Sub-Monolayer Silver Loss from Large Gold Nanospheres Detected by Surface Plasmon Resonance in the Sigmoidal Region

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Abstract

Nanosilver becomes labile upon entering the human body or the environment. This lability creates silver species with antimicrobial properties that make nanosilver attractive as active components in many consumer products, wound dressings, and agricultural applications. Because lability depends strongly on morphology, it is imperative to use a material with constant lability throughout kinetic studies so that accurate lability data can be acquired with efficient detection. Here 2.5 nm thick Ag was coated onto on 90-nm diameter gold nanosphere cores and this surface silver layer was gradually removed by either chemical or X-ray radiation etching. The most sensitive region of a sigmoidal surface plasmon resonance (SPR) response as a function of silver thickness was found for the first time between 0.9- and 1.6-nm thick silver, revealing a new nanosilver standard for lability studies. The SPR peak position detection sensitivity is 8 nm (SPR peak shift)/nm (silver thickness change) within this steepest region of

the plasmon response curve whereas outside sensitivity drops to 1 nm/nm. Since the centroid of SPR profiles can be discerned with 0.25 nm precision, the 8-nm/nm sensitivity means it is possible to detect a 0.3-angstrom or sub-monolayer change in silver thickness. The SPR response simulated by discrete dipole approximation (DDA) was an identical sigmoidal function between 0 and 2 nm of silver coating. These findings were supported by several other analytical measurements, which confirmed no silver recoating during these etching processes.

Keywords: nanosilver; surface silver; silver gold core-shell nanoparticles; surface plasmon resonance (SPR); sigmoidal response; SPR-thickness response; SPR sensing; exponential phase.

Introduction

Silver nanoparticles are generally stable in isolated anaerobic conditions,¹ but upon entering an oxidizing-reducing chemical environment such as the human body, nanosilver is expected to dissolve into silver ions or evolve into small silver nanoparticles (AgNPs).² Silver ions and possibly small AgNPs are toxic and give rise to well-known antimicrobial properties found in many applications such as filter papers for clean water in developing countries and wound dressings.³ To maximize the known antimicrobial capabilities, nanosilver in the form of sprays, liquids, creams, and textiles/fabrics have been developed and used for efficient delivery of large quantities of silver. Due to increasing human exposure and given the differences in toxicity between isolated nanosilver and its labile derivatives, evaluating the lability of nanosilver in different environments is critical.⁴

The difficulty in studying the lability of nanosilver lies in the fact that the lability is highly dependent on nanoparticle morphology (including nanoparticle size and shape). Specifically, the lability usually does not remain constant as surface silver is removed from nanosilver. This

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4 lability-size dependency can be attributed to the surface area-to-volume ratio dependency of
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6 size.⁵ Other factors such as lower coordination numbers for surface silver atoms in progressively
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8 smaller nanosilver may also play a role. It has been shown that as particle size decreases, the
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10 increase in surface free energy causes a decrease in reduction potential, making smaller silver
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12 nanoparticles more easily oxidized.⁶ Morphology has been seen to affect silver loss from
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14 nanosilver as silver near sharp edges and corners or in polycrystalline nanosilver is etched away
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16 more rapidly.^{2,7,8} Hence, silver lability studies require preparing special nanosilver whose lability
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18 is uniform across the whole surface and does not change over a certain size span of interest. In
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20 order to mitigate morphologically variable lability of pure nanosilver, thin silver on a large
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22 spherical substrate is used to warrant the same surface chemistry throughout the entire silver
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24 removal process.
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31 The second complexity is that the loss of surface silver from nanosilver depends on the
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33 environment.^{4,9-12} Real environments such as those in the human body or soil are difficult to
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35 simulate because they contain a complex mix of oxidizing and reducing reagents. A simple, well-
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37 controlled oxidizing or irradiation environment is preferred to benchmark the loss of silver. In
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39 addition, recoating silver species onto the original nanosilver should be avoided.
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43 Finally, consideration must be given to the analytical methods so that silver loss from
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45 nanosilver can be effectively detected. Many existing analytical methods or those being
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47 developed have been used to track silver migration and especially the loss of silver from the
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49 original nanosilver, although most of which require sample purification prior to detection. For
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51 instance, C19 reversed-phase capillary liquid chromatography coupled to diode array detection
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53 and in-tube solid-phase micro-extraction have been used to purify and detect nanosilver.^{13,14}
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58 Mass spectrometry has also been used to investigate the surface of silver nanoparticles¹⁵ or even
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4 of a single silver nanoparticle.^{16,17} Microscopic methods such as TEM, electron tomography, and
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6 acoustic vibrations also require careful purification of samples.^{15,18-20} In contrast, spectroscopic
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8 measurements such as surface plasmon resonance (SPR) measurements may be used to
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10 accurately characterize nanosilver,^{10,21-25} although to date their application to surface silver loss
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12 determination has been limited.^{19,23}
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16 These considerations make evident the importance of developing a holistic approach with
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18 which the loss of surface silver from nanosilver can be conveniently and fiducially detected in
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20 controlled environments without the burden of excessive purification. Detection methods to be
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22 used or developed must be combined with synthetic approaches to completely determine the loss
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24 of silver from the surface of primary nanosilver, making it possible to isolate, identify and
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26 accurately measure silver loss from nanosilver and help elucidate the true sources and extent of
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28 nanosilver toxicity.
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33 Here the study of surface silver loss from nanosilver was made possible by using large
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35 spherical gold substrates coated with thin layers of silver. The spherical symmetry guarantees
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37 that all the surface silver of the same thickness are chemically equivalent and the large gold base
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39 (100 nm diameter) with *thin* (1-2 nm) silver layers ascertain silver at different thicknesses behave
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41 the same chemically. Moreover, using thin layers of silver on large gold spherical nanoparticles
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43 permits quantitative and convenient detection of silver loss without sample purification. This is
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45 possible because the SPR peak position of the specially prepared thin silver layers on large gold
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47 nanospheres (AuNSs) follows a sigmoidal response as a function of silver thickness. This
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49 material will be referred to as sigmoid-silver coated gold nanospheres (S-AguNSs or S-Agu).
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51 The highest sensitivity occurs in the exponential phase of the sigmoid between 0.9 and 1.6 nm
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53 thick Ag on AuNSs. Although silver shell – gold core nanostructures with various silver
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4 thicknesses have been synthesized and studied theoretically in the past, dating back to the
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6 1950s,^{18,20,26-32} S-AguNSs have not been previously reported, experimentally or theoretically. To
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8 mimic real situations, we emulated the loss of surface silver using mild chemical etching and X-
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10 ray irradiation during which no recoating was observed. H₂O₂ was chosen as the etchant because
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12 it mildly mediates the removal of silver from S-Agu, and X-rays were used for their existence in
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14 the environment. Thin silver layers on gold nanorods (AuNRs) were studied as well, although
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16 only a minor sigmoid response was found with regard to silver layer thickness. We call this
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18 material S-AguNRs. Similarly, AguNRs have been made or studied theoretically,³³⁻³⁶ but S-
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20 AguNRs have not been noticed.

26 **Experimental and Theoretical Methods**

28 AuNSs and AuNRs were synthesized following the established seeded growth methods.^{37,38}
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30 Details to achieve reproducible SPR peak positions in the AuNS synthesis, regardless of scale,
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32 are given in the Supporting Information (SI). Protocols for stabilizing and coating silver onto
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34 these nanomaterials, removing silver in H₂O₂ (aq) and with X-ray irradiation, and inspection of
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36 these nanomaterials with several analytical methods including UV-Vis absorption spectroscopy,
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38 atomic absorption spectroscopy (AA), transmission electron microscopy (TEM), energy
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40 dispersive X-ray analysis (EDX) within the TEM, and X-ray absorption spectroscopy (XAS)
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42 using a synchrotron X-ray source are also given in the SI. S-AguNSs with two Ag thicknesses
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44 were made by adding 100 μ L and 200 μ L of AgNO₃ stock solution during coating of AuNSs.
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46 The products are called S-AguNS100 and S-AguNS200. Figure 1 is a schematic showing these
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48 steps. All chemicals were used as received without further purification. MilliQ (MQ) water was
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50 used throughout all experiments.
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Figure 1. Scheme for chemical and X-ray irradiation etching of silver from silver-coated gold nanospheres. 90-nm diameter AuNSs were grown step-wise from 16-nm Au seed nanoparticles. UV-Vis was used to monitor SPR progression and determine the amount of HAuCl_4 needed in the final growth step to achieve AuNSs with SPR at 561 nm (step I). Due to weak citrate stabilization for large AuNSs and ligand loss over purification cycles, AuNSs were coated, purified, and coated again with polyvinylpyrrolidone (PVP) to prevent aggregation during etching experiments (steps II and III). Ag was coated onto AuNSs (step IV), followed by H_2O_2 or X-ray irradiation etching (step V). Finally, the samples were analyzed (step VI).

The samples, including purified AuNSs and S-AguNSs as well as samples mixed with H_2O_2 (various concentrations, 30 min) or irradiated by X-rays, were first measured with UV-Vis absorption spectroscopy. For AgAuNRs, etched samples were not purified; they were measured *in situ* with UV-Vis, and etched continuously in 1 mM H_2O_2 solution for different times. SPR peak positions were obtained by fitting the UV-Vis spectra near the peak of the SPR profiles using an inverse parabolic function. This centroid fitting method can recognize a shift of 0.25 nm. Fittings

for the experimental SPR profiles had R values greater than 0.995 and for simulated SPR profiles the R values were generally greater than 0.998. The corresponding standard deviations for the centroid fitting were less than 0.1 nm. Therefore, the confidence level for being able to recognize a 0.25-nm shift was estimated to be higher than 95%. A fitting example is given in Figure SI-1, showing two SPR profiles whose peaks are separated by 0.25 nm. The SPR-H₂O₂ results were then fitted using a logistic function to produce the sigmoid curves as shown in Figure SI-3. All fittings were performed using OriginPro 8.5.

The centroids of the theoretical simulated SPR profiles for AgNSs or longitudinal SPR (LSPR) spectra for AgNRs were determined from the simulated extinction efficiency spectra using the discrete dipole approximation (DDA) method.³⁹ The open-source Fortran-90 software package (DDSCAT 7.3) was used to compute the extinction coefficient of AgNSs and AgNRs.⁴⁰ While classical Mie theory can be used for homogenous spheres, it is not recommended for computing thin silver coatings and therefore was not used here.⁴¹ A benchmark calculation result was obtained, which agreed with the results reported in the literature. The results are shown in Figure SI-2.

Results and Discussion

The main results given here reveal that SPR peak measurements are superior to TEM and other methods for determining the average thickness of thin silver coatings. For instance, the combined capability of SPR measurements and DDA simulation can differentiate a 0.25-nm SPR peak shift, which corresponds to a mere 0.03-nm Ag thickness change, a feat impossible to accomplish by TEM or other methods.

Figure 2A shows the SPR spectra of AuNSs, S-AgNS200 (purple line), as well as samples mixed with H₂O₂ of different concentrations (mM) for 30 min (light purple and black lines).

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4 TEM images of AuNSs and S-AguNS200 are shown in Figure 2B and 2C. The average AuNS
5 size is 103 ± 13 nm, with an average Ag coating thickness of $5.2 \text{ nm} \pm 2.1 \text{ nm}$ (100 samples
6 counted). Thin Ag layers (light grey) were easily recognizable in Figure 2C, and it is clear that
7 the coating is not uniform. Figure 2D shows the TEM image of S-AguNS200 after 0.175 mM
8 H_2O_2 mixing for 30 min. Silver layers were largely removed and the edge of the particles became
9 fuzzy, possibly due to alloying between the silver layer and gold substrate.

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19 Figure 2E shows the evolution of wavelength of centroids of SPR spectra of S-AguNS200
20 after the particles were mixed for 30 min with H_2O_2 of different concentrations, up to 0.2 mM.
21 Figure 2E also indicates the samples with the corresponding TEM images (C and D). The logistic
22 function (light grey line) fitted to the experimental data (round dots) is shown in Figure 2E. The
23 fitting parameters are shown in Figure SI-3.

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31 Although surface silver loss is a complicated process, it is known that the loss obeys the first-
32 order reaction rate law in either H_2O_2 or Ag if there is no recoating.⁴² For simplicity, silver on the
33 surface of Agu is considered to be removed or etched away by H_2O_2 , even though the real
34 mechanisms are more complicated than this simple picture. Therefore, for adequate
35 concentrations of H_2O_2 and a small amount of surface silver, the loss of surface silver for a fixed
36 mixing time is linearly proportional to the concentration of H_2O_2 . If this is the case, then the
37 sigmoidal response shown in Figure 2E cannot be caused by kinetics of etching but by the
38 thickness change of silver on AuNSs.

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51 Figure 2F shows similar results for S-AguNS100, which also exhibits an incomplete sigmoid.
52 These results suggest that S-AguNS100 has a thinner silver coating because the SPR response
53 does not reach the learning phase of the sigmoid. The saturation phase of the sigmoid for S-
54 AguNS100 is more completely realized in comparison to S-AguNS200. Figure 2E and 2F

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4 together paint a more complete sigmoidal curve (S-curve). The missing learning phase of the
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7 sigmoid shown in Figure 2F proves that the SPR response was not caused by etching kinetics;
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9 otherwise a similar learning phase would exist at low H_2O_2 concentrations. TEM images for S-
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11 AguNS100 and an etched sample are shown in Figure 2G and 2H, though the silver coating is
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13 not readily visible. These results show that TEM cannot differentiate Agu samples with slightly
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15 different etching conditions that SPR can, highlighting the benefit of using SPR over TEM
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21 The H_2O_2 concentrations used for removing silver on S-AguNS are shown in Figure 2E and
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23 2F. The results indicate that less than 0.1 mM H_2O_2 was needed to shift the SPR peak position of
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25 S-AguNS100 from 549 nm to 557 nm, covering nearly the entire exponential growth phase of the
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27 sigmoid. For S-AguNS200, the exponential growth phase also consumed 0.1 mM H_2O_2 , from
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29 0.07 mM to 0.17 mM, the same amount as in the S-AguNS100 case. This suggests that the
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31 thicknesses of silver contributing to the exponential growth phase of the two Agu samples are
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33 about the same, a result that is corroborated by the theoretical simulation shown below. Another
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35 way to compare the two cases is to calculate the rates of silver loss as a function of H_2O_2
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37 consumption, which are 100 nm/mM for S-AguNS200 and 80 nm/mM for S-AguNS100, again
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39 suggesting the two Agu materials share the same sigmoid.
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Figure 2. Results of AguNS characterization and sigmoidal SPR response of AguNSs as a function of silver loss. 2A) UV-Vis results of S-AguNS200. Three samples are shown: S-AguNS200 (dark purple), 0.1 mM H_2O_2 etched (30 min) S-AguNS200 (light purple), and 0.2 mM H_2O_2 etched (30 min) S-AguNS200 (black, identical to original AuNS spectrum). The SPR peak positions are shown with vertical lines. 2B) TEM image of AuNSs. 2C) TEM image of S-AguNS200. 2D) TEM image of etched S-AguNS200 with 0.175 mM H_2O_2 for 30 min. 2E) SPR measurements as a function of H_2O_2 concentration for S-AguNS200 (solid circles). Logistic fit is shown in grey. 2F) SPR measurements for H_2O_2 etching of S-AguNS100 (solid circles). Logistic fit is shown in purple. UV-Vis results of AuNS (black), S-AguNS100 (solid purple), and 0.05 mM H_2O_2 etched (30 min) S-AguNS100 (dotted purple) are shown in the inset. (2G) TEM image

of S-AguNS100. (2H) TEM image of S-AguNS100 after mixing with 0.1 mM H₂O₂. Scale bars are 100 nm.

The centroid peak positions of SPR spectra as a function of the amount of silver coating were predicted by DDA simulation. Figure 3 shows the DDA simulation results of the SPR peak position for S-AguNSs as a function of silver coating of up to 2.5 nm thick on five sizes of AuNS substrates. The results are shown in Figure 3A, and they corroborate the sigmoidal response found in Figures 2E and 2F. The shift of the SPR centroid in the exponential growth phase is most sensitive with an 8 (SPR shift)/nm (Ag coating) slope. This phase of SPR peak change covers a silver thickness range between 0.9 and 1.6 nm for all five different sized AuNS substrates. Outside the steepest region, the sensitivity drops to 1 nm/nm. Being able to determine the SPR peak position with 0.25 nm precision as shown in Figure SI-1 makes it possible to differentiate the average silver thickness with a 0.03-nm or 1/3rd of an angstrom resolution, which is impossible to achieve with conventional TEM. Such a thickness corresponds to a sub-monolayer of silver on large gold nanospheres.

Figure 3B compares the simulated data for 89 nm diameter S-AguNSs with the experimental data, highlighting the transition from the learning to saturation phase between 0.0-2.5 nm. The two sets of data are matched using least-squares fitting within the linear slope regions between 0.9 and 1.6 nm for DDA data and between 0.10 and 0.15 mM H₂O₂ for silver removal data. Because the centroids of SPR spectra respond to the thickness or amount of silver on AguNSs in an identical sigmoidal manner as the H₂O₂ concentration, we conclude that the amount of silver etched away is linearly proportional to the concentration of H₂O₂. Therefore, the sigmoid curves shown in Figure 2 are not caused by etching kinetics. These results indicate no recoating and suggest H₂O₂ etching is a convenient way to produce Agu of different silver thicknesses.

Figure 3C shows the SPR profiles of pure AgNSs (solid black line) and AuNSs (dot-dashed line), which are quite different from the AgAuNS curve (solid line). SPR responses of pure nanospheres do not possess a sigmoidal response. The sigmoidal transition in AgAu occurs within 2 nm Ag thickness because beyond 2-nm the surface dipole from Au quickly falls off.²¹ The SPR central peak wavelength shift for pure particles is gentler, of the order of less than 4 (nm/nm) and in the opposite direction, i.e., red shifting as the size increases. Figure 3D shows the whole SPR spectrum of the simulated S-AgAuNS sample, which agrees well with experimental measurements, again proving the validity of the theoretical simulation.

Figure 2 and 3 show that the loss of Ag is at a constant rate during the etching experiment even though the SPR peak position response is sigmoidal. This proves that Ag in the thin layer coated on large AuNSs bears constant reactivity or lability. The detection sensitivity is the highest within the exponential growth phase of the sigmoid. Even towards the point where all Ag being etched away, the rate of Ag loss is still the same, demonstrating the validity of S-AgAu100 being a nanosilver standard.

Figure 3. DDA simulations. 3A) DDA-simulated extinction efficiency results for AgNSs with five different base AuNS sizes: 70 (solid black), 88 (purple), 89 (dotted), 91 (gray) and 100 nm (long dash). 3B) Experimental SPR peak positions for S-AgNS200 overlaid with DDA simulation results for AgNS using 89 nm base AuNS. 3C) DDA simulation results for pure AgNS (solid black), pure AuNS (dot-dashed line), and AgNS using 88 nm base AuNS (purple). 3D) Experimental UV-Vis spectrum overlaid with DDA simulated extinction efficiency spectrum of AgNS100. The simulated AgNS100 was 88 nm core with 1.875 nm thick Ag. Other simulated results are given in Figure SI-5.

Several other analytical methods were employed to confirm the correlation between the simulated SPR peak position dependency on silver thickness and that on H₂O₂ concentration. Although each of these methods alone is not as accurate, comprehensive or convenient as the SPR measurement, together they help paint a similar picture of the whole silver removal process. Figure 4 shows the high resolution TEM (HRTEM), EDX and EXAFS results of chemical etching of S-AgNS100.

Figure 4A shows the HRTEM image of S-AgNS100, where the silver layers are clearly visible in the high-magnification image before etching. The dark field image shows the AuNSs in white color and coated silver layers in grey color. The average thickness of the Ag layers is 2.25 nm ± 0.77 nm. For S-AgNS100, the SPR peak position reveals a 1.5 nm thick Ag layer according to Figure 3B. Therefore, HRTEM results agree with the thickness determined by SPR measurements/calculations. After etching with 0.1 mM H₂O₂, the silver layers largely disappeared in the bright field image shown in Figure 4B. There is a small amount of small silver nanospheres (AgNSs) around the S-AgNSs/AuNSs after etching. These small AgNSs are not expected to be around AgNS when dispersed in solution during SPR measurements.

Figure 4C shows the EXAFS measurement results of S-AguNS100, and the fitting parameters are listed in Table 1. The average coordination number (CN) of 9.2 at 85 K obtained from this work suggests that the silver layer resembles very large silver nanoparticles.⁴³ The EXAFS results also suggest that silver in S-AguNS100 was largely metallic silver and there was little oxide. Figure 4D shows the EXAFS data of the etched S-AguNS100, and Table 1 shows the fitting parameters. After etching, the average coordination number decreased by 2 and Debye-Waller factor almost doubles compared with that of pre-etched Agu. This implies that after etching, silver layers are more disordered.

Figure 4E shows the EDX/TEM data of etching S-AguNS100. Because EDX is a sampling method, the results may be skewed unless large samples are counted. The EDX results show that there was 9.0 atomic percent (ap) of Ag in the as-made S-AguNS100 and 0.5 ap after 0.1 mM H₂O₂ etching. Using these percentages and the size of AuNSs, the silver thicknesses are calculated to be 1.3 nm and 0.10 nm on 89 nm diameter AuNSs. These values are in qualitative agreement with the results shown in Figures 2 and 3.

Figure 4F shows the AA data of etching S-AguNS200 with H₂O₂. The results show that silver content in the supernatant of S-AguNS200 increased from 3 ppm before adding H₂O₂ to 12 ppm after adding 0.1 mM H₂O₂. Dominating linearity shown in Figure 4F further proves that silver loss is a linear function of H₂O₂ concentration and at constant rate throughout the etching process, again proving the SPR sigmoid shown in Figure 2E is not caused by etching kinetics. The result also affirms that there is little recoating of silver onto S-AguNSs from silver ions in solution.

Figure 4. 4A) TEM image (scanning TEM (STEM) mode) of S-Agu100. 4B) S-AguNS100 after H_2O_2 etching and dilute purification. Scale bars are 20 nm. 4C) EXAFS data of S-AguNS100 in R space from theoretical fitting of $\chi(R)$. 4D) S-AguNS100 after H_2O_2 etching and purification. For both samples, the fit was performed over a k-space from 3 to 14 $\text{\AA}^{-1}$. The R space is from 1 to 6 $\text{\AA}$ and 1 to 4 $\text{\AA}$ for AgNS100 and after H_2O_2 etching and purification, respectively. 4E) EDX data showing the amount of silver on S-AguNS100 and the amount of silver remaining on S-AguNS100 after H_2O_2 etching and dilute purification. 4F) AA data showing linear increase of silver in the supernatant of the etched silver from AgNS200 samples as a function of H_2O_2 concentration.

Table 1. EXAFS fitting results. CN is the coordination number for the absorbing Ag atoms in S-AguNSs. R is the average bond length for the Ag-Ag/Au. σ^2 is the Debye–Waller factor. ΔE_0 is the inner potential correction. Ag foil was measured at room temperature whereas the other two samples were measured at 85 K. S_0^2 of 0.82 was derived from the Ag foil fitting and was used for fitting S-AguNSs. 2.88 Å was used as the reference value for Ag-Ag/Au bond length.

Edge	Sample	Shell	CN	$\sigma^2(\text{\AA}^2)$	E_0	R(Å)
Ag K-edge	Ag foil	Ag-Ag	10.0	0.00886	-2.77	2.85
Ag K-edge	Agu 100	Ag-Ag	9.24	0.00314	-0.54	2.87
Ag K-edge	0.1 mM CE	Ag-Ag	7.36	0.00609	-1.94	2.88

Figure 5 shows the effects of X-ray irradiation. X-ray irradiation of water produces reactive species, including oxidizing agents such as hydroxyl radical ($\bullet\text{OH}$) and H_2O_2 , as well as reducing agents such as superoxide radical and solvated electrons. Therefore, X-rays may etch away silver from AguNSs by H_2O_2 produced in water, or X-rays may reduce silver ions to AgNSs if solvated electrons or superoxide radicals are abundant. Figure 5A/B shows the TEM images of S-AguNS100 before and after 1000 Gy of X-ray irradiation. Figure 5C shows the SPR results after 1500 Gy irradiation. The SPR shift is only 5 nm, which is smaller compared with 0.1 mM H_2O_2 etching. For X-ray irradiation, H_2O_2 is produced from $\bullet\text{OH}$, and 1500 Gy X-ray irradiation is supposed to produce 0.1 mM H_2O_2 in pure water based on the G-values, although the presence of scavengers in solution may lower the actual peroxide concentration.⁴⁴ Figure 5D shows both the EDX (round spots and left Y axis) and AA (triangle spots and right Y axis) results. The EDX results show a linear region in which silver content on the surface of S-AguNS100 decreases from 8.5 to 6 ap when irradiated with up to 400 Gy of radiation. It also shows a clear plateau where silver on S-Agu decreased from 3 to 2 ap as radiation dose

increasing from 1000 Gy to 3000 Gy. AA results show a similar trend to that of H_2O_2 etching, with the supernatant detection of silver changing from 3 ppm without irradiation to 10 ppm after 1500 Gy of irradiation. The linear region of low dose irradiation shown in Figure 5D indicates that the etching process dominates at low X-ray doses, and it is unlikely any recoating of silver occurred below 1000 Gy based on results shown in Figures 5C and 5D. However, saturation of etching was observed at higher doses as shown in Figure 5D. This is probably because silver ions and small AgNPs can scavenge reactive oxygen species, reducing the amount of $\bullet\text{OH}$ and subsequently H_2O_2 .

Figure 5. X-ray irradiation etching results. 5A) TEM image of S-AguNS100 after 1 kGy irradiation etching (inset, S-AguNS100). 5B) TEM image of S-AguNS200 after 1 kGy irradiation etching (inset, S-AguNS200). The scale bars are 100 nm. 5C) SPR results for S-AguNS100 after X-ray irradiation. UV-Vis spectra for AuNSs (solid black), S-AguNS100 (dotted), and S-AguNS100 after 1500 Gy and dilute purification (solid purple) are shown in the inset. 5D) EDX results (round, left Y axis) for S-AguNS100 after X-ray irradiation etching. AA

shows Ag content in supernatant (triangle, right Y axis) as a function of dose to be linear through 1.5 kGy. Lines are fit to the data solely for guidance.

In addition to AuNSs, etching of silver from silver coated gold nanorods (AguNRs) was explored and the SPR response was recorded as a function of etching time with H_2O_2 *in situ* etching. The results are shown in Figure 6. Figures 6A and 6B show TEM images of gold nanorods (AuNRs) and AguNRs in which the silver layer is not easily seen. EDX results for silver coated gold nanorods are given in Figure SI-4. Figure 6C shows the centroid of longitudinal SPR (LSPR) spectra for AguNR100 and AguNR200 as a function of 1.0 mM H_2O_2 etching time. The inset in Figure 6C shows the measured SPR profiles for the AuNRs, AguNRs, and etched AguNRs. 1.0 mM H_2O_2 was used, which should be enough to remove all the silver on AguNRs if there is no recoating of silver ions back to the etched AguNRs. Because the AguNRs were not purified after etching and the concentration of AguNRs was high, silver ion concentrations towards the end of etching were high enough to cause scavenging and reduction of H_2O_2 . At short etching times, movement of the LSPR centroid is linear. The etching rate significantly slowed down after 20 minutes, which is believed to be caused by kinetics. DDA simulation was performed and Figure 6D shows the simulated SPR peak movement. In this case, no obvious S curve is observed, and hence the plateau observed in Figure 6C is caused by etching kinetics. Figure 6D also shows that slopes of the centroids of LSPR movement for pure AuNRs and AgNRs are similar as the sizes grow, although it takes 6 nm of silver thickness for the slope of AguNRs to reach those of the pure nanorods.

Figure 6E shows the calculated SPR response curve (round) in the region of 0-2 nm thick Ag together with the etching data (triangle). The logistic function parameters were given in the Figure SI-5. Upon careful examination of the AguNR DDA calculation results, a minor S

response is observed between 0 and 1.0 nm silver thickness region, as shown in Figure 6E. This sigmoid feature, which is heavily affected by the scavenging and recoating processes, was not observed experimentally. Future work is needed to prepare S-AguNR materials to allow the observation of this feature experimentally.

Figure 6. Etching results of AguNRs. 6A) TEM of gold nanorods (AuNRs). 6B) TEM image of silver coated gold nanorods (AguNR100). The scale bars are 100 nm. 6C) LSPR positions as a function of etch time in 1.0 mM H₂O₂ (aq) for AguNR100 (purple dots) and AguNR200 (black dots). The inset shows UV-Vis spectra of AuNRs (solid black line), AguNR200 (solid purple line), and two etched AguNRs samples at varied times. 6D) DDA simulated LSPR extinction efficiency for AuNRs, AgNRs and AguNRs. 6E) LSPR peak positions for 1 mM H₂O₂ in-situ etching of AguNRs overlaid with DDA simulated AguNRs. For the AguNR200 etching, the zero point of H₂O₂ concentration scale is calibrated using the SPR peak position. Both thickness and

concentration scales are kept the same except for the calibrated zero point. A minor sigmoid is seen in the theoretically simulated LSPR response between 0 and 1.0 nm Ag coating.

Both theoretical and experimental results in the literature have shown that SPR peak position depends on particle diameter, aspect ratio, shape, surface coating, surfactants, surface morphology, volume, coating of other nanoparticles, and aggregates of gold substrates.⁴⁵⁻⁵⁴ Sigmoidal responses were observed as a function of the coverage of proteins (in log scale) on gold particles deposited on flat substrates.⁵⁵ This is similar to the Langmuir-Freundlich isotherm curve,⁴⁷ which is not an intrinsic response of silver layer on gold substrate. Sigmoidal response of silver coating on gold substrate has not been reported.

The less-than-perfect match shown in Figure 3 between theory and experimental data may be caused by morphological distribution of silver species, which leads to SPR profiles deviating from parabolic.⁵² Figure SI-2 shows a small difference between the measured and simulated (using 89 nm dia. AuNSs) SPR profiles.

AguNRs are more intrinsically complex due to non-uniformity of silver surface atoms. For example, the rate for silver loss during etching along the cylindrical surface is different from the end.⁵⁶ Further investigation is needed to uncover whether this non-uniformity plays any role in determining the outcome of silver removal.

It is known that nanomaterials may enhance the X-ray effect.⁵⁷ However, enhanced production of reactive oxygen species due to absorbing gold nanoparticle cores (i.e. physical enhancement) is negligible in this work because the amount of gold is only 0.02 wp of water.⁵⁸ Furthermore, the large nanoparticle size employed means chemical enhancement is minimal since chemical enhancement requires large surface areas.⁵⁹

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4 The gold substrates and the Agu materials shown in this work have a 50-nm full width at half
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6 maximum (FWHM) SPR profile. The narrow spectral width facilitates determination of the peak
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8 position, and the 50-nm FWHM is among the narrowest.^{12,60} In addition, it is experimentally far
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10 more convenient to control the thickness of silver on Agu using H₂O₂ than to make silver-shell
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12 gold-core structures with different thin silver thickness.
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16 This work demonstrates that silver can be coated on and stripped from the gold base by dilute
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18 chemical etchant or X-ray radiation, both existing in nature. The amount of H₂O₂ generated from
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20 X-rays irradiating water is about 0.1 mM for 1500 Gy in pure water. Based on SPR peak shifts
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22 shown in Figure 5C, the shift was about 5 nm after 1500 Gy of X-ray irradiation. This shift
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24 corresponds to 0.06 mM H₂O₂ etching when cross calibrated using results shown in Figure 2F for
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26 S-AguNSs H₂O₂ etching. Because H₂O₂ is produced by •OH in X-ray irradiated water, and •OH
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28 can be scavenged by ligands, silver ions, and even Agu, X-ray irradiation etching of silver on
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30 Agu can still be understood as etching by a reduced amount of H₂O₂.
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36 It is important to point out that the results shown here do not contradict the alloying effect
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38 reported in the literature.⁵ As shown in Fig. 2F and Fig 3, SPR response for 0-1 nm silver coating
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40 is insensitive. It is possible that silver removal is slowed for the last nanometer of silver on the
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42 surface. This further proves that it is desirable to use only well-characterized S-Agu with 1-2 nm
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44 silver coatings for benchmark work.
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48 H₂O₂ has been seen both as an oxidizing or reducing agent, depending on conditions such as
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50 pH.^{4,22,61} Similar to other works,⁶²⁻⁶⁴ H₂O₂ in this work mainly acts as an oxidizing agent. This is
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52 not the case in AguNRs in which recoating occurred due to high silver ion concentrations.
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54 Ionizing radiation in the past has been mainly used as a reducing agent due to the large amount
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56 of silver ions in solution.⁶⁵ In this work, at least for dose below 1000 Gy, X-ray irradiation of S-
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Agu is purely an oxidization process, as shown in both the SPR and AA measurements. The results demonstrate the importance of choosing appropriate starting nanomaterials, detection methods and the etching environment to drive and monitor the desired surface chemistry.

Conclusions

A nanosilver standard with less than 2-nm thick silver coated on large spherical gold substrates has been found, which can be conveniently used to study the loss of surface silver from nanosilver. Such a standard carries fixed amounts of silver on fixed sizes of large gold substrates that can be detected on site using UV-Vis spectroscopy to obtain the SPR peak position and therefore the amount of silver on the S-Agu. For spherical gold nanoparticle substrates, a sigmoidal SPR response is found for the first time between 0-2.5 nm thick silver. The most sensitive detection of silver loss occurs between 0.9 and 1.6 nm thick silver, which can be detected with a sensitivity of a 0.03-nm average silver thickness change, corresponding to a 0.25-nm SPR peak shift. The loss of silver is found to be at constant rate throughout the silver loss process, hence suggesting uniform lability for all the silver in the thin layer. Gold substrates terminate the loss of silver. For gold nanorod substrates, the SPR response has a minor sigmoid between 0-1 nm thick silver. The approach of using thin silver coated large gold nanospheres and SPR measurements enables quick, affordable, and accurate quantification of near-surface silver without requiring purification and expensive/inaccessible instrumentation as is required for existing methods. Future work includes carrying out *in vitro* and *in vivo* studies as well as environmental experiments using this standard nanosilver.

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Graphical Abstract

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