of unaltered nanoparticles and a small number of larger aggregates, Figure 8 (a). Under exposure to 1mM MgSO₄, AgNP-citrate exhibit significant modification, with the particle size distribution dominated by larger aggregates, Figure 8(b). AgNP-PVP particle size is stable when exposed to 1mM NaCl, Figure 8(c). Under exposure to 1mM MgSO₄, AgNP-PVP particle size distribution consists of both unaltered, dispersed particles and micron scale aggregates, Figure 8(d).

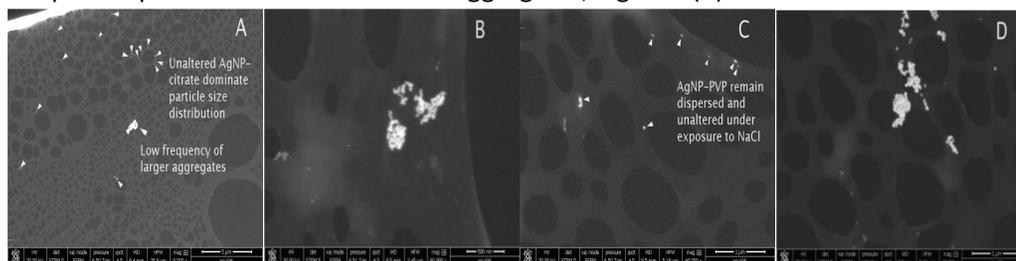


Figure 8: (a) 0.1mM AgNP-citrate in wet state exposed to 1mM NaCl, (b) 0.1mM AgNP-citrate in wet-state exposed to 1mM MgSO₄, (c) 0.1mM AgNP-PVP in wet state exposed to 1mM NaCl, (d) 0.1mM AgNP-PVP in wet state exposed to 1mM MgSO₄.

Results

relevant conditions – dynamic images

Video images capture the fate dynamics of AgNP exposed to environmental media. Figure 9 shows AgNP-citrate aggregating under exposure to 1mM MgSO₄, resulting in the formation of a micron-scale aggregate under wet conditions. Figure 10 shows AgNP-PVP exposed to 1mM NaCl, under which the AgNP-PVP maintain a stable suspension of nano-scale particles.

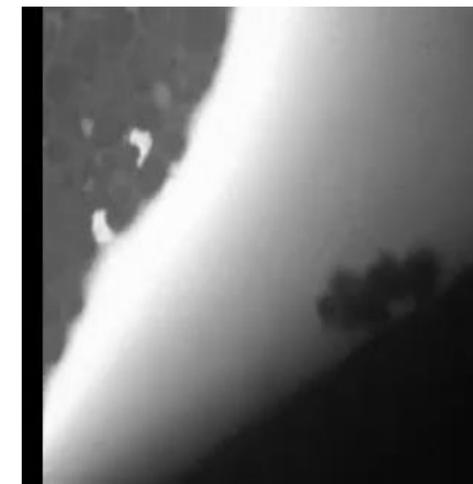


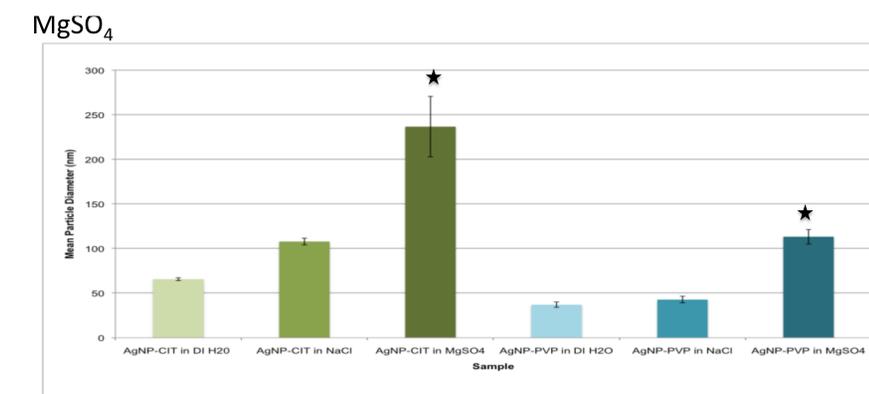
Figure 9: Video images under High Angle Annular Diffraction Field (HAADF) imaging of AgNP-citrate forming an aggregation under exposure to 1mM MgSO₄ solution.



Figure 10: Video images under HAADF imaging of AgNP-PVP exposed to 1mM NaCl solution.

AgNP particle size assessment exposed to environmentally relevant media

Figure 11 displays mean particle size for AgNP prepared in de-ionised water, or exposed to 1mM NaCl or 1mM MgSO₄. Mean particle size is significantly larger for AgNP-CIT exposed to MgSO₄ (p<0.05, ★) when compared to AgNP-CIT in de-ionised H₂O. AgNP-CIT exposed to NaCl record a larger average particle size, but this fails to attain statistical significance (p=0.07). Mean particle size for AgNP-PVP exposed to MgSO₄ is significantly larger (★) compared to AgNP-PVP in de-ionised H₂O.



Results

Comparison of wet-STEM and DLS particle size measurements

Figure 12 shows the mean particle diameters, assessed by DLS and wet-STEM, of AgNP-CIT and AgNP-PVP in de-ionised H₂O, 1mM NaCl and 1mM MgSO₄. The two assessment methods are mostly consistent in their size ordering of the AgNP in the different exposure preparations, the exception being that DLS records the smallest particle size of AgNP-PVP in NaCl rather than in de-ionised H₂O. The absolute size assessments of particles in each exposure solution do exhibit some significant differences between the two methods, using p<0.05 (★) and p<0.01 (★★) thresholds.

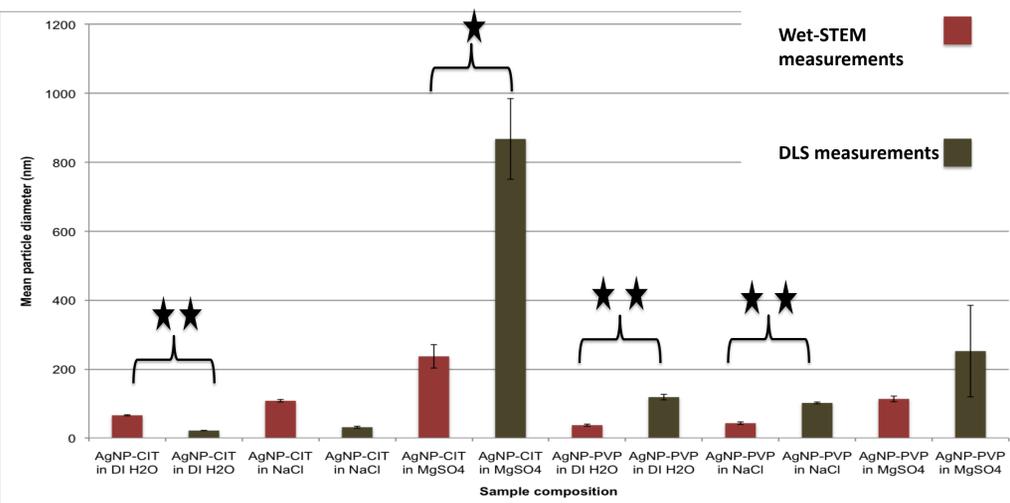


Figure 12: Mean particle diameter of AgNP-CIT and AgNP-PVP prepared in de-ionised water, 1mM NaCl or 1mM MgSO₄, assessed by DLS and wet-STEM.

Discussion

Stability of AgNP can be affected by the physical state of the sample, in particular conversion of a liquid suspension to a dry sample promotes aggregation, as demonstrated for AgNP-CIT in Figures 6 and 7. Particle aggregation can have a significant effect on the reactivity of nanomaterials and how they partition in the environment, and is an important potential outcome in fate modelling. It is therefore critical that the techniques used to investigate the fate of nanomaterials do not artificially promote aggregation and potentially lead to misleading conclusions about environmental fate.

Maintaining samples as suspensions in liquid while performing a visual characterisation can be achieved using wet-STEM and the technique is also shown to have the capability to process samples where the liquid media used can include important environmental components, such as ligand ions, Figure 8. These capabilities of wet-STEM are attractive advantages when compared to existing microscopy methods like TEM and SEM.

Testing of the effects on AgNP of exposure to Cl⁻ and SO₄²⁻ ions is possible with wet-STEM and this can be done on both a static basis by capturing still images and dynamically using video image capture. The images can subsequently be processed to measure particle sizes and to observe the behaviour of particles in suspension. The particle size measurements suggest differentiated aggregation behaviour depending on both the coating applied to the AgNP and the type of ion exposure; PVP is indicated to be more effective than citrate in preventing aggregation, while SO₄²⁻ is more effective at driving aggregation than Cl⁻.

The ordering of AgNP particle sizes arising from exposure across the three test media: de-ionised water, 1mM NaCl and 1mM MgSO₄, is largely consistent between DLS and wet-STEM. These results therefore support the use of DLS and wet-STEM as complimentary techniques in performing assessments of the environmental fate of AgNP. However, the absolute sizes determined by the two methods exhibit some significant differences. Some of these differences may be attributable to differences in measurement definition, for example, DLS assesses the hydro-dynamic radius of particles, whereas wet-STEM measures exclude any hydro-dynamic influence. It is also possible that sample size contributes to the difference; in this study DLS measurements were performed on 1ml samples, while the wet-STEM samples had a volume of 15μ and only the portion of the sample on which stable, 'wet' conditions were established was imaged and assessed.

Conclusions

- Wet-STEM sample chamber conditions can be achieved that allow assessment of AgNP in a 'wet' conditions, under which images can be captured for use in particle size assessment.
- Wet-STEM assessment shows that AgNP-CIT maintain the same physical presentation under wet conditions as under saturated conditions.
- Drying of AgNP promotes particle aggregation and can therefore introduce a misleading distortion in fate assessment. AgNP characterisation techniques that involve sample drying, such as TEM, are therefore likely to be inferior to wet-STEM which avoids this sample preparation treatment.
- Wet-STEM allows evaluation of samples prepared in liquid media with environmentally relevant composition, and can therefore be used in assessment of the fate of nano-materials that may enter the aquatic or marine environment.
- Wet-STEM particle size assessments of AgNP-CIT and AgNP-PVP exposed to different representative environmental media are consistent with those made using DLS.
- Differences in aggregation tendency occur between AgNP coated with citrate and AgNP coated with PVP; PVP is indicated to be more effective at inhibiting aggregation. The type of free ions present in the exposure solution also affect aggregation; SO₄²⁻ ions promote a greater degree of aggregation than Cl⁻ ions.

References:

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