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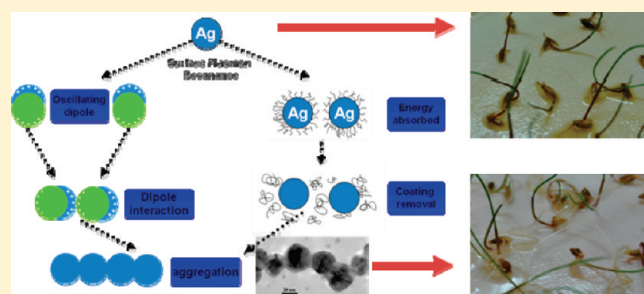
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# Toxicity Reduction of Polymer-Stabilized Silver Nanoparticles by Sunlight

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**ABSTRACT:** The environmental and health impacts of nanomaterials are becoming important topics of research in recent years. The unique advantages offered by these nanomaterials in wide range of applications cannot be realized until these concerns are resolved. Among all the nanomaterials, Ag nanoparticles, due to their existing extensive uses in commercial products, demand immediate attention. Since the nanoparticle suspensions will be exposed to environmental conditions different from a research lab setting, many factors, including light, temperature, salinity, etc., are suspected to affect the stability of the nanoparticle and also their toxicity. In this study, we examined the effect of sunlight on the stability and toxicity of 6 and 25 nm Ag nanoparticles coated with gum arabic (GA) and polyvinylpyrrolidone (PVP). Under sunlight irradiation, all of these nanoparticles irreversibly aggregated to different degrees depending on the surface coating. The UV content of the sunlight is identified to be the driving force of nanoparticle aggregation, and the strong oscillating dipole–dipole interaction is believed to be the origin of the destabilization. Toxicity examinations of the nanoparticles to a wetland plant, *Lolium multiflorum*, indicate that their toxicity is greatly reduced after sunlight irradiation.



## INTRODUCTION

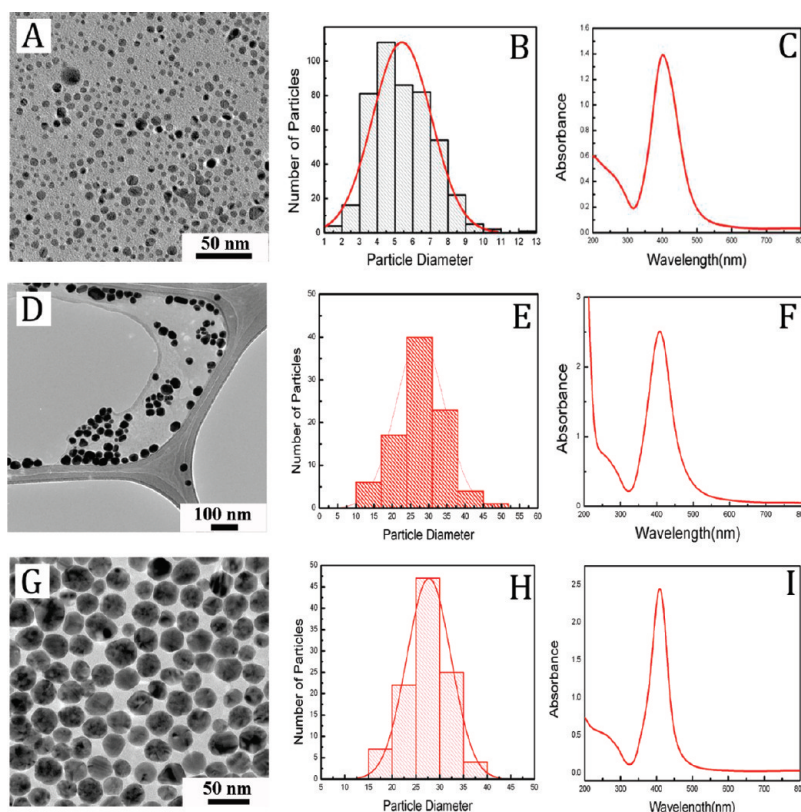
Nanoscale materials, usually defined as materials having at least one of their dimensions ranging from 1 to 100 nm, have attracted considerable interests both fundamentally and technologically because of their unique physical and chemical properties compared to their bulk counterparts.<sup>1</sup> Silver nanoparticles (Ag NPs), in particular, have outstanding properties in optical, catalysis, surface-enhanced Raman scattering (SERS), high thermal and electrical conductivity, and antibacterial applications.<sup>2–4</sup> Such properties have made them attractive in a range of applications and already incorporated in a variety of commercial products. To date, Ag NPs can be found in over 240 consumer products including clothes, personal care products, food and beverage containers, laundry additives, home appliances, baby products, and even food supplements.<sup>5</sup> Such widespread usages may increase the possibility of inadvertent release of the materials into the environment,<sup>6</sup> where little is known about their fate and toxicity. The very properties of Ag NPs that make them promising, such as high surface area, ultrasmall size, and antibacterial function, could lead to unanticipated health and environmental

threats.<sup>7</sup> As a result, assessing the exposure and potential impacts of nanomaterials on health and the environment is becoming an urgent and essential task for the research society.<sup>8</sup>

Toxicity of Ag NPs to a variety of model organisms, such as bacteria, algae, animal cells, and fish, has been examined by various research groups.<sup>9–13</sup> For example, Kim et al. reported that the growth of yeast and *Escherichia coli* was inhibited at low concentration of silver nanoparticle, whereas these growth-inhibitory effects on *Staphylococcus aureus* were only mild, indicating that the toxicity of Ag NPs to different bacteria is different.<sup>14</sup> While these studies presented a partial picture of Ag NPs toxicity to organisms, very little is known about their fate and behavior in the environment. The concern of the environmental impact of Ag NPs is primary considered as a water problem because in water they have a higher mobility and can be transported over longer distance and potentially interact with

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**Figure 1.** Set of images for silver nanoparticles: TEM image of the GA-coated 6 nm Ag NPs (A) and the corresponding particle size distribution, which shows that the particles were within  $6 \pm 5$  nm and the average size was 6 nm. Its UV–vis absorption spectrum (C) shows a single, narrow peak that around 400 nm, which also indicates that the nanoparticles were spherical in shape and monodispersed; TEM image (D) and particle size distribution (E) of GA-coated 25 nm Ag NPs that shows the particles were within  $25 \pm 4$  nm and the average size was 25 nm. TEM image (G) and particle size distribution (H) of PVP-coated 25 nm Ag NPs that shows the particles were within  $25 \pm 5$  nm, and the average particle size was 25 nm. The UV–vis absorption spectra of these two kinds of nanoparticles also indicate that they were monodispersed and uniform in size (F for the GA-coated 25 nm and I for the PVP-coated 25 nm Ag NPs).

more environmental targets.<sup>15</sup> The fate and behavior of the nanomaterials in the environment will be determined by their inherent properties (e.g., size, charge, reactivity) as well as properties acquired through physiochemical reactions in the environment (e.g., degree of aggregation, adsorption, and desorption of natural aquatic chemical species on the particle surface). These acquired properties are expected to vary widely between natural environments as a result of the inherent variation in ionic strength, light intensity, organic matter composition and concentration, and biological activity across ecosystems. Understanding the fate and behavior of nanoparticles is very important since their properties depend significantly on their morphology and size. For example, several recent studies have suggested that only particles with diameter smaller than 30 nm have nonbulk properties because particles below this size are characterized by excess energy at the surface and thermodynamically unstable.<sup>16,17</sup> In other words, any changes in the nanoparticle size or morphology under environmental conditions would potentially affect their toxicity.

The present study aims to examine how exposure to natural light affects the fate, behavior, and toxicity of Ag nanoparticles. Nanoparticles coated with polymer were selectively used due to their most prevalent uses in nanosilver products.<sup>15</sup> Initial experiments showed that Ag nanoparticle suspensions became unstable when left outdoors under sunlight. Control experiments performed on the same materials under ambient conditions in the

lab, UV-light irradiation, and heating ( $60\text{ }^{\circ}\text{C}$ ) showed that the instability is caused by the UV content from the sunlight. TEM and UV–vis absorption analysis of the nanoparticles before and after sunlight treatments were used to elucidate the possible mechanism of oscillating dipole–dipole interaction driving aggregation. The effects of size and coating of the starting nanoparticles were also examined by changing the size and coating of the particles. The toxicity of Ag NPs before and after irradiation was assessed by using a wetland plant *Lolium multiflorum* as a model. The results show that the toxicity of the Ag NPs is significantly reduced after the sunlight treatment.

## RESULTS AND DISCUSSION

The three kinds of silver nanoparticles (Ag NPs) used in this study were 6 and 25 nm gum arabic (GA)-coated Ag NPs and 25 nm PVP-coated Ag NPs. They were freshly prepared for each study and purified with an ultracentrifuge. A set of TEM images, particle size distributions, and UV–vis absorption spectra of the nanoparticles is presented in Figure 1. As can be seen from this figure, these nanoparticles are relatively monodispersed in size. Vials containing nanosilver suspensions of different sizes and coatings were sealed with parafilm, covered by inverted beakers, and placed under sunlight for sunlight stability studies (Figure 2).

What motivated this study is the observation of the instability of the Ag NP suspensions (Figure 3A). After 2 days of exposure



**Figure 2.** Experimental setup for the study of the effect of sunlight irradiation on the stability of silver nanoparticles. The experiments were conducted at the outside of French Family Science Center, Duke University, Durham, NC 27708, during May 2009 and September 2009.

to natural sunlight, Ag NP suspensions cleared, indicating that the nanoparticles had aggregated and precipitated to the bottom of the vial. For comparison, the same suspensions left in the lab have been found to be stable for months. To understand the source of the instability, we designed and performed a series of experiments. On the basis of the assumption that outdoor sunlight may potentially affect the stability of Ag NPs by high-energy UV-light irradiation, lower-energy vis–IR light irradiation, or the thermal energy, control experiments were performed under UV light irradiation, ambient indoor conditions, and heating conditions (60 °C). Figure 3 is a set of digital images of the suspensions and UV–vis absorption spectra of Ag NPs under these different conditions. A comparison of these digital images shown in this figure indicates that the effect of sunlight is similar to that of UV light, and this is particularly evident for the GA-coated Ag NPs: the nanoparticles under both conditions were unstable and gradually precipitated to the bottom of the vial, whereas those samples under ambient and heating conditions were stable. Moreover, this effect of sunlight is independent of nanoparticle concentration, as evidenced from Figure 3A by the four silver nanoparticles suspensions with concentrations of 100, 50, 25, and 12 ppm all became nearly transparent after 7 days under sunlight or UV light irradiation. The similarity of sunlight and UV light is also supported by the UV–vis absorption spectrum acquired after 7 days (Figure 3B). Under sunlight and UV light irradiation, the absorption peak of Ag NPs disappeared completely, but this peak was preserved for nanoparticles under ambient and heating conditions, although a slight decrease in absorbance was observed. The decrease might be caused by the gradual increase in average particle diameter due to the Ostwald ripening process. This process is temperature dependent<sup>18</sup> so a larger decrease was observed for particles under heating conditions.

The similarity of the effect of sunlight and UV light on PVP-coated nanoparticles is also evident from the UV–vis absorption spectrum (Figure 3D) even though the overall stability of the PVP-coated Ag NPs are better than the NPs coated with GA (Figure 3C). Under sunlight and UV light, a new absorption peak appeared at higher wavelength. The appearance of this new peak is an indication of the formation of aggregates.<sup>19</sup> The fact that the wavelength of this new peak is higher for nanoparticles under

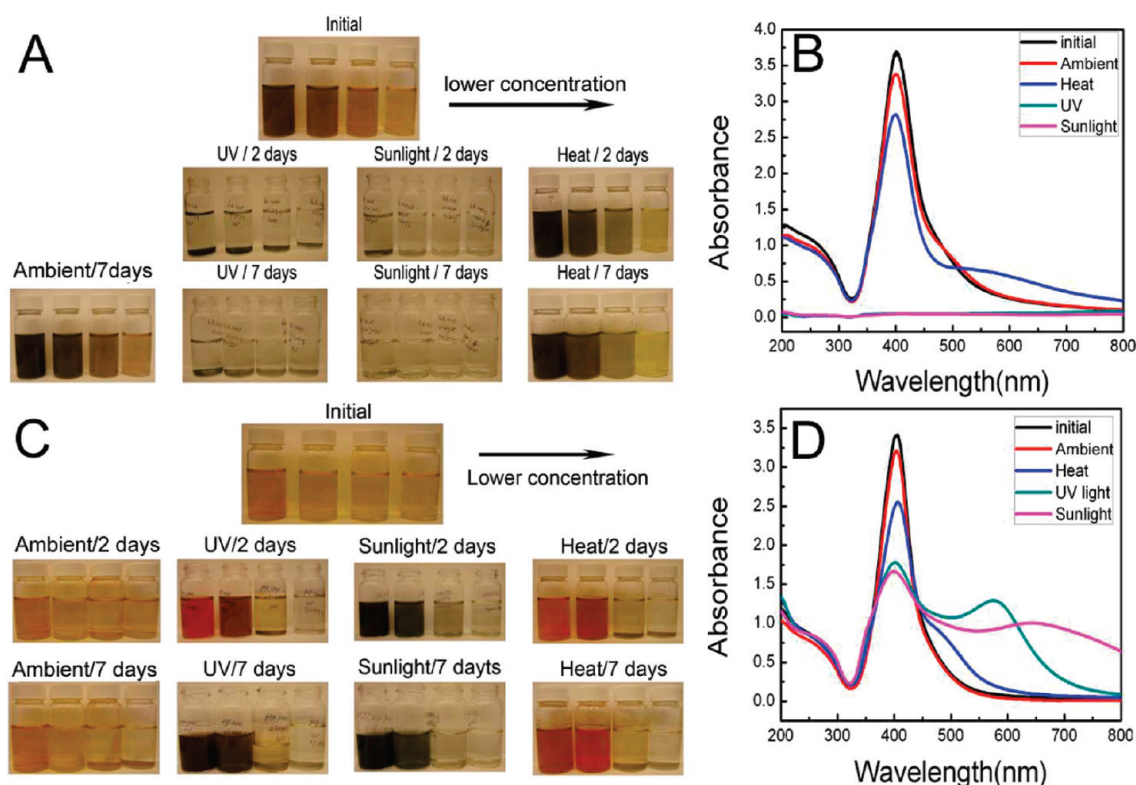
sunlight than under UV light is also understandable because the intensity of the UV light that we employed is much lower than the intensity of sunlight, and thus, nanoparticles under sunlight would experience a higher degree of aggregation. In contrast, there is no obvious change in peak position for nanoparticles under both heat and ambient conditions except for a slight decrease in intensity. Likewise, this slight decrease might be caused by the gradual increase in average particle diameter due to the Ostwald ripening process. On the basis of these experimental evidence, the origin of the effect of sunlight irradiation on the stability of Ag NPs appears from its high-energy UV light content.

However, the coating on the NPs clearly has a strong effect on their stability as demonstrated by the differences in Figure 3. In Figure 4, we further demonstrated the effect of coating by including more experimental data. For the PVP-coated Ag NPs, the new peak that appears in the UV–vis absorption spectrum treated under sunlight (Figure 4A), as discussed previously, indicates the formation of aggregates. Moreover, this new peak is red-shifted as a function of time of sunlight treatment, and this indicates the aggregate growth.<sup>19</sup> The formation of aggregates was also confirmed by TEM images taken after 7 days (C). From this TEM image, it is evident that the nanoparticles aggregated and linked together, forming “chain”-like nanostructures. Meanwhile, although the PVP-coated nanoparticles aggregated, the silver concentrations measured by ICP-AAS were almost constant (B). This means that the aggregates were stable in suspension. For the GA-coated nanoparticles, the absorbance peak at ~400 nm decreased dramatically and disappeared after 4 days (D). This indicates that the GA-coated nanoparticles were unstable and precipitated out. The TEM image (F) taken after 4 days shows that the nanoparticles were aggregated and formed much bigger particles. Furthermore, the change in silver concentration (E) also indicates precipitation of the aggregates with sunlight treatment. These results indicate that sunlight (UV) is capable of initiating and accelerating the aggregation of silver nanoparticles, but the stability of the aggregates depends on the coating. Both of the PVP-coated and GA-coated nanoparticles aggregated, but the aggregates formed from the former were stable in suspension.

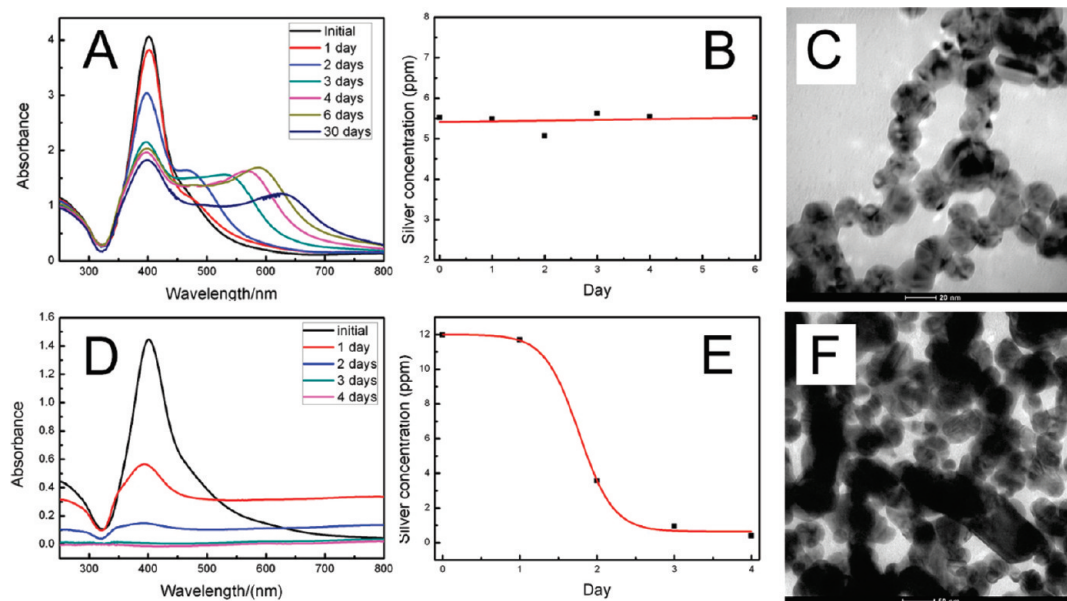
Clearly, the 25 nm GA-coated nanoparticles aggregated under sunlight (UV) irradiation, and the aggregates were unstable and precipitated out from solution. Similar results were also obtained from the 6 nm GA-coated nanoparticles. The UV–vis absorption spectrum shown in Figure 5 (left) indicates that with sunlight irradiation the characteristic absorption peak of Ag NPs also disappeared. Visual examination of the suspension showed that the silver nanoparticles also precipitated out from the solution, and the supernatant was clear after 2 days of sunlight treatment. TEM examination of the precipitates (Figure 5) also identified the formation of large aggregates. These results indicate that for the GA-coated nanoparticles the effect of sunlight (UV) on the nanoparticles is independent of particle size.

Silver nanomaterials possess unique and fascinating optical properties that arise from their nanoscale dimension. At this dimension, silver exhibits strong absorption in the UV and visible range of the spectrum. The origin of this absorption is attributed to the collective conduction band electron oscillation in response to the electrical field of the electromagnetic (EM) radiation of light. This property is termed as surface plasmon resonance.<sup>20</sup> By exciting the oscillation of electrons, energy is transferred from the incident light to the nanoparticles. The energy absorbed initially





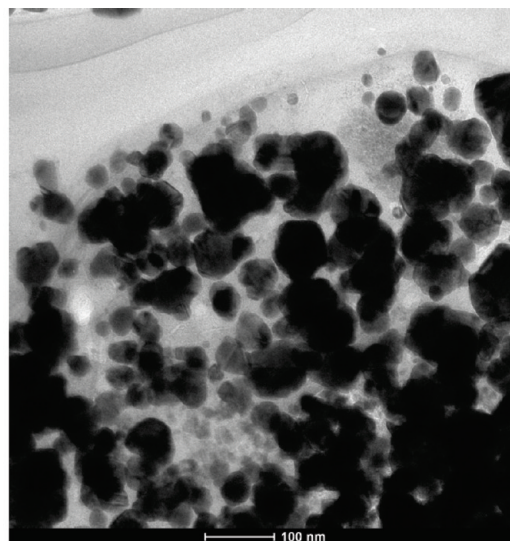
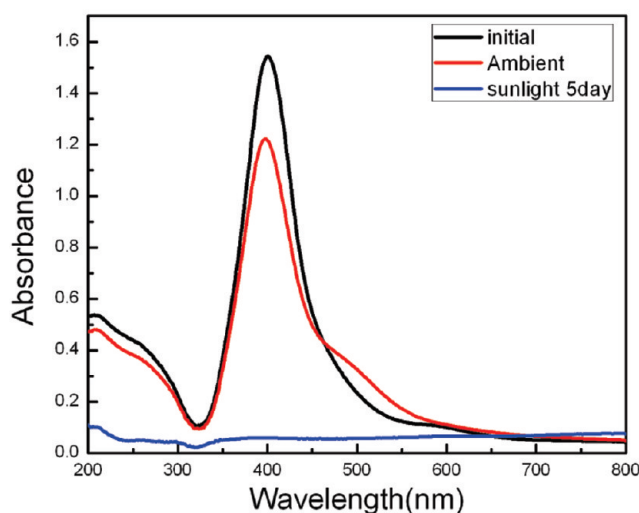
**Figure 3.** Digital images of silver nanoparticles suspensions (A: GA-coated 25 nm Ag NPs; C: PVP-coated 25 nm Ag NPs) under conditions as labeled after 2 and 7 days of exposure (concentration of silver from left to right: 100, 50, 25, and 12 ppm) and the corresponding UV–vis absorption spectra acquired after 7 days (B: GA-coated NPs; D: PVP-coated NPs). Note that the effects of sunlight and UV light are similar.



**Figure 4.** Evolution of (A) UV–vis absorption spectrum and (B) concentration of silver of PVP-coated Ag NPs under the irradiation of natural sunlight and (C) TEM images of nanoparticles after 7 days of irradiation. Evolution of (D) UV–vis absorption spectrum and (E) concentration of silver of GA-coated Ag NPs under the irradiation of natural sunlight and (F) TEM images of nanoparticles after 4 days of irradiation.

generates hot electrons but will quickly disperse through electron–electron scattering and electron–phonon coupling.<sup>21</sup> The created hot electrons have high energy and may be ejected to leave the nanoparticle positively charged and break up to form smaller particles if sufficient charge is accumulated.<sup>22</sup> Because of

this interaction between light and silver nanomaterial, light has been used extensively as a tool in nanomaterial fabrication. For example, light has been used to convert silver nanoparticles from spherical shape to triangular shapes<sup>23–25</sup> and in the synthesis of metal nanoparticles.<sup>26,27</sup>



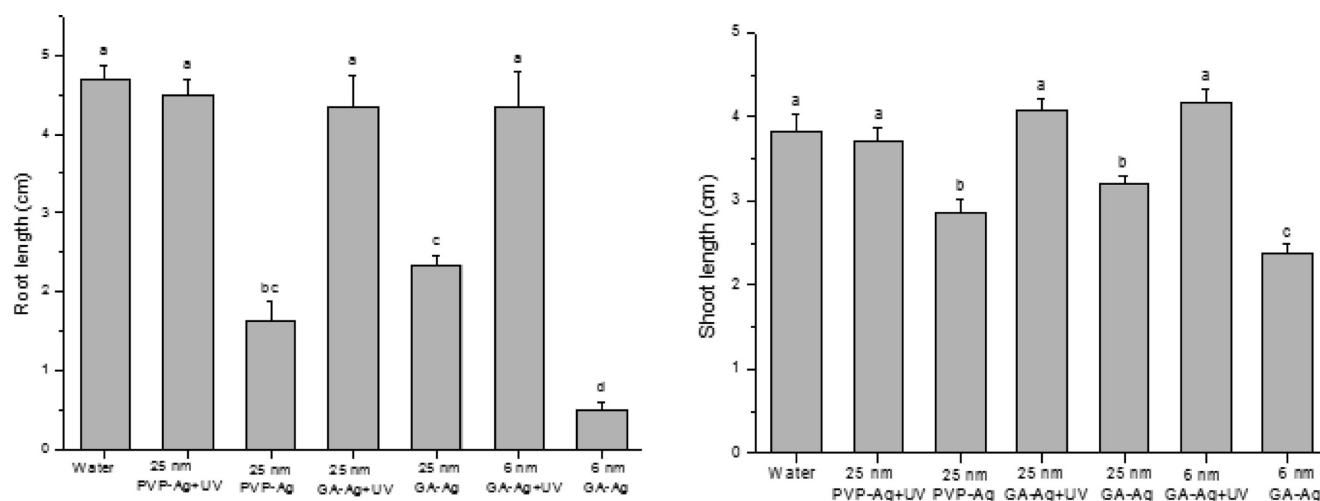
**Figure 5.** Comparison of UV–vis spectrum of 6 nm GA-coated Ag NPs under different conditions (left) and TEM image of nanoparticles after 5 days of sunlight irradiation (right).

The electron resonance condition for spherical silver nanoparticles is the irradiation of EM wave with wavelength around 400 nm.<sup>28</sup> This wavelength is close to the UV light of 365 nm that we used to induce the nanoparticle aggregation. So it is highly possible that the aggregation is driven by the electron resonance. Furthermore, the formation of the aggregates, especially for those with “chain”-like structure (Figure 4C), suggests that the understanding of the interparticle forces is important. One possibility of this force is the van der Waals force that is capable of driving the self-assembly of quantum dot. However, this force would not be the primary force for our system because first this force is estimated to be at the scale of  $0.5kT$  and thus too weak under ambient conditions and second if this force is the driven force for the aggregation, nanoparticles under heating conditions should have the highest degree of aggregation. Other short-range forces, such as hydrogen-bonding force from the polymer coating, are also inefficient in ordering nanoparticles over hundreds of nanometers. Given the fact that the aggregation is observed under sunlight (UV) irradiation, the origin of the interparticle interaction is likely brought by the oscillating of electron at resonant conditions. It is established that under resonance conditions the oscillating electrons result in a nonuniform surface charge distribution and create an oscillating dipole. The dipole–dipole interaction between particles is strong and long-range, capable of driving the formation of aggregates.<sup>29</sup> It is worth noting that the dipole–dipole interaction induced aggregation was also observed for CdTe nanoparticles.<sup>29</sup>

Additionally, the NPs are stabilized in their suspensions via the steric repulsion from the stabilizing molecules coated on their surface. Nanoparticles with sufficient coating are very stable, but they can self-assemble into 2-D nanostructures if the coating is partially removed.<sup>30</sup> In the present study, nanoparticles were purified by three times ultracentrifuge with nanopure water so that they were coated with minimum coating of GA or PVP. The hot electrons results from the strong coupling between the light and nanoparticles at the resonance frequency might transfer their energy to weaken the binding between the coating and the nanoparticle and may lead to partial removal of the coating. GA and PVP have different chemical properties, and thus they are likely having different affinity and binding energy to the

nanoparticles so they would have different susceptibility of removal. As a result, we hypothesized here that the effect of coating on the nanoparticle stability under sunlight could be understood as caused by the differences in their binding energy with the Ag NPs. PVP is a good stabilizer and has been widely used successfully in synthesizing silver nanomaterials with controllable size and shape<sup>31</sup> and is believed to bind strongly to the nanoparticles<sup>32</sup> while GA is rarely used as a stabilizer and might have weaker binding to the nanoparticle surface and thus presumably easier to remove from the NPs.

The aggregates of nanoparticles are suspected to behave differently in their toxicity from pristine nanoparticles.<sup>33</sup> For example, it has been found that increasing the aggregate size of fullerene is associated with decreasing antibacterial activity.<sup>34</sup> In this study, We assessed toxicity of each nanoparticle suspension by exposing seeds of the annual ryegrass (*Lolium multiflorum*) and examining alterations in seed germination and seedling growth relative between plants exposed to Ag NPs and control plants. Prior research<sup>35</sup> has documented significant declines in root and shoot elongation and seedling biomass in these plants when exposed to Ag NPs and higher concentrations of NPs have stronger toxicity effects. A comparison of the average lengths of root and shoot that grew from these seeds in different pristine and UV-light-treated nanoparticle media is summarized in Figure 6. An analysis of the results shown in this figure lead to the following conclusions: (1) Ag NPs exposure leads to greater reductions in root growth than to shoot growth; (2) the level of toxicity varied between Ag NPs solutions that differed in NP size and coating; (3) exposing Ag NPs to UV radiation significantly reduced their toxicity. Indeed, seedlings exposed to irradiated solutions grew to the same mass and length as control seedlings exposed to pure water. Roots of plants exposed to 6 nm GA-coated nanoparticles were the shortest, indicating that 6 nm GA-coated nanoparticles were more toxic than either of the larger 25 nm particles. For these larger Ag NPs, roots grown by plants exposed to 25 nm PVP-coated nanoparticles were significantly shorter than plants exposed to 25 nm GA-coated nanoparticles, indicating that toxicity is affected by particle coating as well as particle size. These observations clearly demonstrated that the existence of toxicity



**Figure 6.** Effect of Ag NPs with and without UV irradiation on the root and leaf length of *L. multiflorum*. Different letters show significant differences ( $P < 0.05$ ).

from Ag NPs toward our model system and the toxicity is greatly reduced by sunlight treatment.

Light microscopy observations of the seedlings revealed that the roots of seedlings exposed to unirradiated AgNPs suspensions did not produce the extensive root hairs observed in control seedlings (Figure 7). Seedlings exposed to UV-irradiated nanoparticles suspensions developed the same number of root hairs as control seedlings; however, our morphological examination revealed subtle evidence of toxicity of these solutions. Although the roots that grew in media with UV-irradiated nanoparticles have comparable length with those grown in water, we discovered that the seedlings grew their roots up away from the growth surface rather than displaying normal geotaxis (Supporting Information). Negative geotactic root growth is a commonly observed indication of toxicity and suggests that either the aggregated nanoparticles or dissolved silver released from these aggregates is affecting plant growth. To elucidate the source of toxicity, we removed the aggregated particles by ultracentrifuge and examined the toxicity of the supernatant. Seedlings exposed to the supernatant alone were identical to controls in morphology leading us to infer that this subtle toxicity is caused by Ag NP aggregates. In addition, the lengths of the roots and the growth form of the roots in all three UV-irradiated particles were very similar (Figure 6). This indicates that the aggregation of Ag NPs reduces the inherent variation in their toxicity. These observations clearly demonstrate that the existence of toxicity from Ag NPs toward our model system and the toxicity is greatly reduced by sunlight treatment. However, the aggregated NPs might still have a different kind of toxicity through a different mechanism. More work is currently underway to understand such mechanisms.

## CONCLUSION

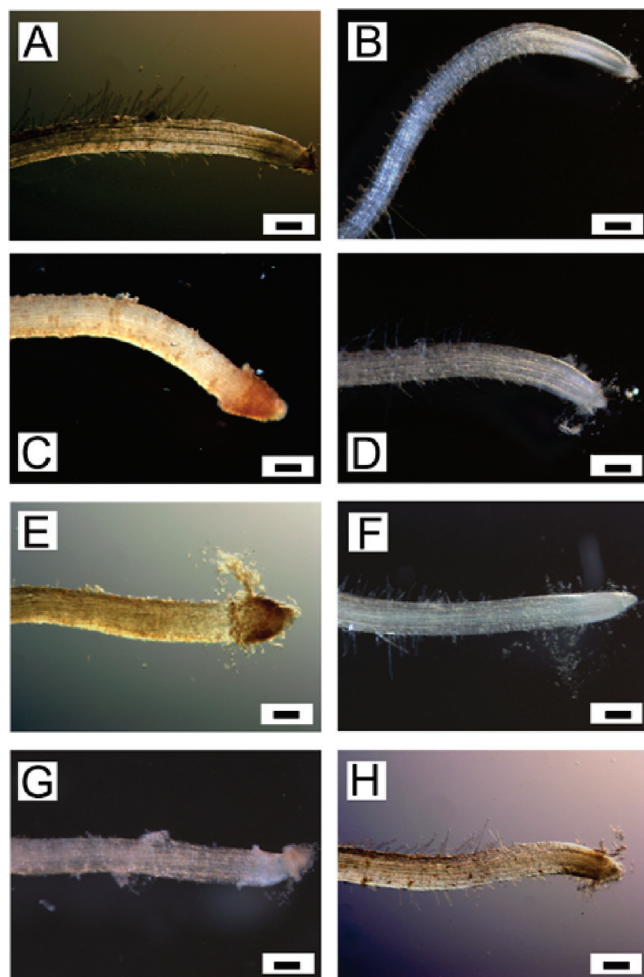
In conclusion, we found that Ag NPs undergo aggregation under sunlight irradiation and the toxicity of the Ag NPs is significantly reduced by sunlight. We identified that exposure to UV light is sufficient to induce aggregation and proposed that the strong oscillating dipole–dipole interaction is the origin. The aggregates that formed from the PVP-coated nanoparticles were stable in solution while those from the GA-coated nanoparticles were unstable. Incubation of the seeds of *L. multiflorum* with

nanoparticles before and after UV-irradiation indicates that the nanoparticle toxicity is significantly reduced by UV-light irradiation. However, there are also indications that even the aggregated Ag NPs lead to altered growth forms but probably through a different mechanism. Our results suggest that the light environment into which nanoparticles are released may be as important a predictor of their fate and toxicity as inherent differences in their size, coating, and reactivity. Given the fact that GA is a natural and relatively nonhazardous chemical and GA-coated particles are more sensitive to natural sunlight in that they undergo aggregation and precipitation, GA might be an excellent candidate as nanosilver stabilizer toward its commercialization. Alternatively, our results might be also helpful for large-scale silver nanoparticles waste treatment by using UV light to induce their aggregation and precipitation.

## METHODS

**Materials.** The three kinds of silver nanoparticles (Ag NPs) used in this study were 6 and 25 nm gum arabic (GA)-coated Ag NPs and 25 nm PVP-coated Ag NPs. All of these nanoparticles were freshly prepared for each study. To prepare the 6 nm GA-coated Ag NPs, 15 mL of 0.08 M  $\text{NaBH}_4$  was quickly injected into the mixed solution of 270 mL of 0.3 g/L GA and 2 mM  $\text{AgNO}_3$  while under vigorous stirring. The reaction was allowed to proceed for 10 min before purification. The 25 nm GA-coated Ag NPs was prepared by injecting 1 mL of 1 M trisodium citrate aqueous solution into the boiling solution made by 10 mL of GA (10 g/L), 5 mL of  $\text{AgNO}_3$  (0.01 M), and 34 mL of nanopure water. The mixed solution was kept under vigorous stirring and boiling for 10 min purification. Both of these two sizes of GA-coated Ag NPs were separated from their synthesis solutions after dilution by water with the volume ratio of 1:10 by ultracentrifugation for 1 h (41K rpm for the 6 nm Ag NPs and 15K rpm for the 25 nm Ag NPs) and redispersed the precipitate in nanopure water by sonication. The centrifugation was repeated three times for sufficient purification. PVP-coated Ag NPs were prepared by the polyol method with slight modifications.<sup>36</sup> Typically, 20 g of PVP (MW 10K) was first dissolved in 75 mL of ethylene glycol, and to this solution 1.5 g of  $\text{AgNO}_3$  was added. After complete dissolution of silver nitrate was achieved, the





**Figure 7.** Typical optical microscopy images of roots grown in different media: (A) 50 ppm  $\text{AgNO}_3$ ; (B) nanopure water; (C) 6 nm 50 ppm GA-coated Ag NPs; (D) 6 nm 50 ppm GA-coated Ag NPs after 3 days of UV light irradiation; (E) 25 nm 50 ppm GA-coated Ag NPs; (F) 25 nm 50 ppm GA-coated Ag NPs after 3 days of UV light irradiation; (G) 25 nm 50 ppm PVP-coated Ag NPs; (H) 25 nm 50 ppm PVP-coated Ag NPs after 3 days of UV light irradiation. Note that those grown in pristine nanoparticle media do not have root hair, but those grown in UV light treated particles have ordinary root hair. (The scale bars are all 200  $\mu\text{m}$ .)

system was heated up to 120  $^\circ\text{C}$  slowly at a rate of 1  $^\circ\text{C}/\text{min}$ , and the reaction was allowed to proceed for 24 h at 120  $^\circ\text{C}$ . To separate the Ag NPs, the growth solution was first dilute by water with a volume ratio of 1 to 10 and then centrifuged at 15K rpm for 30 min. The precipitates after each centrifugation were redispersed in nanopure water with 10 min of sonication and centrifuge again and repeated for three times before stability and toxicity studies.

**Characterization Techniques.** UV–vis absorption spectra were acquired from a Cary 500 scan UV–vis–NIR spectrophotometer (Varian, CA). The morphology and particle size distribution of nanoparticulates were determined using a Tecnai G<sup>2</sup> Twin transmission electron microscope (FEI, Hillsboro, OR). Samples for TEM were prepared by placing a small drop of the suspension on a carbon-coated copper grid, followed by natural evaporation of the solvent of water for 2 h. The silver nanoparticles concentration was measured with ICP-AAS by converting the silver nanoparticles to ionic silver using 5%  $\text{HNO}_3$ . Prior to

the ICP-AAS measurement, Ag NPs suspensions were diluted by nanopure water with the volume ratio of 1:10 (Ag NPs suspension: nanopure water) and then digested with  $\text{HNO}_3$  for at least 24 h. The microscopic images of the *L. multiflorum* roots were taken with a Zeiss Lumar V12 stereoscope.

**Experimental Design.** Glass vials made from potassium-free borosilicate glass were used as containers for the Ag NPs suspension. These vials are nearly transparent for light with wavelength longer than 330 nm (see Supporting Information). Vials containing nanosilver suspensions of different sizes and coatings were sealed with parafilm, covered by inverted beakers and placed under sunlight for sunlight stability studies (Figure 2). For the control experiments, vials were sealed with parafilm for UV irradiation and sealed with caps for heating and ambient conditions. A UV/cryo chamber that has a 365 nm UV light (EMS, 70445-10) was employed to study the stability of nanoparticles under UV light.

We assessed toxicity of each nanoparticle suspension by exposing seeds of the annual ryegrass (*Lolium multiflorum*) and examining alterations in seed germination and seedling growth relative between plants exposed to Ag NPs and control plants. In a typical experiment, 4 mL of 50 ppm silver nanoparticle suspensions was added in a Petri dish (100 mm  $\times$  15 mm) that has a filter paper at the bottom, and 25 seeds were then placed and distributed evenly on the filter paper. The Petri dishes were then covered and sealed with tape and kept under ambient conditions. Three replicate samples were conducted for each nanoparticle suspension tested. After 5 days of incubation, the length of the grown root and germinated shoot were measured, from which the average and standard deviation were calculated.

**Statistical Analysis.** All errors are expressed as standard deviations (SD). Differences between treatments for the different measured variables were tested using one-way ANOVA (SPSS 13.0.1 for Windows), followed by Tukey HSD tests when differences significant at  $p < 0.05$  were found.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Transmission spectrum of the glass vial that was used in this study; images of roots grown in media containing UV-treated particles and the corresponding supernatants. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review, and no official endorsement should be inferred.

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