Surface Immobilisation of Engineered Nanomaterials for *in-situ* Study of their Environmental Transformations and Fate

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ABSTRACT

The transformation and environmental fate of engineered nanomaterials (ENMs) is the focus of intense research due to concerns about their potential impacts in the environment as a result of their uniquely engineered properties. Many approaches are being applied to investigate the complex interactions and transformation processes ENMs may undergo in aqueous and terrestrial environments. However, major challenges remain due to the difficulties in detecting, separating and analysing ENMs from environmental matrices. In this paper, a novel technique capable of *in situ* study of ENMs is presented. By exploiting the functional interactions between surface modified silver nanoparticles (AgNPs) and plasma-deposited polymer films, AgNPs were immobilised on to solid supports that can be deployed in the field and retrieved for analysis. Either negatively charged citrate or polyethylene glycol, or positively charged polyethyleneimine were used to cap the AgNPs, which were deployed in two field sites (lake and marina), two standard ecotoxicity media, and in primary sewage sludge for a period of up to 48 hours. The chemical and physical transformations of AgNPs after exposure to different environments were analysed by a combination of XAS and SEM/EDX, taken directly from the substrates. Cystine- or glutathione-bound Ag were found to be the dominant forms of Ag in transformed ENMs, but different extents of transformation were observed across different exposure conditions and surface charges. These results successfully demonstrate the feasibility of using immobilised ENMs to examine their likely transformations *in situ* in real environments and provide further insight into the short term fate of AgNPs in the environment. Both the advantages and the limitations of this approach are discussed.

INTRODUCTION

Rapid progress in nanotechnology over the last two decades has led to the birth of innovative applications and industries that can potentially improve many aspects of life. However, the inevitable release of engineered nanomaterials (ENMs) into the environment is an increasing concern.¹⁻² A diverse range of ENMs with specifically engineered properties (e.g. optical,³ transport,⁴ and catalytic⁵) has been developed for use in consumer products⁶. These are generally produced with tailored elemental composition, particle size and shape, surface charge and functionality. There are unique properties that arise in the nano-scale due to geometrical (e.g. high specific surface area) and quantum mechanical (e.g. optical resonance) effects, which are not observed within the micro- or macro-scopic domains. However, these special properties are also a reason for the significant concern regarding their potential interactions with the environment, as the associated risks (acute and chronic) may differ from that of their micro- or macro-scale equivalents.

When released to the environment, ENMs may undergo complex physical, chemical and biological transformations. This possibility further complicates risk assessment.⁷⁻⁸ A common approach adopted in evaluating these risks is to study the behaviour of the potential contaminant through selected pathways, systems and endpoints.⁹⁻¹¹ For example, a mesocosm simulating freshwater emergent wetlands was used by Lowry *et al.*¹¹ to study the long-term transformation and fate of silver nanoparticles (AgNPs); whereupon silver sulfide (Ag₂S) and cysteine-bound silver (Ag-cysteine) were found to be the major silver species in the exposed soils and subaquatic sediments. Importantly, sulfidation¹²⁻¹³ of silver (both Ag⁺ and AgNPs) and interactions with natural organic matter¹⁴⁻¹⁵ (NOM) have been reported to reduce effective silver toxicity, and may therefore mitigate the risks associated with AgNPs. These studies form an integral part of the overall risk assessment framework as they offer valuable insight into the processes that may take place in the environment, but allow them to be studied under controlled conditions. At the same time, transferring knowledge and techniques beyond these boundaries is a major challenge, partly because the retrieval and analysis of ENMs dispersed in environmental media at relevant concentrations (aqueous or otherwise) is difficult at best.¹⁶ Extensive separation steps are required to extract and prepare environmentally exposed ENMs for analysis, and may not always be feasible.

Uniquely, X-ray absorption spectroscopy (XAS) can be performed within the host matrix (i.e. without separation), enabling the detection and investigation of ENMs deployed in the real environment. For example, XAS has been applied to detect and study the fate of AgNPs and zinc oxide (ZnO) nanoparticles through wastewater and sewage treatment processes *in situ*¹⁷⁻¹⁹ and found them to yield their respective sulfides in the freshly treated sludge. This is in agreement with the previous

identification of Ag₂S nanoparticles in sewage sludge products.²⁰ However, the concentrations need to be high enough for reliable characterisation by XAS (typically tens of mg.kg⁻¹) which may exceed realistic environmental concentrations other than in specific endpoints such as sludge. Further complications arise in the real, open environment as it is a complex and dynamic system that cannot always be simulated accurately in a closed environment. On the field scale, a major project is underway where AgNPs are being released into the Experimental Lakes Area under the Lake Ecosystem Nanosilver (LENS) project to determine ecosystem level impacts.²¹ This is a highly valuable investigation, but is a unique approach that cannot be performed repeatedly in different environments.

Some previous studies on mineral reactions at the micron scale have shown discrepancies between laboratory and field experiments, with thermodynamic²² or temporal²³ factors suggested as possible reasons. Several tools have been developed to address these issues for larger particles. For example, Birkefeld *et al.*²⁴ fixed mineral microparticles on to polymer supports using epoxy resin, and directly inserted them into soils to monitor their transformations. Fakih *et al.*²⁵ developed a similar tool without the epoxy resin through a process of synthesizing, centrifuging and drying the mineral in the presence of striated polymers as supports. Others have used porous bags to keep the particles localised (e.g. 26). While some of these tools may be adaptable for ENMs, the combination of strong attachment and maximal nanoparticle exposure cannot be easily achieved. For instance, due to the small size of ENMs, thick epoxy resin coatings may simply engulf them and block them from interactions with the environment; while achieving surface adhesion of adequate strength without appropriate “glue” may not be simple for ENMs of different surface charges. In one study, Kent and Vikesland developed a method to study dissolution applicable to ENMs based on nanosphere lithography and atomic force microscopy (AFM).²⁷ However, AFM on field-deployed substrates would be challenging with high levels of contaminants on the surface, and access to alternative analytical techniques would be necessary to provide further information such as speciation.

In this paper, we present a novel approach designed to track the chemical transformations of ENMs deployed in the environment by attaching them to a solid support via strong surface interactions. This was achieved by plasma polymerisation; a technique that can deposit thin polymer films on to solid surfaces and that can immobilise nanoparticles via electrostatic or covalent bonding.²⁸⁻²⁹ This approach has a unique advantage in that the charge, functionality and density of the polymer coatings can be easily controlled by varying the monomer composition,³⁰ so that the coated surfaces can accommodate ENMs of different surface charge and concentration. ENMs often adsorb and

accumulate at various interfaces (e.g. liquid-solid interface, bacterial/algal cell walls); therefore, this also provides a realistic model design of where they may be found in natural environments.

By exploiting this technology, an inert polyimide film was coated with a functional plasma-polymer film which was then used to immobilise AgNPs with different capping agents of opposing charges. These were directly deployed into selected freshwater and seawater locations as well as in laboratory based media for a period of time, after which, they were easily retrieved and analysed directly on the substrate by XAS and SEM. AgNPs and their derivatives are very common in the consumer market.⁶ However, ionic silver (Ag^+) is toxic to prokaryotes and simple eukaryotes and AgNPs will likely impact the ecosystem by releasing Ag^+ , especially given their high surface area. Some studies have indicated a direct contribution to ecotoxicity from AgNPs as well,³¹ raising concerns about their overall potential. Furthermore, Ag is a reactive metal and its speciation in natural waters will influence its solubility and bioavailability,¹²⁻¹³. Such transformations may also be affected by other physical interactions such as adsorption, aggregation and/or sedimentation. For these reasons, AgNPs are one of the most actively researched ENMs in the field of nanotoxicology and were hence the ideal candidate to demonstrate the use of this novel approach. This study aims to demonstrate the feasibility of immobilised ENMs as a means for investigating their physicochemical transformations in the field; present the advantages and limitations of the method; and provide further insight into the chemical fate of AgNPs in natural waters.

Experimental

Design and preparation of an XAS substrate for nanoparticle immobilisation

A Perspex (polymethylmethacrylate) sheet of 2 mm thickness was cut into small rectangular pieces (25 × 8 mm) and windows of 15 × 5 mm were made in each piece as shown in Figure 1. This window size is sufficiently large to allow an X-ray beam of 1-2 mm in the largest dimension to pass unhindered with an incident angle of 45°, while still retaining structural rigidity. Adhesive polyimide tape of *ca.* 63.5 µm thickness (2.5 mil, Cole-Palmer, EW-08277-82) was used to cover the window on both sides, with one surface used to immobilise the AgNP and the other to seal the window and prevent contaminants from adsorbing onto the reverse side of the immobilisation substrate. All substrates were rinsed in ethanol prior to plasma coating.

A custom-built plasma reactor was used to perform plasma polymerisation.²⁸ This approach has been used successfully to coat a variety of substrates with thin, functional films capable of nanoparticle immobilisation.²⁹⁻³⁰ The substrates were first cleaned using air plasma (40W, 2×10^{-1}

mbar) to eliminate organic contaminants from the surface. Subsequently, allylamine vapour was introduced into the plasma chamber at a pressure of 2×10^{-1} mbar. Deposition was carried out using a power of 40 W for 100 seconds. These conditions resulted in the deposition of amine-rich film of 23 nm thickness as measured by ellipsometry. The same conditions were used for the deposition of films rich in aldehyde functional groups using propanal vapour instead of allylamine. All reactions were carried out at room temperature and the coatings were allowed to stabilise overnight in air prior to the immobilisation of AgNPs.

Figure 1. Schematic and photo (inset) of Perspex supported polyimide film as XAS substrate for nanoparticle immobilisation. The dimensions given are approximate and vary slightly between each substrate.

Synthesis and immobilisation of silver nanoparticles

Silver nanoparticles (AgNPs) were prepared by the reduction of 1mM silver nitrate (AgNO_3 , Sigma Aldrich, 99.99%) with 2 mM sodium borohydride (NaBH_4 , Sigma Aldrich, 98%), in a similar manner to that described previously in the literature.^{29, 32} Three different capping agents were used to stabilise the AgNP dispersions: trisodium citrate ('CIT', $\text{Na}_3\text{C}_3\text{H}_5\text{O}(\text{COO})_3$, Ajax Finechem, 99%), polyethyleneglycol ('PEG', $\text{MW}_{\text{av}} = 6000 \text{ g.mol}^{-1}$, Technical Grade, VWR) and polyethyleneimine ('PEI', $\text{MW}_{\text{av}} = 1300 \text{ g.mol}^{-1}$, Sigma Aldrich). In the case of CIT and PEG, the capping agent was added to the reaction mixture after the reduction step, whereas PEI was added before the reduction step. All particles had relatively broad size distributions in the range 2 – 50 nm as estimated by SEM imaging and ImageJ³³ (Figure S1). As measured after synthesis using a Zetasizer Nano ZS (Malvern Instruments), CIT and PEG coated AgNPs (CIT- and PEG-AgNPs, respectively) had a negative surface

charge (-39 mV and -27 mV, respectively) while PEI coated AgNPs (PEI-AgNPs) carried a positive surface charge ($+28$ mV).

Nanoparticle immobilisation was carried out by direct immersion of the plasma polymer coated substrates into solutions containing AgNPs. CIT- and PEG-AgNPs were immobilised on the positively charged polyallylamine coated substrates, while PEI-AgNPs were immobilised on to aldehyde-rich polypropenal coated substrates. The latter immobilisation is achieved by the reaction of primary amines on the PEI branches with the carbonyl groups to form an imine via nucleophilic substitution. The nanoparticles were allowed to undergo immobilisation for 24 h in the dark, and subsequently rinsed 3 times with ultrapure water to remove any weakly bound AgNPs and contaminants. A slight coloration of the substrate indicated the attachment of AgNPs, and SEM images confirmed their presence (Figure 2). The variability in the AgNPs attachment between the substrates prior to exposure was *ca.* 10 %, as characterised previously by XPS for similar depositions.³⁰

Figure 2. a) Schematic representation of AgNPs immobilisation on polyimide film surface via electrostatic attraction with the deposited polymer (allylamine in the figure), and, b) SEM image of the XAS substrate confirming the attachment of CIT-AgNPs on the surface.

Field and laboratory deployment of immobilised AgNPs

Two sites in South Australia were selected for the field deployment of immobilised AgNPs: a suburban lake ('lake') forming part of a recycled-water circulation scheme in the northern suburbs of Adelaide, and a jetty abutting a marina in the Gulf of Saint Vincent ('marina'). The substrates were housed in perforated stainless steel cages to protect them from severe weathering and/or inquisitive fish or fauna. As a laboratory-based comparison, substrates were also immersed in 15 mL of standard OECD³⁴ and f/2³⁵ ecotoxicity testing media without shaking (to minimise uncertainty due to possible detachment). These solutions are commonly used as growth media for freshwater and marine organisms, respectively. Finally, two controls were used in this study: untreated substrates (negative control), and substrates deployed in ~ 10 g of primary sewage sludge (SS) obtained from a municipal wastewater treatment plant in Adelaide, Australia (positive control). The positive control for AgNP transformation was selected on the basis of an experiment in which AgNP added to SS were shown to sulfidise over time.¹⁸ Deployment lasted 48 hours for all treatments apart from the SS, for which 24 hours was deemed sufficient for positive control purposes. All substrates were rinsed 3 times with ultrapure water (18.2 MΩ) upon retrieval, dried with N₂ gas and kept at 4 °C under argon until analysis. The time between initial AgNP synthesis and field deployment was less than 3 days, and aside from the experimental exposure period, all samples were kept under the same storage conditions. The exposure conditions (temperature, pH, Eh, EC, Cl⁻ levels and TOC) are summarised in Table 1.

X-ray Absorption Spectroscopy

XAS data was collected at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10, at the Advanced Photon Source (APS) of the Argonne National Laboratory (ANL), USA. The storage ring operated at 7 GeV in top-up mode. A liquid N₂ cooled double crystal Si(111) monochromator was used to select the incident photon energies and a platinum-coated mirror was used for harmonic rejection. Calibration was performed by assigning the first derivative inflection point of the absorption K-edge of Ag metal (25514 eV) and each sample scan was collected simultaneously with a Ag metal foil. The substrates were affixed to a 20-hole sample holder and mounted for analysis without any further modifications. Following sample alignment, the lights in the beamline hutch were switched off and data collection took place in the dark. Various Ag standards were used to collect reference spectra, including silver-oxide (Ag₂O), -sulfide (Ag₂S), -chloride (AgCl), -sulfate (Ag₂SO₄), and -carbonate (Ag₂CO₃), as well as Ag sorbed to humic acids (Sigma Aldrich), cystine, glutathione, ferrihydrite and acetate. These spectra were acquired on the same beamline with identical scan parameters but on separate occasions to the samples. However,

the corresponding Ag metal reference spectra were consistent and provided a good fit, hence the spectra were deemed suitable for use in the analysis. All spectra were normalised over the -100 to +200 eV range relative to the K-edge prior to analysis.

The X-ray Absorption Near-Edge Structure (XANES) region of the Ag K-edge, defined for this study as -50 to +100 eV relative to the calibration energy, was analysed by linear combination fitting (LCF). In the first instance, principal component analysis (PCA) of the normalised sample spectra was used to estimate the number of species contributing to the overall spectra, and target transformation (TT) was used to identify the likely species required for LCF. However this procedure, which has been found appropriate in previous studies, did not produce satisfactory fitting of the sample spectra as it did not identify some forms of Ag which were highly likely to be present. A wider range of likely species were therefore selected for LCF analysis, representing the insoluble forms of Ag species (Ag_2CO_3 , AgCl and Ag_2O) and those based on the expected sulfidation/thiolation of Ag (Ag_2S , Ag-cystine and Ag-glutathione), together with the corresponding control. The SixPack program³⁶ was used to perform PCA and TT, while Athena³⁷ was used to perform data normalisation and LCF.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) images of substrate bound AgNPs were acquired after deployment and compared to the controls in order to survey for simple morphological changes. An FEI Quanta 450 FEG-ESEM equipped with an EDAX Apollo X Energy Dispersive X-ray (EDX) spectrometer was used for the analysis. The SEM was operated at 20 kV with a typical working distance of 7 mm, and images were acquired directly from the substrate in both secondary (SE) and backscattered electron (BSE) modes. Sputter coating of the substrate was avoided so as not to disturb the nanoparticles' surface morphology. Thus, all analysis was performed under low vacuum conditions (*ca.* 130 Pa) to minimise the charge accumulation on the surface.

Results and Discussions

SEM examination of the immobilised AgNPs

SEM analysis and EDX spectra of the control treatments for all types of AgNPs confirmed the successful immobilisation of AgNPs with even coverage on the substrates. PEI-capped AgNPs showed a lower particle density compared to the CIT- and PEG-AgNPs, but the signal to noise levels in the XANES spectra were still sufficiently high for use in the LCF procedure.

Morphological changes were observed in all AgNPs exposed to the primary SS, lake or marina, while no significant changes were visible in those exposed to the laboratory based media. This is largely in agreement with the XAS analysis where substantial chemical transformations were observed in the SS, lake and marina exposed samples, but not in those exposed to laboratory media. Some chemical changes were also measureable by EDX: sulfur was detected from the SS samples, indicative of thiolation and/or sulfidation of the AgNPs. As can be seen in Figure 3, AgNPs exposed to SS have a variable amount of poorly defined structures adhered to the surface, which are likely to be organic matter within the SS. Deployment in the lake/marina also induced clear physical changes (Figure 3), with poorer particle definition (lake) as well as the appearance of very small nanoparticles (*ca.* < 10 nm, marina) and larger nanoparticles (lake and marina). In some cases, larger crystalline deposits were also seen (e.g. CIT-AgNPs following marina exposure) which are likely to be inorganic Ag salts (e.g. AgCl as detected by XAS) or other elements, but an accurate elemental composition could not be determined in this case due to a low EDX yield. SEM images of all samples are shown in Figure S2.

Figure 3. SEM images of selected samples: a) CIT-AgNPs exposed to primary SS, $\times 150,000$, b) CIT-AgNPs deployed in the marina showing inorganic/crystalline contaminants $\times 80,000$, and c) higher magnification image of b) $\times 300,000$. (SE/BSE image overlay, 20 keV, WD = 7mm) SEM images of all samples are included in the Supporting Information section, Figure S2.

Silver speciation by XANES and LCF

XANES spectra of the AgNP controls (CIT, PEG and PEI) all showed some minor differences compared to the XANES profile of polished Ag metal. This did not affect the quality of the LCF analysis (R-factor < 0.0002 in all cases) as the spectra from the control AgNPs were used in the LCF analysis of the exposed AgNPs as one of the standards instead of Ag metal, to account for any differences. Although they were stored at 4°C under argon after exposure, some chemical

transformations may have occurred during transport (air, ambient temperatures) due to reaction with atmospheric gases such as H₂O, O₂ and CO₂. However, it should also be noted that ultimately, similar fits were acquired regardless of the choice of AgNP vs. Ag metal, with the majority of transformations leading to thiolation or sulfidation.

Figure 4 shows the XANES spectra of all samples, including the controls, and their corresponding profiles produced by LCF of reference spectra. It can be seen that there are significant changes in the spectral profiles of AgNPs deployed in the field and those exposed to primary SS. The speciation of the AgNPs after exposure as determined by LCF are summarised in Table 2, expressed in percentage of contributions of the listed Ag standards. AgNPs deployed in the lake and marina show a change in the overall speciation of Ag, having transformed to cystine (an oxidised cysteine -S-S- dimer) and glutathione-bound Ag (a tripeptide containing cysteine, the reduced thiol form), with additional minor contributions from AgCl and Ag₂S in almost all the samples. Both organic ligands have a known affinity and stability with silver.³⁸⁻⁴⁰ Their formation can be rationalised by a thermodynamic narrative: Levard *et al.*⁷ calculated a Pourbaix diagram including Ag, S, Cl, H₂O and either CO₂ or free cysteine (reduced form of cystine), under typical freshwater (low Cl⁻ concentration) and seawater (high Cl⁻) conditions. In the absence of the organic ligand (cysteine), the major species that are thermodynamically favourable were found to be AgCl, Ag₂S and Ag⁰ in freshwater, whereas AgCl₂⁻(aq) and Ag⁰ are favoured in seawater. It should be noted that AgCl₂⁻(aq) would be dissolved in the seawater and is thus unlikely to be present on these substrates. This is a limitation of the present method as will be discussed later. On the other hand, Levard *et al.*⁷ show that in the presence of cysteine, the thiol-bound Ag will be equally dominant with the other phases particularly at higher Cl⁻ concentrations and pH. This is consistent with the results of the LCF. Although glutathione was not included in the calculation of the Eh-pH diagram, it is expected to bind preferentially to Ag over free cysteine given its higher stability constant³⁹ (log K_f = 15.1 cf. 11.9 for cysteine⁷).

All AgNPs deployed in the lake retained a significantly greater metallic character in comparison to those exposed to primary SS or the marina environment. Despite the higher TOC content (Table 1), organically bound Ag was not as prevalent as it was after marina exposure. Moreover, all types of lake-exposed AgNPs underwent a similar extent of chemical transformation, unlike the AgNPs in the SS or the marina where PEI-AgNPs transformed to a lesser extent. This suggests that the transformation rates and pathways are dependent on both the surface charge and the surrounding environment. It has previously been observed that gold nanoparticles (AuNPs) interact with NOM (5 mg.L⁻¹) regardless of the type of capping agent (cationic, neutral or anionic),⁴¹

and this interaction was found to be the major factor for their stability against aggregation,⁴¹⁻⁴² although it can also be influenced by the strength of the coating and cation concentration.⁴³ Similarly, interaction with NOM can reduce dissolution⁴⁴ and sulfidation rates.⁴⁵ Thus, in the lake water which contains high NOM (16.5 mg.L⁻¹), all AgNPs may similarly interact with NOM and undergo slower transformation, whereas in the low NOM environment of the marina (1.8 mg.L⁻¹), the differences resulting from surface charge may be more dominant despite the greater charge screening afforded by the high EC. For example, with low NOM concentrations, the higher electrostatic attraction towards NOM (generally negatively charged⁴⁶) may be important enough to result in a thicker accumulation of NOM on the positively charged PEI-AgNPs, resulting in the slower transformation.

Figure 4. Measured (blue) and fitted (red) XANES spectra of CIT-, PEG- and PEI- capped AgNPs after a) no exposure, and exposure to: b) sewage sludge, 24h, c) OECD freshwater media, 48h, d) freshwater (lake), 48h, e) f/2 saltwater media, 48h, and f) seawater (marina), 48h.

By comparison, only slight changes were observed for those AgNPs exposed to laboratory-based ecotoxicity media. According to the LCF procedure, all but one sample maintained $\geq 95\%$ of its metallic Ag⁰ core, with negligible contributions from other Ag species. Tejamaya *et al.*⁴⁷ previously studied the physical stability of citrate-, PEG-, and PVP-capped AgNPs in OECD media used

for *Daphnia magna* ecotoxicity testing, and found that CIT-AgNPs quickly aggregated upon exposure to the media. Dissolution was also presumed to take place but to a lesser extent, and no quantification of its rate was attempted. In contrast, the major physical transformation processes relevant to this study are AgNP dissolution and/or detachment, as rapid aggregation is effectively prevented by their immobilisation. In the case of sulfidation by inorganic sulfur (e.g. Na₂S), an initial dissolution and re-precipitation has been suggested as a mechanism, with the new phase forming nano-bridges between the existing AgNPs.⁴⁸ If the AgNPs are not allowed to aggregate, this may not promote Ag₂S retention and thus may not provide a complete view of the transformation processes.

The major species found after AgNPs exposure to SS were Ag bound to cystine and glutathione. As can be seen, primary SS with a high organic carbon content (TOC = 9749 ± 2471 mg.L⁻¹) caused a transformation of AgNPs to thiolated forms with no apparent formation of Ag₂S. This is in contrast with the previous study¹⁷ where Ag₂S dominated the speciation of added AgNPs after only 2 hours of exposure to non-aerated sludge, and almost no organically bound forms (7 hours in aerated sludge). There is insufficient data to conclusively resolve this discrepancy, but the difference observed may be attributable to one of two possibilities. In the previous experiment, the AgNP samples were kept frozen in the sludge after exposure until shortly before the XAS measurement, and thus would have remained in contact with sulfurous compounds and dissolved oxygen, which may have led to oxysulfidation.⁴⁵ This is often unavoidable as there may be a delay between the time of the experiment and, for example, access to synchrotron facilities for XAS analysis. The present method overcomes storage and preservation issues since the AgNPs can be completely separated from the SS thereby minimising further reactions, and once they are formed, organo-sulfur Ag are known to be stable against oxidation.^{38, 49} This is a major advantage of this method. The other possibility is that the Ag₂S formed does not stay bound to the substrate. For instance, experiments using PVP-capped AgNPs revealed that PVP is lost in the sulfidation process.⁴⁸ If similar losses occur with the CIT, PEI or PEG capping, this could alter the surface charge or functionality of the AgNPs, thereby leading to detachment of the particles. Nevertheless, this is unlikely to have occurred in this experiment because the raw K-edge absorbances of SS-exposed AgNPs are still comparable to the controls (Figure S4), indicating that the overall Ag concentration on the surface has not changed significantly.

The findings discussed above highlight some limitations of the current technique that should be recognised. Nonetheless, this technique offers significant advantages and benefits for *in situ* study of AgNP fate. For instance, at the low concentrations of AgNPs relevant in real environments, homoaggregation is unlikely to be as significant as it is in laboratory experiments using high

concentrations of AgNPs, and the ability to eliminate this effect while still allowing detection and analysis is in fact, an important advantage of this method. However, as Gondikas *et al.*⁵⁰ have suggested, there is a complex interplay of chemical, physical and biological processes that may take place, which need to be examined in concert. Formation of larger clusters or particles can have an impact on dissolution rates,⁵⁰ transport,⁵¹ thiol binding and surface structures,⁴⁰ and should also be considered to provide a complete understanding.

There is also a possibility that the charged plasma polymer coating may attract other contaminants from the environment that may affect the AgNPs transformation processes. For example, many naturally occurring colloids have negative surface charges,⁴⁶ which may be attracted by the positively charged polyallylamine plasma-polymer and interact with AgNPs. Given the reactive nature of Ag, some transformations may be enhanced by their presence and this will need to be examined in future studies. An approach that could overcome this issue may involve capping the charged sites on the polymer with strongly binding counter-ions or inert polymers after AgNPs immobilisation so that unintended interactions may be controlled.

It is important to appreciate that including dissolution and/or detachment of AgNPs in the interpretation will require a multi-faceted approach. For example, lake and marina samples show slightly lower raw XAS absorbances, suggesting that some Ag has been lost from the surface. However, this information alone cannot distinguish whether this occurs as a result of dissolution or detachment of AgNPs, and SEM examination is required for these purposes. Conversely, SEM alone cannot distinguish whether the NPs remaining on the surface are still AgNPs or, for example, Ag₂S nanoparticles. EDX will not have enough spatial resolution or sensitivity to determine whether sulfur, if detected, is co-localised with Ag as As₂S or is present on the surface with other contaminants. Appropriate interpretation of these finer points can only be made when SEM is combined with a technique such as XAS. In this way, incorporating multiple analytical methods can maximise the insight gained towards both physical and chemical transformations of AgNPs.

Implications for environmental risk assessment of ENMs

As clearly demonstrated in this study, immobilisation of ENMs opens the door to new experimental models and methodologies that can be used to understand the fate and transformation of ENMs in the environment. Most importantly, it offers easy retrieval of ENMs following deployment in the field; thereby allowing direct, *in situ* assessment of their reaction processes and products. This largely addresses the current need for ENM investigations to be conducted under more realistic environmental conditions.⁷ The use of surface-bound ENMs is also a

realistic scenario for many of the nanoparticles that are released, as they tend to accumulate onto interfaces, and this approach thus offers a way to study their behaviour with relevant models.

The ease of this technique also allows for it to be used as a screening method to assess the transformation not only of AgNPs but also of other ENMs, as long as they have sufficient binding capacity for immobilisation. In this work, plasma-polymer films were used to attached AgNPs via electrostatic or covalent means but this may be modified to allow ENMs to be bound by alternative mechanisms (e.g. hydrogen bonding). The applications of ENMs are continuously and exponentially expanding and the effect of surface-functionalisation on environmental fate is one of many key properties that is being explored. Quantifiable differences were observed between negatively and positively charged AgNPs in this study, and similar differences may also manifest themselves across the range of functionalities. Quick and accurate screening methods are required to assess the likely transformations and potential risks of ENMs in the environment, so that safety and regulatory decisions can keep pace with the rapid development and commercialisation of new ENMs. This technique thus provides a practical and efficient approach to help meet the demands of this highly active field.

Finally, this study also highlights the apparent discrepancy between unstirred laboratory media and actual environmental waters with regards to their potential to chemically transform AgNPs. In this respect, media based experiments such as standard laboratory ecotoxicity testing or stability testing may need to be interpreted with caution.

SUPPORTING INFORMATION

Additional information is available including: SEM images, PSD histograms, and EDX spectra of the CIT-, PEG- and PEI-AgNPs control substrates are shown in Figure S1, accompanied by a description of the method used for estimating the PSD using ImageJ, and SEM images of all substrates (Figure S2). The XANES spectra of all silver standards used in the LCF procedure are shown in Figure S3 and the XANES spectra of all samples pre-normalisation are shown in Figure S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Table 1. Fundamental parameters of the exposure conditions.

	T (°C)	pH	Eh (mV)	EC (mS)	Cl ⁻ (mg.L ⁻¹)	TOC (mg.L ⁻¹)
Freshwater Media (OECD)	22	7.7	192	0.18	40.6	0.26
Saltwater Media (f/2)	22	8.4	112	56.8	22.7×10 ³	2.8
Freshwater (Lake)	23	9.1	90	5.9	1.1×10 ³	16.5
Seawater (Marina)	22	8.1	94	55.8	20.5×10 ³	1.8
Sewage Sludge	22	6.7	-257	5.1	393	9749

Table 2. LCF results of the Ag K-edge XANES spectra using the standards listed, as percentage composition and variability (in parentheses) of the total. The low residual (R-factors) indicate the quality of the fit.

	Freshwater (Lake)			Seawater (Marina)			Sewage Sludge		
	CIT	PEG	PEI	CIT	PEG	PEI	CIT	PEG	PEI
AgNP* (Ag ⁰)	58 (2)	65 (2)	64 (3)	15 (1)	21 (1)	57 (2)	14 (1)	27 (1)	60 (2)
Ag-glutathione	--	19 (3)	27 (5)	58 (4)	52 (4)	22 (4)	62 (2)	54 (3)	38 (3)
Ag-cystine	11 (3)	16 (3)	--	19 (3)	10 (3)	--	24 (2)	19 (2)	2 (3)
Ag ₂ S	9 (3)	--	4 (3)	4 (2)	8 (2)	17 (2)	--	--	--
AgCl	23 (4)	--	5 (3)	5 (2)	9 (2)	4 (3)	--	--	--
Ag ₂ O	--	--	--	--	--	--	--	--	--
Ag ₂ CO ₃	--	--	--	--	--	--	--	--	--
<i>R-factor</i>	0.0002	0.0001	0.0002	0.00006	0.00003	0.0001	0.00004	0.00009	0.00013

	Freshwater Media (OECD)			Saltwater Media (f/2)		
	CIT	PEG	PEI	CIT	PEG	PEI
AgNP* (Ag ⁰)	100 (0)	98 (0.5)	95 (2)	100	99 (1)	87 (2)
Ag-glutathione	--	0 (0.6)	5 (2)	--	0 (2)	6 (4)
Ag-cystine	--	--	--	--	1 (1)	--
Ag ₂ S	--	2 (0.4)	--	--	--	--
AgCl	--	--	--	--	--	--
Ag ₂ O	--	--	--	--	--	5 (3)
Ag ₂ CO ₃	--	--	--	--	--	3 (2)
<i>R-factor</i>	0.00007	0.00005	0.0002	0.0001	0.0001	0.0001

*AgNPs here refers to the control substrate with the corresponding capping agent.