# Role of humic acid in the stability of Ag nanoparticles in sub-oxic conditions

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## ABSTRACT

Stability and temporal changes in size distributions have been observed for citrate- and polyvinylpyrrolidone- (PVP) capped silver nanoparticles (AgNPs), in the presence or absence of sulfide and natural organic matter (NOM – as humic acid), whilst under sub-oxic conditions. There were substantial differences in the influence of the two capping agents, with PVP-AgNPs showing few or no significant changes in apparent stability or particle size distribution under the conditions examined, whilst the apparent size distributions of citrate-capped AgNPs changed rapidly. Sulfide and humic acid each individually caused immediate increases in cit-AgNP size distributions which were then relatively stable over 60–145 days. This may be due to sulfide bridging and cation bridging respectively. However in competition it was the influence of the humic acid which dominated that of the sulfide. These observations have implications for

environmental fate and toxicity of AgNP. The increased stability in the presence of even low concentrations of NOM may limit the rapidity of Ag dispersal, but may also concentrate the dose received by organisms which subsequently ingest the stabilized particles.

## INTRODUCTION

The significant expansion over recent years in the use of engineered nanoparticles (ENPs) in an increasing range of commercial applications, including consumer textile and healthcare products, has inevitably led to a corresponding increase in concern and attention regarding the eventual environmental fate of the nanomaterials used [1]. Multiple studies have now demonstrated how ENPs can be released into the aquatic environment, for example through personal hygiene or laundry into wastewater streams [2, 3]. Considerable attention has been paid to the environmental behaviour of silver nanoparticles (AgNPs) in particular, partly because they represent a substantial proportion of the industrial ENP use, but also because of the potential Ag toxicity [4, 5]. It is well documented how, under appropriate environmental conditions, AgNPs exhibit toxic effects to soil bacteria and a range of other microfauna (see for example, compilation of toxicity evidence in Fabrega et al, [6]). Indeed, one of the primary reasons for use of AgNPs in the first place is their antibacterial properties.

In aqueous conditions pure AgNPs are generally not stable and may oxidatively dissolve releasing $Ag^+$, which is considerably more toxic, or will exist in a stabilized form. Preparations of AgNPs are frequently stabilized using capping agents. Two of the most widely used are citrate, a small negatively charged ligand which provides electrostatic stabilization of the AgNPs, and polyvinylpyrrolidone (PVP), a large hydrophilic polymer which stabilizes the AgNPs through steric effects [7]. It has been shown that the nature of the capping can profoundly influence the stability and behavior of the AgNP across a range of conditions. Electrosterically stabililized AgNPs (such as PVP-) are less susceptible than electrostatically-coated (or indeed uncoated) AgNPs to variation in conditions such as pH, ionic strength, or electrolyte composition [8-10]. This was recently exemplified in microcosm experiments where it was shown that PVP-AgNPs remained unaltered for periods of 28 days with migration being diffusion-dominated, while cit-AgNPs in the same conditions exhibited conformational changes with sedimentation- and precipitation-influenced migration processes [11]. However, it is interesting to note that despite these established differences in characteristic, some studies of environmental behaviour are undertaken using only a given type of capped NP and not all appear to consider the impact that variation in capping agent may have on the observations made.

Understanding the processes controlling the balance between AgNP stability or decomposition, particularly in environmental contexts, is therefore of major concern. In natural aqueous systems a major influence on AgNP stability is the ubiquitous presence of natural organic matter (NOM) and this has been the subject of considerable attention, recently reviewed by Phillippe and Schaumann [12]. There is significant evidence to suggest that NOM interactions with AgNP increase colloidal stability and reduce the extent of homoaggregation of the AgNPs e.g. [13] although there are some contradictory observations. The nature of the NOM itself is also important and marked differences have been observed between different fractions of NOM. For example, in one study using the widely-studied Suwannee River Humic and Fulvic Acids (SRHA and SRFA) the HA was reported to increase stability and persistence while the FA increased deposition and removal of AgNPs [14]. Yet in other work, also using synthetic cit-AgNPs with SRFA, the FA was found clearly to enhance stability of the particles [15]. A third study found that both SRHA and SRFA increased the colloidal stability but that the degree of sulfur- and nitrogen-group functionality within the NOM structure was a more important control of release of $Ag^+$ [16]. The picture remains complicated and our understanding of the role of NOM in AgNP stability still not fully developed.

Several studies have considered the impact on AgNP behavior and the interaction with NOM of fundamental environmental parameters including pH and hardness [17], or ionic strength and electrolyte composition [15, 18]. Redox conditions are also likely to play an important role. It is widely regarded that one of the primary mechanisms for mobilization of $Ag^+$ from AgNPs is oxidative dissolution. However, not all environments where the AgNPs occur are oxidizing and it is possible for the AgNPs to be deposited or transported in suboxic environmental conditions such as waste water treatment effluents, landfill leachate plumes hyporheic zone sediments or potentially confined groundwater environments [19, 20]. Indeed some of these are potential hot-spots for AgNPs in the environment (e.g. [19]).

One of the significant processes affecting AgNPs which is influenced by redox conditions is sulfidation. AgNPs can become sulfidized by sulfide species present in anaerobic conditions in the wastewater stream[3] and the sulfur-stabilized particles are able to persist right through the treatment process to the point where $Ag_2S$ NP have been identified in final sewage sludge products [19]. In laboratory studies sulfidation appears to inhibit the dissolution and the release of $Ag^+$ ions, potentially therefore moderating toxicity effects. Increasing sulfur concentration forms $Ag_2S$ nanobridges between the coated AgNPs creating chain-like structures; the larger aggregate particle size and altered surface charge reduce the dissolution rate [21]. Given that both sulfidation and NOM have been shown to influence stability and toxicity of AgNPs, recent attention has focused increasingly on the competitive interaction between the two, with studies conducted on uncapped [22], PVP-capped [23] and citrate- and Tween-capped AgNPs [24]. Whilst it was shown that the sulfidation in itself reduced the apparent toxicity of the AgNPs [22], the introduction of NOM appeared to suppress sulfidation [23] and reduce aggregation rates [24] potentially without modulating the toxicity.

Sub-oxic conditions are, of course, intrinsically more difficult to maintain. Lapworth et al [20] considered the effectiveness of a range of analytical techniques for characterization of AgNP in sub-oxic groundwaters but also clearly demonstrated that aeration of previously sub-oxic groundwater samples produced rapid and dramatic changes in aggregation and the observed size distribution of natural colloids. This confirmed that the absence or exclusion of oxygen can lead to significant variation in NP behavior – at least for NP present in natural samples. The relative impact of sub-oxic conditions on the multi-component or competitive interactions of other major influences on AgNP stability and degradation including capping-agent, NOM and sulfidation remains a focus for attention.

In this study we examine these interactions by observing the stability and temporal changes in size distributions of citrate- and PVP-capped AgNPs in the presence or absence of sulfide and NOM whilst under sub-oxic conditions at environmentally realistic concentrations.

## MATERIALS AND METHODS

### Preparation of NPs

Synthesis of citrate-capped AgNPs was carried out by the method used previously at the Facility for Environmental Nanoscience Analysis and Characterisation (FENAC) and described elsewhere [24]. In summary, silver nitrate was reduced by sodium borohydride in the presence of trisodium citrate and heated to boiling for 3 hours, then cooled and stored for 16 hours at room temperature in the dark for the particle size to stabilize. The resulting NPs were cleaned repeatedly by ultrafiltration (1 kDa) before redispersion in trisodium citrate to stabilize them and avoid further growth. Details on the preparation of PVP-capped AgNPs can be found in Tejamaya et al. [10] using a modified method developed by Mulfinger et al. [25]. In brief a portion of the stabilized citrate NP suspension was repeatedly reacted with 3% PVP solution and volume reduced by ultrafiltration. Further details of procedure and quantities are provided in the Supporting Information.

Fresh suspensions of AgNPs were prepared for this work. The stock suspensions were stored at 4°C with exclusion of light and did not display any evidence of deterioration for the duration of the work. The hydrodynamic size distributions of the pure capped AgNPs were 36.2 nm and 50.6 nm for cit-AgNP and PVP-AgNP respectively with zeta-potential of the purified NP in deionized water of -35 mV and -25 mV respectively.

**Stability experiments**

Experiments were carried out by mixing aliquots of capped Ag NPs (1 mg/L) with different ratios of sulfide (0, 0.01 and 0.1 M) and humic acid (0, 2.5, and 25 mg/L as HA; i.e. 0, 1.3 and 13.4 mg/L DOC) solutions in a batch mode (see Table 1), and then observing the stability over time. For the sulfide reagent, 0.331 g of sodium sulfide ($Na_2S$) were dissolved in 25 mL deionized water to give a stock solution of 0.10 M. Sodium sulfide was preferred to hydrogen sulfide because of the easier handling of solid reagent over toxic gas. For the humic acid, 7.6 mg of Suwannee River Humic Acid (SRHA, from IHSS) was dissolved in 25 mL of deionized water to yield a stock solution of approximately 300 mg/L (actual 304 mg/L as HA, 161 mg /L as DOC). The resulting SRHA solution was passed through a 0.45 µm membrane filter.

All the mixtures were prepared in a synthetic matrix of 1 mM $CaCl_2$ to represent the calcium concentration typically observed in Chalk rivers and groundwater in southern and eastern England e.g. [26]. To obtain sub-oxic conditions the deionized water used to prepare reagents was first thoroughly deoxygenated by sparging with an oxygen-free nitrogen bubble stream. The experimental batch mixtures were then prepared under a light nitrogen flow inside a pre-equilibrated glove bag. These conditions cannot be considered totally anoxic as air was not fully excluded from the glove bag, but the positive pressure of nitrogen in the glove bag was sufficient to ensure a predominantly nitrogen atmosphere during handling and exposure of the reagents and hence maintain a sub-oxic environment. Measurements on previous similar experimental solutions prepared using this technique showed DO in solution of <0.05% saturation.

Calcium matrix, sulfide and humic stock aliquots were diluted to final volume with sparged DI water with the Ag NP aliquot added last to avoid transient exposure of the NP to more concentrated conditions which may have altered the stability prematurely. The pH of each tube was adjusted by addition of 30 μL droplets of either $HNO_3$ (0.003 M) or NaOH (0.03 M) until the observed pH stabilized to pH 7±1. Required doses of acid or base varied slightly according to the proportions of humic (slightly acidic), sulfide (strongly basic) or citrate (buffering) which were involved but the total volume adjustment was never greater than 2.3%, so the overall impact on the nominal concentrations was small relative to the variation of concentration ranges under consideration. Completed test mixtures were kept in screw-top tubes, immediately encased in aluminium foil to exclude light and stored in a refrigerator at 4°C when not actively undergoing further analysis or sampling.

## Characterization

Size distributions of the Ag NPs in pure synthesized form and in the batch mixtures (Table 1) were measured by dynamic light scattering (DLS) using a Malvern Zetasizer NanoZS instrument with a 1 cm optical cell. Each measurement included five replicate scans of 2–3 min each, on which the zetasizer software was used to determine the size-average hydrodynamic diameter.

Measurements were also made to determine zeta potential. However, changes in the observed zeta potential scans over a period of 10s of minutes during multiple acquisitions suggested gradual poisoning of the cell electrode, associated with those solutions containing $Na_2S$. Therefore only the initial scans for each sample have been used in interpretation and comparison between experimental mixtures.

Transmission Electron Microscopy (TEM) was used in order to assess the size and shape of the NPs without reliance on the hydrodynamic behavior.  Cu TEM grids (200-mesh , two per tube, held horizontally)  were covered with an aliquot of diluted sample mixture (0.5 mL, in 10.5 mL of deionized water) and ultracentrifuged at 35000 rpm for 1 hr to deposit Ag NP and particles onto the substrate. The grids were rinsed by immersing twice in suboxic deionized water for a few seconds to remove residual matrix, before being allowed to dry overnight under nitrogen. Characterization of the prepared Cu grids used a JEOL 1200EX instrument with 80 keV electron generation. Images were acquired at magnifications between 100,000 and 300,000.

## RESULTS AND DISCUSSION

Addition of fresh citrate-capped Ag NPs to the calcium experimental matrix, without the presence of either sulfide or humic acid, produced rapid and significant changes in the apparent size distribution of the NPs (Fig 1 and Table 2). Whereas the pure cit-AgNP stock (maintained in citrate matrix) displayed a single, monomodal, clean and relatively narrow particle size distribution with a z-$d_h$ of 36 nm (mode 44 nm), the introduction of the $CaCl_2$ matrix rapidly produced a much broader and asymmetric bimodal size distribution. Although the z-$d_h$ reduced to 28 nm, the shape of the distribution suggested a significant proportion of the particles were now apparently smaller (peak at 11.3 nm), but the majority now larger (peak at 67.7 nm) than in the pure material. TEM imaging (Fig. 2; C1, 24 hr) showed the AgNPs in small aggregated clusters, which could be attributed to the suspension destabilisation and subsequent aggregation in previously observed in the presence of $Ca^{2+}$ as electrolyte [8]. This aggregation, which was established within 15 min of mixing, remained relatively stable for at least 24 hours, but over an extended period the size distribution evolved to larger particles. After 64 d the bulk of particles formed a uniform distribution between 100 and 1000 nm with a z-$d_h$ of 245 nm. This peak then remained largely unchanged for a further 80 days, although there was some evidence of a small increase in the small number of much larger agglomerates at approximately 4000 nm.

The behavior observed in the presence of sulfide (Fig 1, expt C2) was entirely different, with the size distribution shifting rapidly to higher values with a peak at 208 nm. This then remained unchanged for at least 24 hours. Over the longer term to 64 days the particle size remained similar, although there was some evidence of ageing of the particles as shape of the distribution deteriorated slightly from the pseudo log-normal observed for fresh material. These larger aggregates, which were clearly observed by TEM (Fig 2; C2, 24 hr), are believed to be due to the formation of sulfide nanobridging between the particles comparable to that reported by Levard et al (2011). EDX elemental analysis confirmed pronounced co-location of S with the AgNP (see Fig S1, Supporting Information). Variation in the concentration of the sulfide produced corresponding variation in the degree of shift of the NP size distribution: the stronger the sulfide, the stronger the shift. Thus the size distribution peaked at 122 nm for 0.001 M $S^{2-}$ (expt C3), compared to the 208 nm for 0.01 M $S^{2-}$ (Fig 3b).

The presence of humic acid (Fig 1, expt C6) also appeared to stabilize the cit-AgNP over a prolonged period, but at a size much closer to the original Ag(cit) NP dimensions. On addition of HA to Ag(cit) NP, within an hour the peak of the distribution had shifted from the initial 44 nm for the pure Ag(cit) NP to a mode of 52 nm, but this then remained relatively stable and consistent for the entire duration of the experiment up to 145 days, with very little change in the polydispersity index. The shape of the distribution was also largely unchanged, which combined with the stability of the peak suggests that in the presence of the HA the NP structures remained effectively constant.

The influence of the humic acid was relatively insensitive to the concentration of HA within the concentration range studied (Fig 3a). Using HA concentration 10× more dilute (2.5 mg/L, expt C4) showed very similar behavior to the 25 mg/L with a shift of the size distribution peak to 51 nm, the same as for the higher concentration to within the uncertainty of the measurements.

Fig 1 also shows the observed size distributions for Ag(cit) NP in the presence of both sulfide and SRHA (expt C7). It is clear that when in competition the influence of the humic acid dominated that of the sulfide. There was a small shift observed in the size distribution of the AgNP from the pure Ag(cit) NP to around 56 nm, similar to the presence of SRHA without sulfide. Again the distribution then remained relatively stable for the remainder of the experiment up to 145 days, with no apparent change in polydispersity. The much larger 200 nm particles produced by sulfide without humic acid, were not observed in the presence of humic acid. The TEM evidence for the experiments containing SRHA (Fig.2; C6 and C7, 24 hr) was consistent with the observations from DLS in that even though HA was present the AgNPs were not observed to be aggregated or bridged but rather exhibited as single particles closer in appearance to the original pure AgNP preparation.

In complete contrast to the Ag(cit) NP, experiments using PVP-capped AgNPs showed few or no significant changes in apparent stability or particle size distribution of the Ag(PVP) NP under any of the conditions examined (Fig 4). Not $CaCl_2$ matrix, introduction of sulfide or presence of SRHA caused a shift or change in the shape of the observed distribution. It is clear that the steric stabilization of the AgNP by the PVP capping agent leads to profoundly different behavior of the AgNP compared to the citrate or other more weakly complexed capping agents. This observation has significant implications for the interpretation of experimental investigations where only one variety of capping has been studied. Consequently it also has significant implications for understanding the ageing, degradation and hence release of the different varieties of AgNP in the environment. Studies based on PVP-capped AgNP might tend to suggest far greater stability and, in the absence of competing effects, slower release of Ag than studies based on citrate-capping. However, caution must therefore be taken to ensure that generalized conclusions are not drawn from a single type of AgNP which may not adequately reflect the behavior of other forms.

Fig 5 shows zeta potential results for experiments C1, C1, C6 and C7. These results support the results obtained by DLS and show a progressive/additive stability of the cit-AgNP particles for experiments C1 through to C6. There is a clear shift to a negative zeta potential observed on the addition of SRHA (C6) compared to mixtures with no NOM (C1) implying increased NP stability due to NOM-cit-AgNP interactions. While this method provided useful insights which support the DLS results the presence of $S^{2-}$ ions rapidly poisoned the electrodes and this method is therefore poorly suited to explore AgNP stability under sulfide reducing conditions.

The mechanism of cation bridging has been observed in previous studies [27], and is a likely explanation for the cit-AgNP aggregation shown in this study in the presence of Ca and absence of NOM. In the absence of NOM and the presence of sulfides the DLS and TEM data show a significant increase in particle size, consistent with NP aggregation though sulfide bridging. This suggest pseudo-stable and large AgNP-S complexes, the size of which was limited by the availability of $S^{2-}$ ions. While this study supports existing findings from laboratory studies and models for AgNP stabilization in the presence of $S^{2-}$ under reducing conditions [21, 24], there are few actual environmental settings where NOM is likely be absent. Where a mixture of NOM and sulfides are present, including waste water treatment works where AgNPs are likely to be concentrated, this work has shown that there is a dominant effect in rapid NP stabilization from NOM at a smaller size than observed due to the sulfide-bridging. DLS size distributions, supported by zeta potential results and TEM imaging (Fig 2, samples C6 and C7) suggest that this may occur through the formation of a NOM layer on the surface of the citrate capped AgNPs which stabilizes the NP for at least 145 days, limiting the potential for AgNP aggregation and altering the surface charge characteristics and hence mobility and persistence of the AgNPs in the environment[28]. This effect of rapid NOM stabilization of AgNPs is observed even at relatively low NOM concentrations (e.g. 2.5 mg/L SRHA) which are typical for many environmental matrices such as surface waters and groundwaters [29, 30].

The evidence in the DLS indicates traces of smaller particles below 10 nm appearing over time.. It is striking that this does not occur in the presence of sulfide, with or without the presence of NOM, reinforcing that the sulfide-bridging provides enhanced stability against break-up or dissolution of the AgNPs. In the absence of sulfide, the evidence of scattering and hence small particles at <10 nm becomes significantly lower in the presence of NOM. This may be attributable to the stabilizing influences of the NOM reducing dissolution[24], although if the smaller distributions are due to dissolution under conditions of low-oxygen it is also possible that some rapid dissolution occurs until the limited oxygen in the sub-oxic conditions is used up. Such oxygen would be rapidly scavenged and removed in the presence of sulfide. Dissolution of the AgNP is normally considered oxidative so significant dissolution would not be expected in sub-oxic conditions. This was confirmed by ICP-MS analysis of the supernatant from the TEM ultracentrifugation which showed residual Ag in solution of 23 μg/L for one replicate of C7 (from an intial AgNP concentration of 1mg/L), with C1,C2 and C6 all yielding <2 μg/L Ag in solution.

These findings are significant when considering the environmental toxicity of AgNP. While NOM and $S^{2-}$ can be effective in stabilizing the Ag in the NP form and preventing the release of Ag in the dissolved phase for considerable time [31], it does concentrate the potential dose for organisms that can assimilate the all or part of the AgNP if they are ingested whole and start to dissolve at a later date [32]. NOM stabilization which reduces the potential for AgNP breakdown and Ag dilution in the environment could equally enhance the persistence and transport of AgNP away from point sources such as waste water treatment works or as sludge that is applied to the soil surface [26], and facilitate adsorption on to biological surfaces in the environment [33].

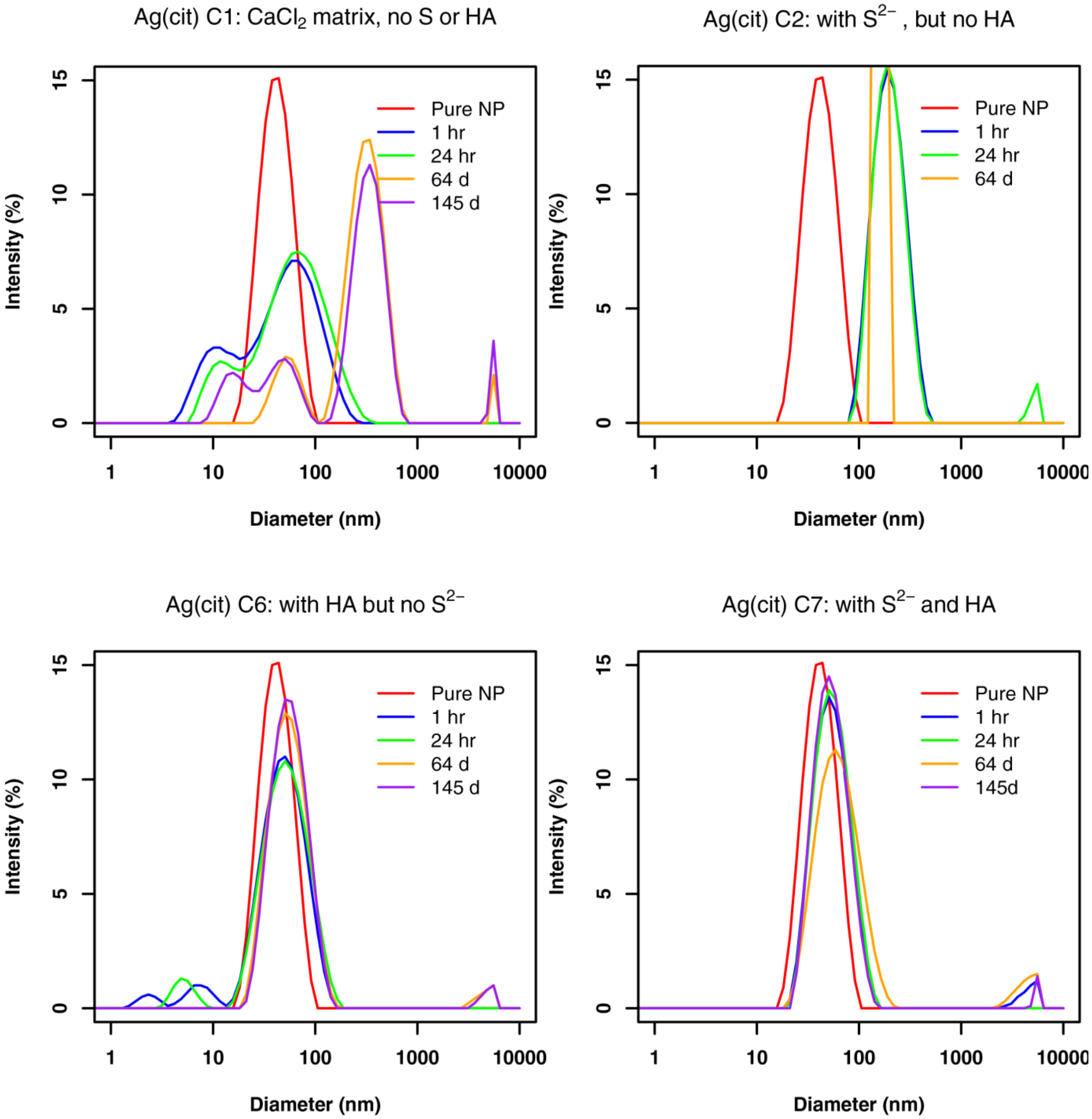
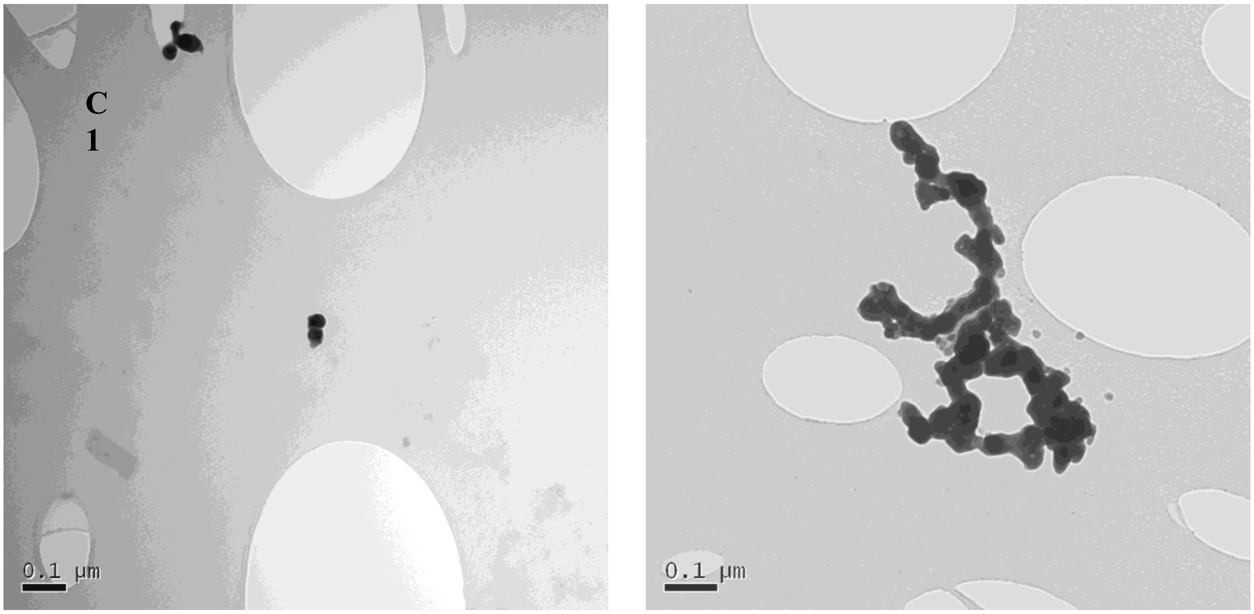
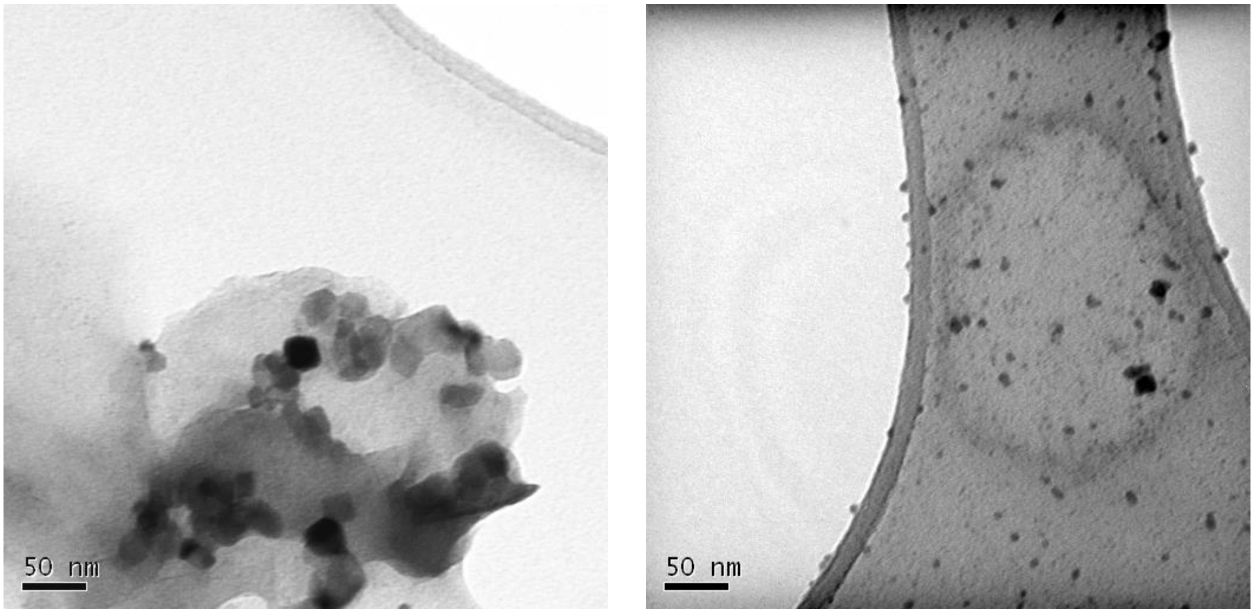


Figure 1. Evolution with time of size distributions for Ag(cit) NPs in calcium chloride matrix with varying sulfide and humic acid concentrations. C1 – CaCl2 matrix only; C2 – addition of sulfide; C6 – addition of humic acid; C7 – both sulfide and humic acid.



Ag(cit) C1: CaCl2 matrix, no S or HA

Ag(cit) C2: with S2–but no HA



Ag(cit) C6: with HA but no $S^{2-}$

Ag(cit) C7: with $S^{2-}$ and HA

Figure 2. TEM images of Ag(cit) NPs prepared after 24 hr in calcium chloride matrix with or without presence of sulfide and/or humic acid.

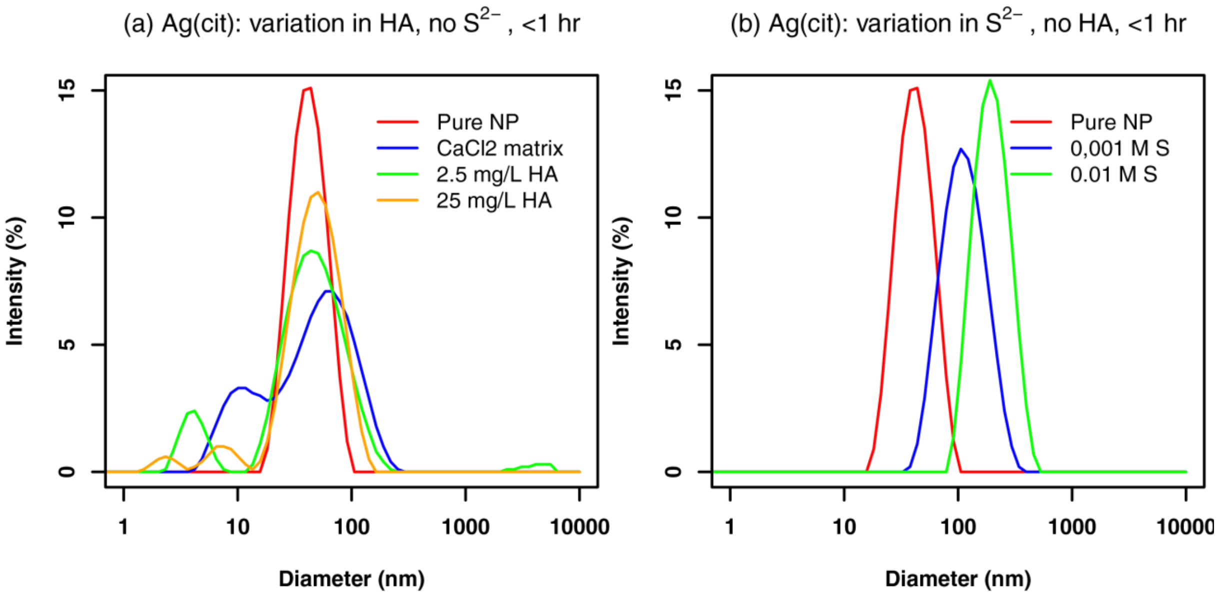


Figure 3. Impact of variation in (a) concentrations of humic acid (HA) and (b) sulfide matrix concentrations on size distributions of Ag(cit) NPs.

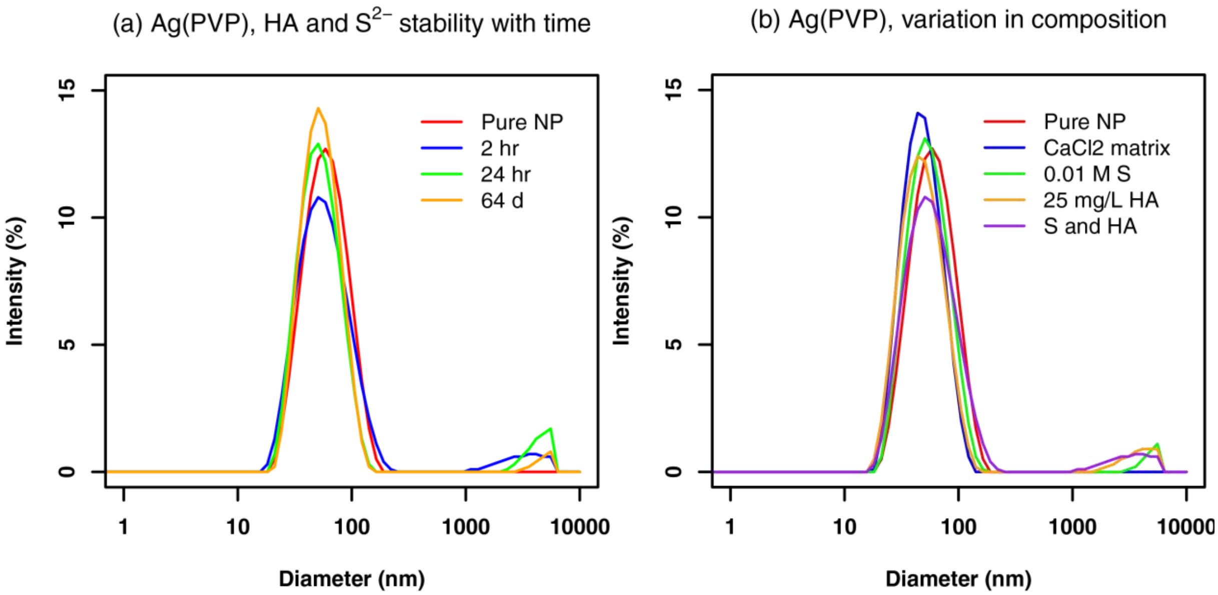


Figure 4. Impact of (a) evolution over time and (b) variation in sulfide and humic acid concentrations on observed size distributions of Ag(PVP) NPs in calcium chloride matrix.

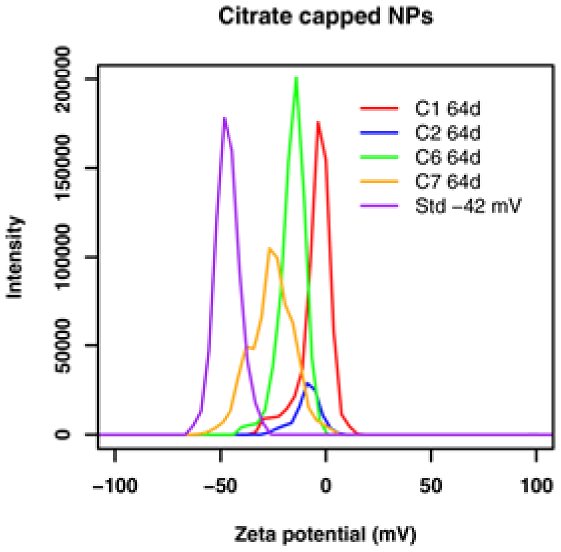


Figure 5. Zeta potential results for selected mixtures of citrate-capped AgNPs after 64 days. Due to the degradation of the electrodes discussed in the text, the first scan for each mixture is shown. The peak for the standard has been reduced in scale by a factor of 2 to provide clearer comparison.

Table 1. Summary of experimental reagent concentration used in test mixtures.

| Expt No. | NP type | NP conc. (mg/L) | SRHA (mg/L) | Na2S (M) | Adjusted pH |
|---|---|---|---|---|---|
| C0 (pure NP) | Cit | | | | Deionised water |
| C1 | Cit | 1 | 0 | 0 | 7.4 |
| C2 | Cit | 1 | 0 | 0.01 | 7.9 |
| C3 | Cit | 1 | 0 | 0.001 | 7.0 |
| C4 | Cit | 1 | 2.5 | 0 | 6.6 |
| C5 | Cit | 1 | 2.5 | 0.01 | 7.5 |
| C6 | Cit | 1 | 25 | 0 | 7.0 |
| C7 | Cit | 1 | 25 | 0.01 | 8.0 |
| C8 | Cit | 1 | 2.5 | 0.001 | 6.3 |
| C9 | Cit | 1 | 25 | 0.001 | 6.3 |
| P0 (pure NP) | PVP | | | | Deionised water |
| P1 | PVP | 1 | 0 | 0 | 7.1 |
| P2 | PVP | 1 | 0 | 0.01 | 7.9 |
| P6 | PVP | 1 | 25 | 0 | 7.1 |
| P7 | PVP | 1 | 25 | 0.01 | 7.9 |

Table 2. Observed z-average hydrodynamic diameter (z-$d_h$ in nm) and polydispersity index (PdI) measurements for citrate- and PVP-capped Ag NPs in HA and sulfide mixtures, usually derived from average of 5 replicate scans, except where unreproducible outlier scans have been excluded. Also shown are the mode values for the dominant distribution in each case; modes have not been tabulated for secondary peaks in polymodal dispersions.

| | **1 hr** | | | **24 hr** | | | **64 d** | | | **145 d** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **mode** | **z-$d_h$** | **PdI** | **mode** | **z-$d_h$** | **PdI** | **mode** | **z-$d_h$** | **PdI** | **mode** | **z-$d_h$** | **PdI** |
| C0 | 44.5 | 36.2 | 0.19 | | | | 45.8 | 37.8 (81d) | 0.18 | | | |
| C1 | 67.7 | 27.9 | 0.50 | 71.2 | 38.8 | 0.45 | 315 | 245 | 0.42 | 298 | 410 | 0.78 |
| C2 | 208 | 190 | 0.15 | 208 | 209 | 0.27 | | 3400 | 1.00 | | | |
| C3 | 122 | 100 | 0.17 | | | | | | | | | |
| C4 | 51.1 | 28.6 | 0.52 | | | | | | | | | |
| C5 | 66.3 | 59.9 | 0.28 | | | | | | | | | |
| C6 | 52.2 | 36.1 | 0.30 | 56.0 | 39.3 | 0.28 | 61.2 | 47.6 | 0.23 | 58.1 | 50.1 | 0.24 |
| C7 | 56.2 | 52.3 | 0.23 | 57.9 | 52.9 | 0.20 | 62.3 | 63.7 | 0.35 | 66.5 | 68.4 | 0.23 |
| C8 | 56.8 | 53.1 | 0.25 | | | | | | | | | |
| C9 | 53.6 | 49.8 | 0.20 | | | | | | | | | |
| P0 | 63.7 | 50.6 | 0.21 | | | | 63.1 | 51.6 (29d) | 0.20 | | | |
| P1 | 50.9 | 42.0 | 0.21 | 49.9 | 42.4 | 0.23 | 46.7 | 53.13 | 0.26 | | | |
| P2 | 60.1 | 50.1 | 0.24 | 58.8 | 49.5 | 0.21 | 57.5 | 53.1 | 0.24 | | | |
| P6 | 51.7 | 45.1 | 0.30 | 52.9 | 42.4 | 0.24 | 52.5 | 45.1 | 0.29 | | | |
| P7 | 61.0 | 51.4 | 0.24 | 56.4 | 51.9 | 0.25 | 53.4 | 48.7 | 0.20 | | | |

## ASSOCIATED CONTENT

### Supporting Information

Details of preparation of citrate- and PVP-stabilized AgNP; and Energy-dispersive X-ray spectroscopy (EDX) data for experiment C2, cit-AgNP with 0.01 M (320 mg/L) sulfide ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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GRAPHIC FOR TABLE OF CONTENTS AND ABSTRACT