

Storage Wars: how citrate-capped silver nanoparticle suspensions are affected by not-so-trivial decisions

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Abstract A critical but often overlooked component of silver nanoparticle (AgNP) suspensions involves their behavior following short- and long-term storage. The current study investigates the integrity of citrate-capped AgNP suspensions, nominally 20 nm in average diameter, in a series of distinct storage conditions, based on possible combinations of reasonable decisions researchers make, both nanoparticle-based (AgNP and relative citrate concentration) and environmental-based (solution oxygenation and ambient light or dark). AgNP integrity was determined by monitoring single particle stability, aggregation/agglomeration, and oxidation for 104 days. We demonstrate that AgNP suspensions lose their physical and chemical integrity by two distinct processes: (1) oxidation only (light-independent) and

(2) oxidation followed by photo-reduction (light-dependent), following initial dilution from a concentrated (and newly synthesized) AgNP stock solution. Optical spectroscopy indicates that the effects of oxidation are readily observed while the effects of photo-reduction are less obvious, leading to a greater increase in average particle diameter, the formation of new, metallic nanoparticles, and the oxidation of the parent citrate capping agent. In general, the overall integrity of citrate-capped AgNP suspensions are best maintained when these solutions are purged with nitrogen gas and stored in the dark at the highest AgNP and citrate concentrations. This study outlines a strategy for both assessing and monitoring the integrity of AgNP suspensions in an effort to harmonize long-term experiments and promote inter-laboratory consistency.

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Introduction

Silver nanoparticles (AgNPs) have gained a significant amount of attention over the past two decades due to their novel applications, including their use in field bandages, water filtration, commercial products, anti-microbial surface coatings, and printing of electronic devices (Rai et al. 2009; Singh et al. 2010). As a result, researchers have devoted tremendous resources to

develop AgNP applications and investigate their long-term environmental, health, and safety (EHS) risks (Benn and Westerhoff 2008; Akaighe et al. 2011; Kaegi et al. 2011). For example, both the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) have formed task forces and scientific advisory panels to identify and promote key research areas that address nano-EHS concerns (FDA 2007; EPA 2010). A primary goal in these panels is to better understand the EHS risk potential over the entire AgNP lifecycle.

AgNP suspensions are subject to oxidation, dissolution, aggregation, and agglomeration transformations under various conditions during their lifecycle (Meyer et al. 2010; Chinnapongse et al. 2011; Huynh and Chen 2011; Stebounova et al. 2011; Zook et al. 2011a, b, c; Levard et al. 2012; Poda et al. 2013). This strong in situ transformation potential requires that the physico-chemical properties of the stock AgNP dilutions be well characterized and continuously monitored, whether for a single experiment (e.g., toxicological) or over the lifecycle of a AgNP product, in order to correctly attribute the cause of changes or degradation to the act of aging or the experimental variable. Typical standard practice includes core chemical characterization of the AgNP material immediately after synthesis/purification, although which techniques necessary for characterization vary depending on the material (Stone et al. 2010; MacCuspie et al. 2011b). Very little is known about the relative integrity of common-place stock suspensions post synthesis, during both short- and long-term storage periods, with limited studies found investigating the effect of storage on particle integrity (Kittler et al. 2010; Kennedy et al. 2012). Integrity in this context is defined as any measureable change in the single particle stability (optical properties), aggregation/agglomeration (sizing properties), and/or oxidation to any component of the AgNP suspension.

The focus of this study is to investigate how distinctive storage conditions affect the overall integrity of nominally 20 nm citrate-capped AgNP suspensions. The storage condition variables examined included: (A) AgNP concentrations, (B) citrate concentrations, (C) exposure to laboratory light, and (D) purging with air or nitrogen gas for an oxygen rich/deficient suspension (Fig. 1). In total, 36 individual AgNP suspensions were monitored for single particle stability over 104 days of storage while select time

points were chosen to study aggregation/agglomeration and oxidation. The methods chosen for this study were made with a focus on having the capability of high throughput with readily-accessible instrumentation to enhance the applicability of the technique to other research projects. The objectives of this work are: (1) to identify what nanoparticle- and environmental-based variables most strongly affect the integrity of AgNP suspensions, (2) to provide storage condition recommendations to maintain overall integrity of citrate-capped AgNP suspensions, and (3) to discuss potential transformation mechanisms and products of citrate-capped AgNP suspensions appropriate for commonly used storage conditions.

Methods¹

AgNP synthesis

The AgNP suspensions were synthesized by previously reported procedures designed for synthesizing 20 nm NPs [see supporting information (SI) for more details] (MacCuspie 2011; Zook et al. 2011a; MacCuspie et al. 2011a). To purify the synthesized AgNP suspensions, stirred-cell ultrafiltration was employed on the entire volume using a 10 kDa, molecular mass, regenerated cellulose cut-off filter (Millipore, Bedford, MA, USA) until ≈ 65 mL of retentate remained, removing $\approx 97.7\%$ of impurities. The retentate was then diluted to 3.150 L for a final silver concentration of $\approx 43.2 \text{ mg}_{\text{Ag}} \text{ L}^{-1}$ with a citrate concentration of $\approx 11.66 \text{ mg}_{\text{cit}} \text{ L}^{-1}$. This AgNP suspension was stored in a sealed, glass container in the dark overnight.

Experimental design/matrix

After the suspensions were stored overnight (<24 h), the AgNP suspension ($\approx 43.2 \text{ mg}_{\text{Ag}} \text{ L}^{-1}$) was divided into 36 “stock” suspensions, each with a different sets of storage conditions at room temperature (24°C).

¹ Certain trade names and company products are mentioned in the text or identified in illustrations in order to specify adequately the experimental procedure and equipment used. In no case does such identification imply recommendation or endorsement by National Institute of Standards and Technology, nor does it imply that the products are necessarily the best available for the purpose.

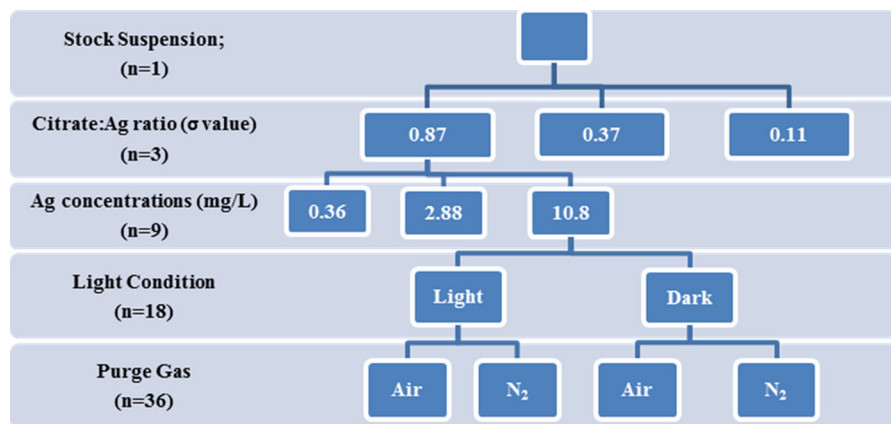


Fig. 1 Schematic representation of the approach for developing a complete matrix of storage conditions for AgNP suspensions. (Top to bottom) The purified stock suspension is first split into three different relative citrate concentrations which are determined by maintaining three different molar ratios with the [Ag] to realistically simulate a dilution. The stock

The conditions included three concentrations of AgNPs (in $\text{mg}_{\text{Ag}} \text{L}^{-1}$); three citrate:AgNP molar ratios (σ values); absence or presence of laboratory light; and absence or presence of oxygen (Fig. 1). Initially, the AgNP suspension ($43.2 \text{ mg}_{\text{Ag}} \text{L}^{-1}$) was divided into three containers, one remained at the initial citrate concentration ($11.66 \text{ mg}_{\text{cit}} \text{L}^{-1}$) while additional trisodium citrate ($37.97 \text{ mg}_{\text{cit}} \text{L}^{-1}$ and $89.50 \text{ mg}_{\text{cit}} \text{L}^{-1}$) was added to the remaining two solutions to yield σ values of 0.11, 0.37, and 0.87, respectively. Each of these three AgNP suspensions was separately diluted into (0.36 , 2.88 , and 10.8) $\text{mg}_{\text{Ag}} \text{L}^{-1}$ suspensions with milli-Q water. Each of these nine suspensions was divided into two groups: those exposed to laboratory, fluorescent lighting, and those shielded with aluminum foil (dark condition). Lastly, these 18 suspensions were divided once more for gas purging by bubbling either house air or nitrogen for ≈ 8 min. All 36 suspensions consisted of at least 100 mL of AgNP suspension in a sealed, clear glass bottle placed on the bench top at room temperature for a 104 day period. Measurements on select samples made within 30 days of synthesis demonstrated a pH range of 7.5–8 for samples of intermediate citrate concentration. Samples were periodically removed for analysis from the sealed bottles. For consistency, all suspensions were purged with the appropriate gas for an additional 6 min following each sample removal period. Aliquots obtained from the different storage conditions

suspensions are then diluted into three different concentrations of AgNPs for storage. Each of these nine different suspension conditions is split into a light or dark environment in a laboratory chemical hood. Each of these 18 suspensions is divided one final time to purge with air or nitrogen to adjust the dissolved oxygen (36 storage conditions)

were always stored in the dark prior to UV analysis (within 24 h of sampling).

Analytical Techniques

Ultraviolet–visible (UV–Vis) spectroscopy

AgNP suspensions were analyzed using a Shimadzu (Columbia, MD, USA) UV-1800 UV Spectrophotometer (double pass), which was calibrated with two standard reference materials (SRM 2034 and SRM 2031) from the National Institute of Standards and Technology (NIST) to confirm accuracy of the wavelength and absorbance measurements, respectively (Mavrodineanu and Baldwin 1980; Weidner 1986). All samples were measured from 200 to 800 nm using quartz cuvettes with a 1 cm path length and manually background subtracted using Milli-Q water. For brevity, absorbance values are reported at three wavelengths for each AgNP sample at 389, 455, and 650 nm (henceforth referred to Abs_{389} , Abs_{455} , and Abs_{650}), respectively. Although the initial maximum absorbance ranged from 387 to 389 nm, Abs_{389} was chosen as the position of the single AgNP SPR. Values at Abs_{455} and Abs_{650} were chosen to indicate potential changes in size (Gorham et al. 2012) and/or agglomeration/aggregation state (Zook et al. 2011a), respectively. All three measured values were normalized to Abs_{389} at 0 days for a given set of storage conditions.

The time ($t_{97\%}$) required for a 3 % loss at Abs_{389} was arbitrarily chosen as a reference point for suspension integrity for a given set of conditions and determined via best fit analysis using Sigma Plot.

Dynamic light scattering (DLS)

DLS measurements were performed using a Malvern Instruments (Westborough, MA, USA) Zetasizer Nano in 173° backscatter mode with the instrument controlled temperature fixed at $(20.0 \pm 0.1)^\circ\text{C}$. The cumulants analysis algorithm was applied to obtain qualitative information on the intensity based, average hydrodynamic diameter (D_H). Measurements were acquired initially for three, 2.88 mg L^{-1} AgNP samples at the three different σ values to be representative of all $t = 0$ samples, with D_H measuring at 21.5, 21.7, and 24.6 nm for high to low citrate concentrations, respectively. All stored samples were measured separately after 104 days of storage. Results reported are the mean and 1 SD based on four or more consecutive measurements of each sample. This uncertainty represents the repeatability of the measurement, and not the width or polydispersity of the size distribution. All samples were prepared in a particle-free hood with clean cuvettes (Hackley and Clogston 2007). For further discussion on DLS analysis and data interpretation, please refer to the SI.

Nuclear magnetic resonance (NMR) analysis

NMR experiments were conducted on a Bruker Avance II 600 MHz spectrometer, operating with Topspin (Version 2.1.5, PL1) software, using a 5-mm broadband inverse (BBI) detection probe. ^1H spectra were acquired on aqueous solutions (containing $\approx 10\%$ D_2O) at 300 °K with water suppression using excitation sculpting with 256 scans for acquisition and without spinning the sample tube.

^1H spectra were processed by smoothing with an exponential window function with a line-broadening factor of 0.3 Hz, zero filled to 32,000 points and then converted from time domain to frequency domain by Fourier transformation. Spectra were then manually phase corrected across the spectral region containing all peaks of interest. Selected sections of the spectrum were separately baseline corrected using a 5° polynomial to obtain peak integration areas.

Additional initial characterization techniques

Additional chemical and physical information regarding the initial composition of the AgNP suspensions can be found in the SI.

Results

Data analysis and presentation

In this work, suspension integrity is comprised of three individual components: single particle stability, aggregation/agglomeration, and oxidation. While these variables are interconnected, we make these distinctions based on the analytical techniques used throughout this investigation. The single particle stability, which refers to the retention of the initial AgNP suspension characteristics, was monitored by observing changes in the surface plasmon resonance (SPR) absorbance. The SPR absorbance is a sensitive surrogate parameter, with shifts in the peak position indicative of changes to the physical diameter or surface coating while decreases in absolute absorbance value indicative of a loss of single metallic particle concentration (e.g., through oxidation or agglomeration) (Lok et al. 2007; Kuzma et al. 2012; Power et al. 2013). At other wavelengths, UV–Vis provided an indication of aggregation/agglomeration within the AgNP suspension with additional, more qualitative evidence being provided by DLS. Oxidation of metallic silver can be confirmed through the addition of a strong reducing agent and examination of the SPR absorbance, while oxidation of the citrate stabilizer was measured using NMR. Changes in these three components, occurring separately or concurrently, can indicate degradation to the overall integrity of AgNP suspensions and therefore need to be monitored.

Over 500 UV–Vis spectra were collected to screen for regions of interest across many different storage conditions (36 conditions; Fig. 1) with variables including silver concentrations, citrate:silver molar ratios (σ value), light exposure, and dissolved gas content. To more clearly present and analyze the temporal trends in the UV–Vis spectra, Fig. 2a provides an example of select data to demonstrate the temporal variation over 104 days for one specific storage condition, specifically 2.88 mg L^{-1} ,

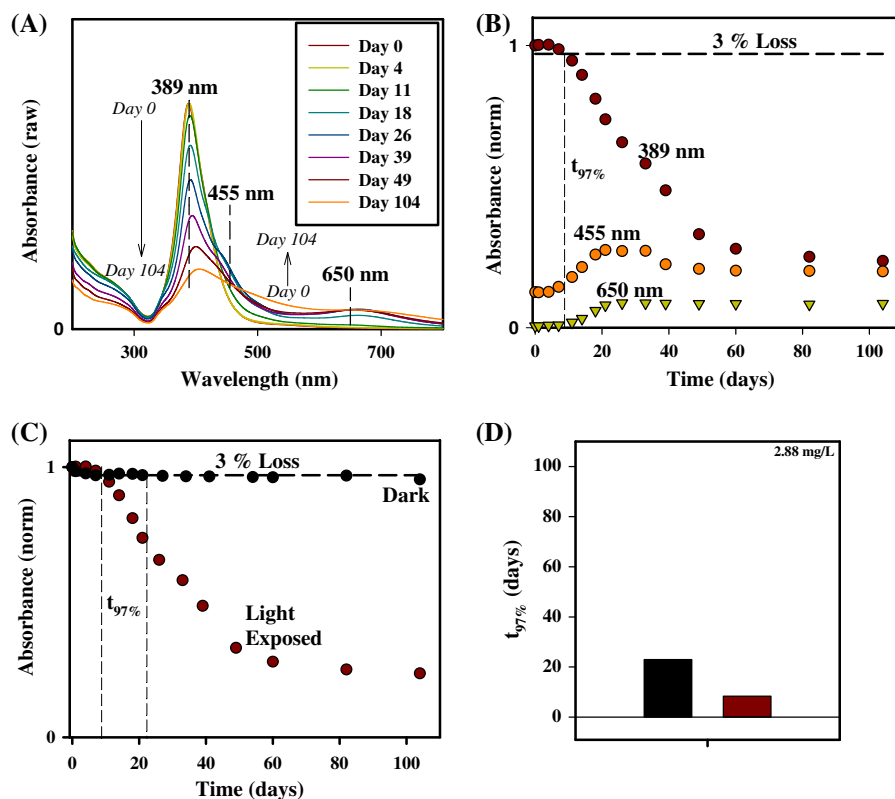


Fig. 2 a Select UV-Vis spectra of the AgNP suspensions monitored through 104 days of storage. Storage conditions consisted of AgNP suspensions diluted to 2.88 mg L^{-1} , a relative citrate concentration of 0.87, air purged and exposed to laboratory light. **b** Designated wavelengths were chosen to be monitored from the spectra in **a**. The SPR was monitored at 389 nm (red), 455 nm for particle diameter increases (orange), and 650 nm for

$\sigma = 0.87$, air purged and exposed to light. Over this period, Abs_{389} decreased while Abs_{455} and Abs_{650} values increased. This variation is further illustrated in Fig. 2b, where the absorption values at each specific wavelength are plotted as a function of storage time. The horizontal dotted line in Fig. 2b represents 97 % of the initial SPR absorbance; absorbance values that fell below the dotted line were designated as compromised (e.g., changing) AgNP suspensions. The $t_{97\%}$, or the time it takes for a AgNP suspensions to drop below 97 % of its initial absorbance, is identified by the vertical line in Fig. 2b. The $t_{97\%}$ value was determined by best fit analysis using sigmoidal or exponential regressions in the presence and absence of light, respectively (Fig. 2c). As discussed later, these two distinct decay processes proved important to understand the unique transformation mechanisms caused

the presence of aggregates (green). The horizontal, dotted line displays the point where 3 % of the SPR absorbance at 389 nm is lost. **c** Comparison of the SPR loss from storing AgNPs in the presence (red) and absence (black) of light. **d** The single particle stability, or $t_{97\%}$, for 2.88 mg L^{-1} AgNP suspensions (σ value = 0.87, air purged) shielded (black/left) and exposed (red/right) to laboratory lighting. (Color figure online)

by the presence of light. For the remainder of the results section, the temporal variations of the single particle characteristics will be represented by their $t_{97\%}$ values for each storage conditions in bar charts as illustrated in Fig. 2d. Suspensions with a $t_{97\%}$ value estimated to be over the 104 days experimental time were plotted only as being stable through the duration of the study. All raw spectra and plotted SPR absorbance intensities over time can be found in the SI (SI Figs. S3–S12).

Impact of storage conditions on single particle stability ($t_{97\%}$)

The temporal impact on SPR absorbance differed substantially between samples stored in the light and in the dark. Figure 3 summarizes the effect of storing

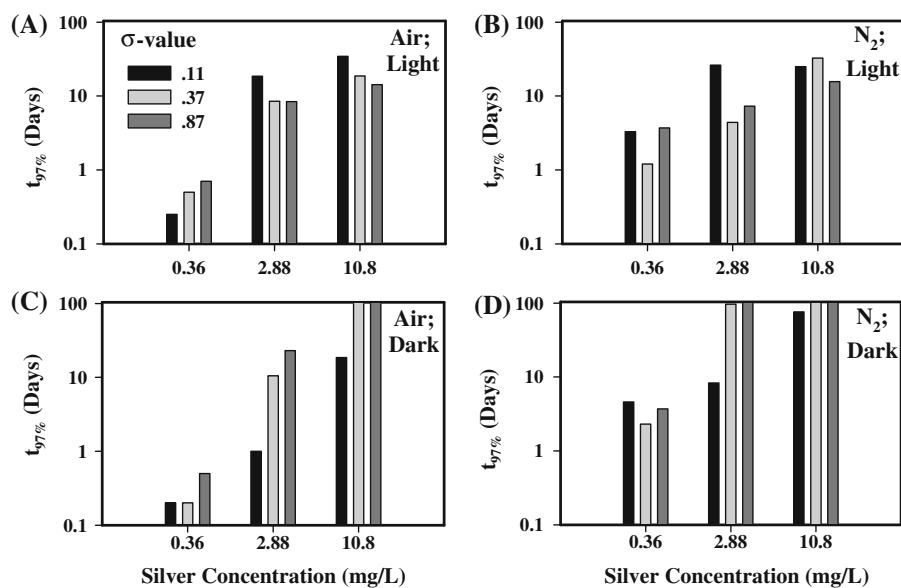


Fig. 3 Extracted $t_{97\%}$ values are plotted for 18 different storage conditions including **a** air-purged AgNP suspensions stored in the presence of light, **b** nitrogen-purged AgNP suspensions

stored in the presence of light, **c** air-purged AgNP suspensions were stored in the absence of light, and **d** nitrogen-purged AgNP suspensions were stored in the absence of light

AgNP suspension in both light and dark conditions after purging with air or nitrogen for a total of four sets of environmental conditions (individual spectra and SPR degradation curves in Fig. S3–S8). The clearest trend observed across all environmental conditions was that increased AgNP concentrations led to the greatest single particle stability (i.e., maintained highest $t_{97\%}$ value), with all other storage conditions held constant. Indeed, some AgNP suspensions stored at the highest silver concentration in the dark retained suspension integrity throughout the entire 104 days of storage and would retain stability, based on best fit analysis, >6 months.

The impact of the σ values varied depending on the other storage conditions. For air-purged AgNP suspensions exposed to the light (Fig. 3a), higher σ values typically decreased the single particle stability for the highest two silver concentrations while the exact opposite trend was observed for lowest silver concentration. Nitrogen-purged AgNPs stored in the light, however, exhibited no conclusive trend in improving sample integrity with respect to σ values (Fig. 3b). The storage conditions in the absence of light were the most consistent, with nearly all storage conditions, except at low silver and low σ , exhibiting increased single particle stability with increasing σ value (Fig. 3c, d).

Overall, UV–Vis confirms that the stability of AgNP suspensions is not solely dependent on environmental variables (purge and light exposure conditions), but is also dependent on other solution/particle specific variables. For example, enhanced stability was consistently observed when silver concentrations and σ values were high and samples were stored in the dark while no clear trends based on light exposure emerged when silver concentrations and σ values were low. When comparing purge conditions in the dark for a given AgNP concentration and σ value, nitrogen-purged AgNP suspensions generally had greater single particle stability ($t_{97\%}$ values) compared to air-purged samples (Fig. 3c, d). AgNPs suspensions stored in the light and nitrogen purged did not consistently show improved single particle stability over their air-purged counterparts.

To summarize, the $t_{97\%}$ values nearly uniformly increased with AgNP concentrations (holding all other variables constant). Increasing σ values generally increased the $t_{97\%}$ for suspensions stored in the dark (for both purge gases), but decreased stability when samples were stored in the light (air only). More often than not, the presence of light decreased $t_{97\%}$ when compared to dark conditions. Lastly, nitrogen purging greatly increased long-term single

particle stability for a wider range of AgNP suspensions (different AgNP concentration and σ values) when stored in the dark.

Impact of storage conditions on average AgNP hydrodynamic diameter (D_H)

DLS was employed to make qualitative measurements used to identify conditions that may promote changes to the size distribution (i.e., aggregation/agglomeration, diameter change). These qualitative measurements are reported as changes to the D_H (ΔD_H) (measured individual values provided in SI Tables 1, 2). These values are representative of the average of four or more measurements $\pm 1SD$. When stored in light, the ΔD_H increased in the majority of storage conditions for both air (Fig. 4a) and nitrogen gas (Fig. 4b) purged suspensions. While overall trends of size increase measured by DLS were less clear than those observed by UV–Vis, predominantly due to the polydispersity of the samples and/or the low particle concentrations, the highest σ values tended to have greater ΔD_H when stored in light exposure conditions (exceeding a 30 nm increase in some, N_2 -purged cases). This observation is consistent with the single particle stability ($t_{97\%}$) trends, which suggests that light-exposed AgNP suspensions are incapable of maintaining sample integrity at higher σ values, especially in air-purged conditions (Fig. 3a). For AgNP suspensions stored in the dark, increased σ values generally resulted in decreased ΔD_H for both air (Fig. 4c) and nitrogen-purged (Fig. 4d) samples. There was also a decrease in the ΔD_H with increasing AgNP concentrations for a given σ value when stored in the dark, especially in nitrogen-purged conditions. Dark storage conditions exhibited a more constant D_H , with the AgNP average D_H generally increasing modestly, if at all, compared to suspensions stored under laboratory light conditions. Generally, D_H data was consistent with changes in Abs_{455} and Abs_{650} of the UV–Vis data (SI Figs. S9–S12). While most suspensions exhibited only an increase in D_H or no change at 104 days of storage, some suspensions did exhibit a small D_H decrease at early storage times (See SI Fig. S13 for example). With the exception of low AgNP concentrations (SI Fig. S13), this early D_H decrease was observed under dark storage conditions at higher σ values.

Impact of storage in light on silver speciation (oxidation)

To detect the presence of oxidized silver, selected AgNP suspensions were subjected to chemical reduction of the supernatant following centrifugation (Gorham et al. 2012). Light-exposed AgNP suspensions ($2.88 \text{ mg}_{Ag} \text{ L}^{-1}$, $\sigma = 0.37$, air purged) were chosen as representative material since the SPR absorbance had deteriorated below 50 % of the initial value by day 36 for the air-purged samples (Fig. 5). Centrifugation removed the majority of SPR-active AgNPs from the supernatant, as evidenced by the small (or non-existent) peak at 389 nm (Fig. 5). Following the addition of sodium borohydride (NaBH_4), a common reducing agent used to synthesize AgNPs from Ag^+ , a color change from nearly clear to yellow was observed. More importantly, SPR absorbance recovery was observed (Fig. 5), which indicates that oxidized silver species were formed during storage in the light. The same test was performed on the nitrogen-purged equivalent AgNP suspension stored for 39 days and SPR recovery was also observed (SI Fig. S14), albeit to a lesser extent.

The impact of storage conditions on citrate at high silver concentrations

$^1\text{H-NMR}$ was employed to probe the behavior of the citrate capping agent during storage. 10.8 mg L^{-1} AgNP suspensions stored at the highest σ values (0.87) were analyzed after 60 days of storage under all four environmental conditions (presence/absence of light; air-/nitrogen-purged). These suspensions were chosen to maximize the citrate signal in the NMR in order to best detect citrate degradation and new product formation. The four methylene protons of citrate were evident as a AB quartet ($J = 15 \text{ Hz}$) centered at 2.52 ppm, consistent with the literature in D_2O (Anet and Park 1992; Xue et al. 2008) and in-house reference measurements in water (SI Fig. S15, top spectra) when the study was initiated (Fig. 6a, bottom spectra). After 60 days of storage in dark conditions, the relative citrate concentration remained present regardless of the purge conditions with some trace degradation peaks. By comparison, no detectable citrate signal remained when the suspensions were stored under light exposure conditions for 60 days. Several unique $^1\text{H-NMR}$ signals evolved as the citrate quartet

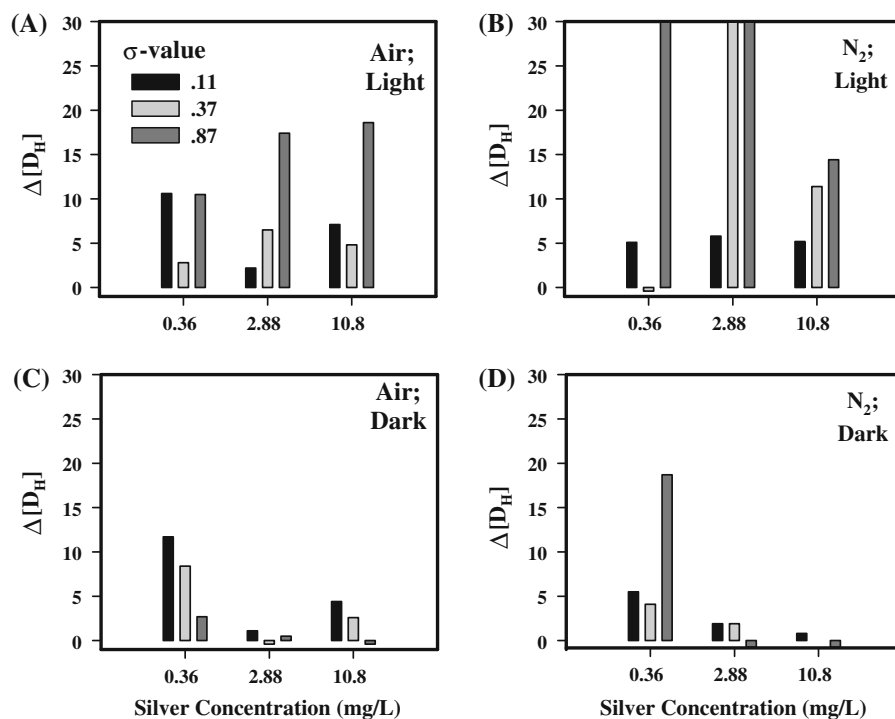


Fig. 4 DLS measured increases in the hydrodynamic diameter (ΔD_H) as a function of AgNP concentration and σ value for the following environmental conditions: **a** air purged and laboratory

light exposed, **b** nitrogen-purged and laboratory light exposed, **c** air purged and dark, **d** nitrogen purged and dark

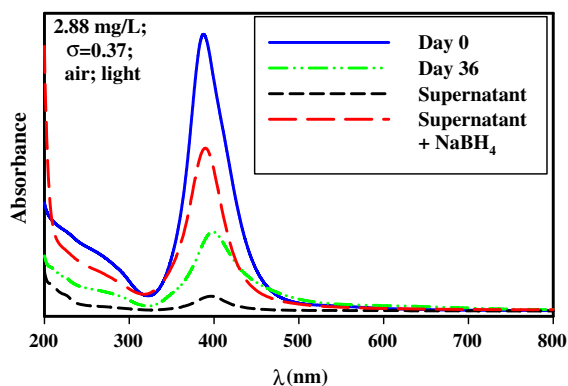


Fig. 5 Results from NaBH_4 testing for the presence of oxidized silver with UV-Vis spectroscopy represents the change between day 0 (blue) and day 36 (green) of storage for the air purged, light exposed 2.88 mg L^{-1} AgNPs with a σ value of 0.37. After centrifuging, the supernatant was measured alone (black) and with the addition to NaBH_4 (red) to reduce any oxidized silver present. (Color figure online)

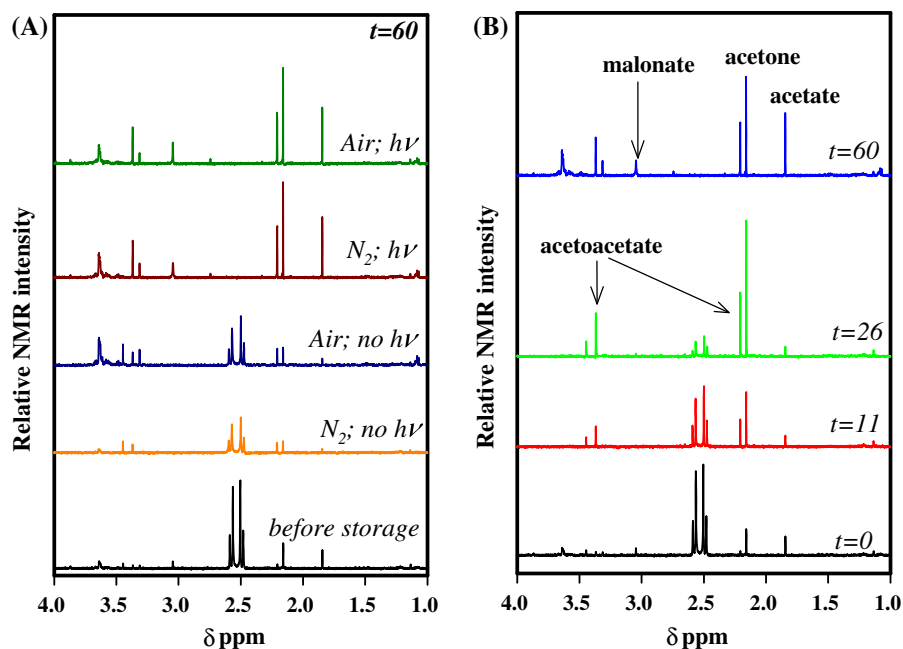
disappeared and were identified as acetoacetate (3.37 ppm (2H, s) and 2.21 ppm (3H, s)), malonate (3.05 ppm (2H, s)), acetate (1.84 ppm (3H, s)), and

acetone (2.16 ppm (6H, s)) and confirmed by reference compounds (SI Fig. S15).

Since light exposure appeared to cause citrate's degradation, the light exposed, nitrogen-purged AgNP suspensions were further scrutinized and subjected to periodic $^1\text{H-NMR}$ analysis during the 60 days storage period (see Fig. 6b for select spectra). As storage time increased, the concentration of citrate decreased; by 60 days, the quartet centered at 2.52 ppm was no longer detectable (Fig. 6b). Concomitant with the disappearance of citrate was the evolution of the acetoacetate and acetone peaks. The intensity of the malonate and acetate (1.842 ppm) peaks began to increase as well.

Except at short storage times ($t < 11$ days), the disappearance of citrate as measured by $^1\text{H-NMR}$ corresponded well with a decrease Abs_{389} measurements from the UV-Vis spectra (Fig. 7). At shorter storage times, the Abs_{389} values remained largely unchanged while citrate concentrations decreased. The appearance of the acetoacetate and acetone signals (again measured by $^1\text{H-NMR}$) was accompanied by an increase in Abs_{455} values, similar to the

Fig. 6 Select ^1H -NMR spectra of 10.8 mg L^{-1} AgNP suspensions with 0.87 relative citrate concentrations which were **a** stored for 60 days under four different sets of conditions and **b** nitrogen purge and exposed to light at representative storage times over 60 days of storage



mirroring of malonate and acetate with Abs_{650} values (Fig. 7b, c). These observations will be discussed further in the discussion.

Discussion

This study demonstrates that the overall integrity of citrate-capped AgNP suspensions is strongly affected by the conditions in which they are stored, including both particle (silver concentration and σ values) and environmental (presence/absence of light and gas purging) variables. The schematic representation in Fig. 8 is a representation of how these transformations occur and the products that are subsequently formed. In the first step of the transformation process (Fig. 8, step 1), the AgNP suspensions undergo surface oxidation. The presence of oxidized silver in this study was clearly demonstrated through the recovery of SPR absorbance following the addition of a strong reducing agent (Fig. 5). Though SPR absorbance recovery experiments were limited to light-exposed samples in the current work, oxidation and dissolution of AgNP suspensions in the dark have been previously demonstrated (Liu and Hurt 2010). We also observed SPR absorbance loss in samples stored in the dark without significant increases in the higher wavelength absorbencies (SI Figs. S3, S5, S6, and S8), suggesting

that surface oxidation and subsequent dissolution does occur, albeit at a slower rate compared to samples stored in the light. If the mechanism of degradation involved dissolution, one would expect a decrease in average particle diameter during storage. While this was qualitatively observed for early storage for some samples stored in the dark (SI Fig. S13), by 104 days of storage there were no AgNP suspensions that exhibited any significant decrease (Figs. 4, S13; SI Tables S1, S2). This suggests that if there were any size reduction for AgNP suspensions stored in the absence of light, the loss was completely masked by day 104 by either aggregation or surface layer adsorption.

The rate of both surface oxidation and dissolution depends on the quantity of species necessary to facilitate oxidation in suspension. Therefore, surface oxidation is inhibited when the concentration of capping agent (citrate) is increased, which blocks oxidation sites and may slightly decrease $[\text{H}^+]$, and when suspensions are purged with nitrogen, which limits oxygen availability (Elzey and Grassian 2009; Liu and Hurt 2010). Consistent with this assertion, virtually no SPR absorbance degradation (surface oxidation) was observed when suspensions were maintained with high σ values (excess citrate) and higher AgNP concentrations (2.88 mg L^{-1} and above) in the dark (Fig. 3). Similarly, in the dark, the nitrogen

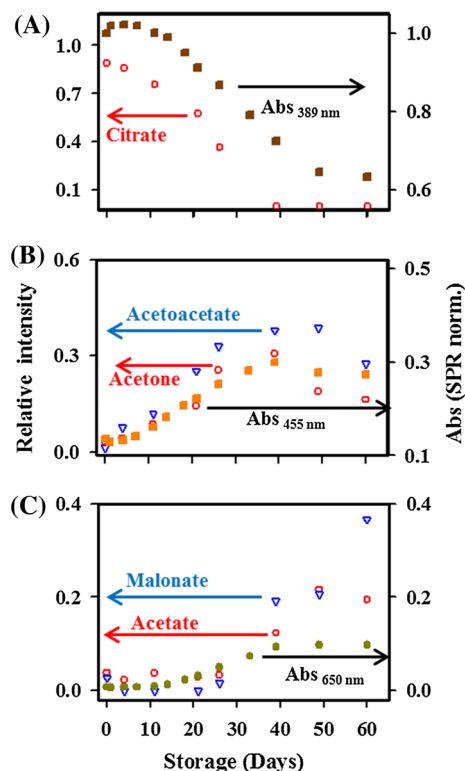


Fig. 7 Plots of the $^1\text{H-NMR}$ detected compounds associated with citrate and its degradation products as well as the different UV–Vis spectral features associated with AgNP transformations over the first 60 days of storage for the 10.8 mg L^{-1} AgNP suspensions with a 0.87 relative citrate concentration, exposed to light and purged with nitrogen. **a** The citrate intensity and the absorbance at 389 nm, **b** the acetoacetate and acetone intensities and the 455 nm absorbance, and **c** the malonate and acetate intensities and the 650 nm absorbance

gas-purged suspensions maintained suspension integrity for longer than air-purged samples in most cases (Fig. 3, SI Figs. S5, S8). Regardless, many of the nitrogen-purged suspensions ultimately lost integrity. It is important to note, however, that the nitrogen-purged suspensions are not claimed to be oxygen free, merely oxygen deficient.

In the absence of light and independent of gas purging conditions, oxidation (with subsequent dissolution) is most responsible for the overall integrity loss of the AgNP suspension. These processes should continue until: (A) all metallic AgNPs are oxidized, (B) all species related to surface oxidation (O_2 , $[\text{H}^+]$) are consumed from solution, and/or (C) a low solubility surface layer is formed and inhibits additional oxidation through the “bulk” of the NP. Since

the SPR absorbance is present after 104 days of storage (Fig. 3) and has slowed in its rate of degradation, it appears that oxidation and/or aggregation of all metallic AgNPs (option A) does not occur to completion within the time frame of the current study. Option B, the consumption of all oxidizing species, is unlikely since all stored suspensions were purged after each sample was removed, providing a renewable source of oxidizing species. The consumption of protons and slowing down the reaction, however, is a possibility, as has been previously demonstrated (Zhang et al. 2011). Therefore, if the process at 104 days has been completed, then by elimination, the most probable explanation is the formation of a low solubility surface layer (option C). However, if oxidative dissolution at 104 days has slowed down due to proton depletion and the rate of SPR loss is undetectable by UV–Vis, then option B becomes a possibility.

In the presence of laboratory lighting, however, the combination of AgNPs, excess sodium citrate and newly oxidized species (Ag^+) allows additional transformations to proceed. Photo-reduction of dissolved silver species by citrate is the second transformation process (Fig. 8, step 2) and leads to the further degradation of the AgNP suspensions. It has been observed that Ag^+ interacts with citrate to form Ag^0 and acetoacetate when exposed to 150 W halogen lamps (Xue et al. 2008) or when heated by microwaves (McClary et al. 2013). Indeed, the presence of acetoacetate in the present study (Figs. 6, 7) suggests that Ag^+ is undergoing photo-reduction to metallic silver in a similar manner. We postulate that the newly formed metallic silver could become part of the AgNP suspension by at least two mechanisms: (1) the reduced silver atoms can become part of an existing nanoparticle (re-deposition), thus altering the original AgNP’s size and/or shape, and/or (2) the silver atoms may nucleate and form new AgNPs (reductive nucleation).

Evidence for both pathways has been observed in the present study. For example, the increase in normalized SPR absorbance to values greater than 1.0 during the induction period strongly suggests a growing number of individual single AgNPs in suspension (SI Fig. S5E, S5F, S8E, and S8F). Reductive nucleation of new AgNPs with a diameter large enough to contribute to the SPR could explain the observed increase in Abs_{389} values. Long-term

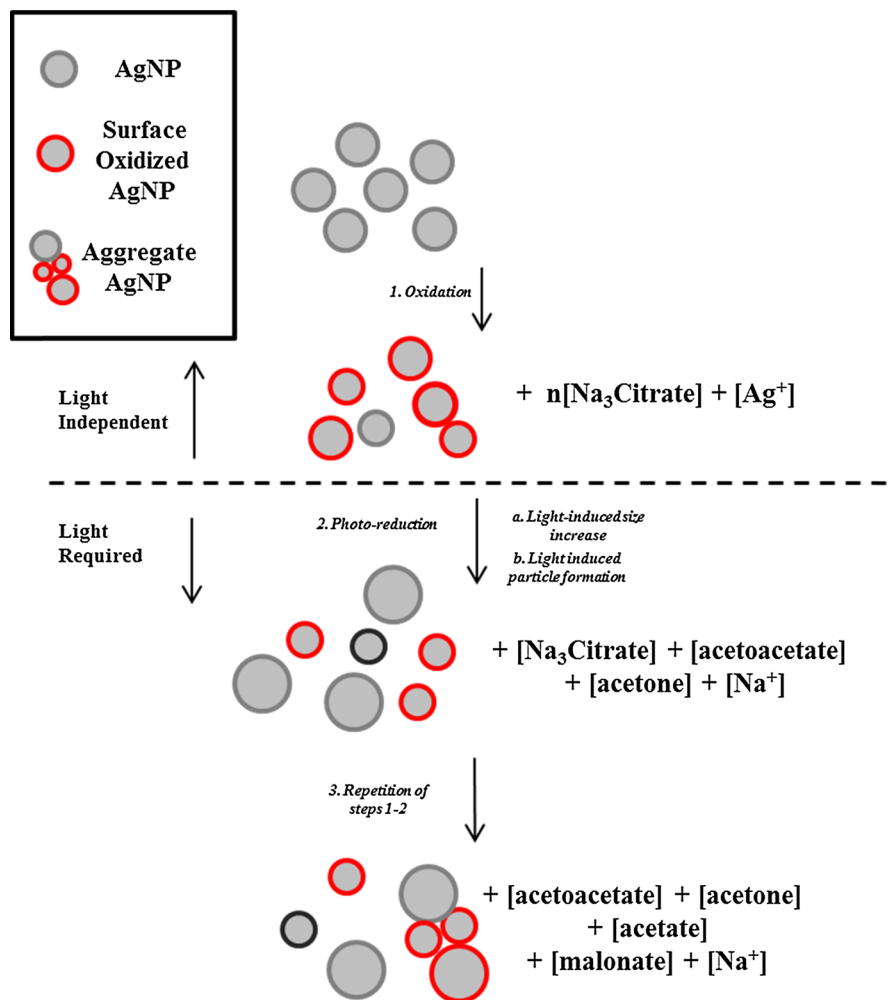


Fig. 8 Scheme of early destabilization of citrate-capped AgNPs suspensions. The postulated process occurs through the following steps: 1 Surface oxidation with or without light, a result of oxidative, solution phase species resulting in some dissolution. 2 Light required, photoreduction of the dissolved silver species with citrate reducing agent generating metallic silver and acetoacetate. Reduced silver atoms may deposit onto existing particles to increase particle diameter (a) or nucleate to

form new AgNPs (dark gray) (b). Additionally acetoacetate and acetone byproducts form from citrate. 3 After repeated oxidation followed by photoreduction steps, the citrate is fully consumed and there is a mixture of organic byproducts from citrate's degradation. At 104 days of storage there will be a mixture of small, single AgNPs, size enhanced AgNPs, and aggregated AgNPs in suspension

exposure to light, however, resulted in increases of both Abs_{455} (Fig. S11, S13) and D_H (Fig. 4). Both of these observations can be explained by an increase in the AgNP particle diameter or shape as a result of re-deposition.

Furthermore, since citrate is essential in photoreduction, one would expect the greatest size changes to occur in the AgNP suspensions stored in the light with the highest σ values. Consistent with this hypothesis, we observed that AgNP suspensions exhibited a

greater size increase at the highest σ values when stored at constant silver concentration and in the light (Fig. 4a, b). As this storage time increases, the oxidation, dissolution, and reduction cycle of the stored AgNPs continue until eventually all of the available citrate is consumed (Fig. 8, step 3) at which point, the total single particle stability can no longer be maintained. For example, when approximately 50 % of the citrate concentration is lost within 14 days storage ($10.8 \text{ mg}_{\text{Ag}} \text{ L}^{-1}$, σ value = 0.87, light,

nitrogen purge), SPR decay and aggregation commence (Fig. 7, SI Figs. S5, S8). SPR decay (Abs_{389}), size increase/shape change (Abs_{455}), and aggregation (Abs_{650}) continue as the citrate is degraded. As citrate degrades, four degradation products were detected during the photoreduction of Ag(I) species from stored AgNP suspensions in the light. Specifically, acetoacetate, acetone, acetate, and malonate were identified and confirmed with control compounds (Figs. 6, 7, SI Fig. S15). While acetone is a known decomposition product of acetoacetate (Hay and Bond 1967), the mechanism of formation for the other two compounds is currently unclear. What is clear, however, is that these organic molecules are not effective electrostatic stabilizers. Consequently, citrate-capped AgNP suspensions stored in the light for long periods will undergo some aggregation and contain little of the original capping agent.

Clearly, the products of the storage of citrate-capped AgNP suspensions are varied and depend greatly on the conditions under which they are stored. Indeed, all environmental storage conditions appeared to retain greater integrity at greater silver concentrations suggesting that low concentration storage is a poor strategy. Surface oxidation followed by dissolution for all conditions appeared to be the most probable mechanism, although the degree to which this mechanism took place varied. When stored in the dark, all techniques utilized suggest that overall integrity of the AgNP suspension was maintained best at high AgNP concentrations and high σ values; nitrogen purging appeared to provide some additional benefits as well (Figs. 3, 4).

When exposed to light, however, overall integrity typically decreased when compared to equivalent conditions in the dark, although multiple factors contributed to the AgNP suspension integrity. Specifically, short storage times (<15 days) resulted in minimal optical absorbance transformations (little change to Abs_{389} values) yet led to major changes in the state of the citrate capping agent (Figs. 6, 7). It was only following long exposures to light that all techniques recorded large changes to average particle size, stability, and oxidation (Figs. 3, 4, 5, 6, 7, SI Figs. 2–12, 14). This study highlights the importance of using multiple techniques when assessing the overall integrity of AgNP suspensions, employing proper storage conditions throughout the entire experimental period, and identifies a need for better reporting of storage conditions.

Conclusions

Based on the results of conditions examined in this study, the following general storage recommendations for maximizing the overall integrity of citrate-capped AgNP suspensions are as follows: (in order of importance):

- (1) Maintain high AgNP concentrations.
- (2) Ensure that suspensions are kept in the dark.
- (3) Elevate σ values.
- (4) Purge with nitrogen gas (most applicable for storage >30 days).

Additional recommendations for utilizing any AgNP suspensions, or any reactive NP suspension, in research studies can also be made. These include the following:

- Employ multiple analytical techniques providing different information to adequately characterize the synthesized suspensions and the initially prepared stock suspensions that may include optical, size, and chemical information (e.g., UV–Vis, DLS, Ag⁺ tests, ¹H-NMR, and/or other techniques).
- Utilize the same techniques to assess the overall integrity of the AgNP suspensions over the duration of a research project.
- Report the integrity or instability in published findings.

Following these recommendations can be quite helpful in determining the consistency of integrity for AgNP stock solutions used throughout the course of an experiment. The benefits of “high throughput” techniques include easy access to instrumentation, rapid acquisition of data, and feasible interpretation of results. Tracking characterization parameters using a periodic mentoring approach allows for the identification of material transformations at the instance they occur.

Supporting information

In the SI, there are additional initial characterization techniques available for secondary electron microscopy, energy dispersive spectroscopy, and X-ray photoelectron spectroscopy. Many representative spectra are provided for each of the storage conditions tested. Additionally, full plots over the 104 days of storage are

provided for all 36 conditions for the Abs₃₈₉, Abs₄₅₅, and Abs₆₅₀. Further DLS data and DLS analysis information as well as another example of the Ag⁺ test are available along with representative NMR spectra of reference compounds.

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