Monitoring the Seed-Mediated Growth of Gold Nanoparticles Using \textit{in Situ} Second Harmonic Generation and Extinction Spectroscopy


Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, United States

\section*{Supporting Information}

\section*{ABSTRACT:} \textit{In situ} second harmonic generation (SHG) coupled with extinction spectroscopy is used for real-time monitoring of seed-mediated growth dynamics of colloidal citrate-stabilized gold nanoparticles in water. The time-dependent \textit{in situ} SHG results capture an early stage of the growth process where a large enhancement in the SHG signal is observed, which is attributed to the formation of plasmonic hot spots from a rough and uneven nanoparticle surface. The temporal peak in the SHG signal is followed by a decay that is fit to an exponential function to characterize the size-dependent nanoparticle growth lifetime, which varies from 0.45 to 1.7 min for final nanoparticle sizes of 66 and 94 nm, respectively. This early growth stage also corresponds to a broadening of the plasmon spectra, as monitored using time-dependent \textit{in situ} extinction spectroscopy. Over the course of the seed-mediated growth reaction, the nanoparticle becomes more thermodynamically stable through surface reconstruction resulting in a smoother, more uniform surface, corresponding to lower, stable SHG signals and narrower plasmon spectra. With real-time monitoring of nanoparticle formation, \textit{in situ} SHG spectroscopy combined with \textit{in situ} extinction spectroscopy provides an important insight for controlling nanoparticle synthesis and surface morphology for potential nanoscale engineering of different colloidal nanomaterials.

\section*{INTRODUCTION}

Colloidal gold nanoparticles have received considerable attention due to their potential applications in molecular sensing, photovoltaics, catalysis, imaging, nanomedicine, and photothermal therapy.\textsuperscript{1−14} These applications rely heavily on the localized surface plasmon resonance (LSPR) from coherent oscillations of free electrons that lead to optical field enhancements at the nanoparticle surface.\textsuperscript{15} The optical properties of gold nanoparticles can be tuned depending on the desired application by changing the nanoparticle size, shape, and surrounding medium.\textsuperscript{16,17} Varying the nanoparticle size causes only minor changes in the extinction spectra for gold nanospheres of diameters ranging from approximately 12 to 200 nm.\textsuperscript{13} However, varying the gold nanosphere shape by elongating it into a nanorod\textsuperscript{18} or by creating a bumpy “urchin-like” surface\textsuperscript{19} leads to pronounced changes in the associated optical properties. Therefore, developing synthetic protocols for greater control and tunability over the associated surface chemistry is critical for developing gold nanoparticle-based applications.\textsuperscript{15,16}

Citrate-stabilized gold nanoparticles prepared by the reduction of a gold chloride salt (HAuCl\textsubscript{4}) with sodium citrate (Na\textsubscript{3}C\textsubscript{6}H\textsubscript{5}O\textsubscript{7}) in water remains one of the most common synthetic methods for producing spherical gold nanoparticles.\textsuperscript{20} By varying reaction conditions, such as the citrate to gold salt ratio and the aqueous pH, it is possible to obtain gold nanoparticles ranging from 5 to 150 nm in diameter.\textsuperscript{21} However, the gold nanoparticle sample polydispersity is often very large with nonuniform surfaces when using this method to prepare nanoparticles larger than 30 nm in diameter.\textsuperscript{22,23} To gain better control over the nanoparticle size and shape distributions, an additional seed-mediated growth step can be used where additional gold salt is selectively reduced on the surface of the colloidal citrate-stabilized gold seeds using hydroquinone. This seed-mediated nanoparticle growth procedure results in spherical gold nanoparticles with sizes ranging from 50 to 200 nm while minimizing unwanted secondary nucleation for improved monodispersity and uniform surface morphologies.\textsuperscript{24}

Characterization techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) are excellent for studying nanoparticle size and shape distributions \textit{ex situ} and postsynthesis. However, monitoring nanoparticle surface formation \textit{in situ} can provide important additional insight into the growth dynamics for better control over surface and optical properties. The nucleation and growth of gold nanoparticles by citrate reduction in water have been studied using \textit{in situ} extinction spectroscopy\textsuperscript{25} as well as X-ray absorption near-edge spectroscopy (XANES) and small-angle X-ray scattering (SAXS),\textsuperscript{25} where the results indicate that the gold nanoparticle formation occurs by a four-step mechanism. Previous work has also used \textit{in situ} extinction spectroscopy combined with \textit{in situ} SAXS to monitor the growth of gold nanoparticles synthesized in
nanoparticles made of gold, silver, and gold−silver al−lloys. Additionally, gold nanoshells grown on silica microparticles have been studied using in situ nonlinear second-harmonic scattering (SHS) where the large SHS signal during the growth process is attributed to electric-field enhancements in gaps of incomplete or uneven nanoshells. Here, large nonlinear optical field enhancements are dependent on the metal nanoparticle shape as well as the surface roughness. For example, fundamental studies have investigated the SHG electric field enhancements from rough metal surfaces and sharp metal tips, both experimentally and theoretically, where the localized plasmon resonance contributes to the electrostatic “lightning-rod effect”. Another nonlinear spectroscopic technique using in situ four-wave mixing has been used to investigate growth lifetimes of gold nanoparticles in water using borohydride and citrate reduction. Obtaining real-time information about nanoparticle growth in situ poses a challenge in that the synthesis must be scaled to a suitable volume for sufficient optical detection which typically utilizes a specialized experimental setup.

Second-harmonic generation (SHG) is a noninvasive nonlinear spectroscopic technique in which two incident photons of frequency ω coherently add to produce a third photon of frequency 2ω. This process is forbidden in bulk media with a center of inversion symmetry. However, SHG is allowed at interfaces, making SHG a powerful tool for studying colloidal nanoparticle surfaces. Previous work has used SHG to investigate TiO₂ microparticles, liposomes, and metal nanoparticles made of gold, silver, and gold−silver alloys. Additionally, in situ SHS or SHG coupled with in situ extinction spectroscopy provides important information that can be used to characterize the nanoparticle growth dynamics for potential advances in colloidal nanoparticle engineering.

In this article, we demonstrate the versatility of in situ SHG and extinction spectroscopy by monitoring the seed-mediated growth process of gold nanoparticles in water under varying synthesis conditions. Changes in the SHG signal as a function of the reaction time are fit to exponential functions to obtain the associated growth lifetimes under different initial gold seed concentrations. The final nanoparticle sizes are determined using extinction spectroscopy and TEM measurements. The measured growth lifetimes depend on the final nanoparticle diameter where larger nanoparticles have corresponding longer growth lifetimes. By comparing the in situ SHG results with in situ extinction spectroscopy, an intermediate growth stage attributed to a rough, nonuniform nanoparticle surface is observed which is characterized by inhomogeneous spectral broadening followed by a narrowing and blue-shifting of the plasmon spectrum. This correlates with the in situ SHG results where a size-dependent maximum in the SHG signal is measured at early times and is attributed to a rough, bumpy surface during the initial nanoparticle growth process. Combining the in situ SHG and extinction spectroscopy results with the ex situ TEM and Mie theory analysis provides important information about the size-dependent reaction dynamics and the associated mechanism, which cannot be obtained from ex situ measurements alone. The versatility of in situ SHG measurements coupled with in situ extinction spectroscopy in combination with conventional ex situ techniques enables a wide range of investigations for studying nanoparticle growth and functionalization in real time for developing new nanomaterials and associated applications.

**EXPERIMENTAL SECTION**

**Gold Nanoparticle Synthesis.** The colloidal gold nanoparticle samples are prepared by a seeded-growth technique using hydroquinone (HQ) as the reducing agent. The gold seeds are prepared by adding 900 μL of 34 mM sodium citrate (Na₃C₆H₅O₇) to 30 mL of 290 μM gold tetrachloride (HAuCl₄) in water under boiling and vigorous stirring conditions. Five gold nanoparticle samples of different sizes are synthesized by adding 25, 30, 35, 40, and 50 μL of the prepared gold seeds to 25 μL of 29 mM HAuCl₄ which are all diluted to a final volume of 2.5 mL with ultrapure water. The addition of the reducing agents using 25 μL of 0.03 M HQ and 5 μL of 34 mM sodium citrate initiates the growth process. For each sample, the final solution is left to stir at room temperature for 60 min.

**In Situ Second Harmonic Generation and Extinction Spectroscopy.** The SHG setup uses an ultrafast oscillator laser, an optical setup, and a high-sensitivity spectroscopy detector, modified from our previous work. A titanium:sapphire oscillator laser produces 75 fs pulses centered at 800 nm with a repetition rate of 80 MHz and an average power of 2.7 W. The laser beam is attenuated to 600 mW and is focused into a 1 cm quartz cuvette containing the gold seeds, HAuCl₄, HQ, and sodium citrate in the various gold nanoparticle
reaction conditions. An optical filter is placed in front of the cuvette to remove any residual SHG prior to the sample. Another filter is placed after the sample to remove the fundamental light while transmitting the SHG, which is collected in the forward direction and refocused into a high-sensitivity charge-coupled device (CCD) detector coupled to a monochromator-spectrograph. This optical setup allows for the detection of the SHG signal as a function of time to capture nanoparticle reaction dynamics. At the same time, in situ extinction spectroscopy is measured using a low-intensity broadband tungsten filament lamp which is collimated using a pair of lenses and focused orthogonal to the SHG beam and into the quartz cuvette with the resulting spectra detected using a fiber-optic spectrometer detector. For each trial, the precursor gold seeds and HAuCl4 are added in ultrapure water for a baseline SHG measurement. At time zero \((t = 0)\), HQ and sodium citrate are added simultaneously to initiate the nanoparticle growth process. For each sample, 5 in situ SHG spectra and background are taken followed by 10 in situ extinction spectra at 0.5 and 1 s acquisition times, respectively, in repeating iterations to track the nanoparticle growth dynamics. A schematic diagram of the in situ SHG and extinction spectroscopy setup is shown in Figure S7 of the Supporting Information.

## RESULTS AND DISCUSSION

After the nanoparticle synthesis is completed, TEM images are acquired surveying \(~200\) nanoparticles for each nanoparticle sample. The nanoparticle sizes are measured and fit using a log-normal distribution to obtain the average nanoparticle diameter, as shown in the Supporting Information, for each sample. Figure 1 shows TEM images that are representative of each gold nanoparticle sample with sizes of 92.2 ± 4.0, 86.1 ± 4.3, 72.6 ± 5.3, 71.3 ± 4.8, and 65.6 ± 5.0 nm synthesized from 25, 30, 35, 40, and 50 \(\mu L\) of precursor gold seeds, respectively. The average nanoparticle size is also confirmed by fitting each final extinction spectrum using Mie theory with corresponding sizes of 94, 89, 76, 72, and 66 nm, respectively, which are within the standard deviations of the sizes measured from TEM. Additional TEM images from the other nanoparticle samples are shown in the Supporting Information.

The in situ extinction spectra from gold nanoparticle growth provide important information about the seed-mediated nanoparticle reaction. Representative extinction spectra at various times during the reaction for the 25, 35, and 50 \(\mu L\) precursor gold seed samples are shown in Figure 2. Prior to the start of the nanoparticle growth reaction, a baseline extinction spectrum is taken showing the absorption and scattering profile of the 15 nm gold precursor seeds along with the added gold chloride solution. Immediately after the addition of the reducing agents, a broad plasmon peak centered near 600 nm is observed, which is attributed to a polydisperse distribution and an uneven, rough nanoparticle surface.19,22 As the nanoparticle growth reaction proceeds, this broad plasmon peak narrows to a wavelength centered near 575 nm, indicating a more uniform surface and reduction in polydispersity. During the final stage of the reaction the plasmon peak continues to narrow and blue-shifts as the nanoparticle surface reaches its final, smooth morphology with a final spectrum that is very stable over time. A similar trend in the in situ extinction spectra is observed for the remaining nanoparticle samples, as shown in the Supporting Information.

The final extinction spectrum using 25 \(\mu L\) of precursor gold seeds is shown in Figure 3 with the corresponding Mie theory fit which is in agreement to the sizes measured from TEM.

Previous studies have investigated the formation of an “urchin-like” or “blackberry-like” intermediate stage in gold nanoparticles, which is characterized by a red-shifted plasmonic spectrum.19,46 This spectrum blue-shifts as the rough nanoparticle surface becomes more smooth during the nanoparticle growth reaction,19 which is consistent with our in situ extinction spectroscopy and SHG results, as discussed in more detail below.

The in situ SHG results provide additional and complementary surface-sensitive information about the seed-mediated nanoparticle growth process. The temporal evolution of the SHG electric field for each nanoparticle sample is shown in Figure 4. Before the growth process is initiated at time zero, a baseline SHG intensity is measured for all samples, which contains contributions of SHG from the precursor seeds41 as well as hyper-Rayleigh scattering47 from the aqueous solvent.
After adding the reducing agents HQ and sodium citrate, a sharp increase in the SHG electric field is observed followed by a size-dependent exponential decay for all samples. To separate the time-dependent linear extinction response from the nonlinear response of the nanoparticles, a correction to the in situ SHG signal is applied, where 

\[
I_{\text{SHG,corr}} = I_{\text{SHG}} \exp \left( \varepsilon_{800} + \frac{1}{2} \varepsilon_{400} \right),
\]

and where \( I_{\text{SHG}} \), \( \varepsilon_{800} \), and \( \varepsilon_{400} \) are the measured SHG intensity, extinction at 800 nm, and extinction at 400 nm, respectively.\(^{27}\) The SHG electric field is taken as the square root of the integrated SHG signal, with 

\[
E_{\text{SHG}} = \sqrt{I_{\text{SHG,corr}}}
\]

where \( E_{\text{SHG}} \) allows for precise fitting for the exponential profile. Representative in situ SHG spectra of the 25 \( \mu \)L precursor gold sample at selected reaction times are shown in Figure 5.

**Figure 4.** SHG electric field (red squares) as a function of reaction time of gold nanoparticles using (a) 25, (b) 30, (c) 35, (d) 40, and (e) 50 \( \mu \)L of precursor gold seeds compared to the fits (black lines).

Additional in situ SHG spectra from the other samples are displayed in the Supporting Information. The in situ SHG spectra are fit with a Gaussian function to obtain a central wavelength of 400 nm and a full width at half-maximum of 4.8 nm, which are constant values throughout the growth process for each nanoparticle sample.

The SHG electric-field time profile allows for the determination of the seed-mediated nanoparticle growth lifetime for the different prepared samples. For each nanoparticle sample, a maximum in the SHG electric field is observed in the initial stage of growth, shortly after time zero. The peak SHG near time zero shows approximately an order of magnitude increase in SHG intensity, which is attributed to the initial stage in the reaction that is characterized by inhomogeneous growth of the nanoparticles with rough surfaces giving rise to plasmonic hot spots.\(^{19,27−29,46}\) As the reaction proceeds, the sharp SHG feature exponentially decays where the characteristic growth lifetime is observed to be longer for larger nanoparticles. This indicates that the second stage in the reaction is described by the nanoparticle surface becoming more smooth and uniform over time, leading to a decrease in plasmonic hot spots and a lower SHG intensity. Finally, the nanoparticles reach their final morphology characterized by a relatively smooth surface where the reaction is complete and the SHG signal remains constant over time.

The size-dependent growth dynamics of the gold nanoparticles are analyzed in more detail to obtain the corresponding growth lifetimes. The SHG electric field time trace for each nanoparticle sample is fit using an exponential function given by 

\[
E_{\text{SHG}}(t) = \gamma_0 + Ae^{-t/\tau},
\]

where \( t \) is the reaction time after the addition of the reducing agents, \( \tau \) is the growth lifetime, \( A \) is the amplitude, and \( \gamma_0 \) is the offset. The fits for each nanoparticle sample are shown in Figure 4, and the best fit parameters are tabulated in the Supporting Information. The resulting growth lifetimes are plotted as a function of the final nanoparticle diameter (Figure 6a). These growth lifetimes decrease as the final nanoparticle diameter decreases with values of 1.67 ± 0.03, 1.57 ± 0.03, 1.05 ± 0.08, 0.95 ± 0.03, and 0.45 ± 0.04 min for the 94, 90, 75, 72, and 66 nm nanoparticles, respectively. The general trend in these lifetimes is in agreement with previous studies observing that the growth rate is proportional to the amount of precursor seeds.\(^{48}\) Because the SHG signal decreases as a function of time for each of the seed-mediated nanoparticle growth
concentrations of Au\(^0\) in the presence of HQ become less. These uneven surface energy facets that form under high

reactions, the dominant contribution of the change in SHG

signals is attributed to the surface morphology becoming more smooth over time, corresponding to a decrease in plasmonic hot spots.

The in situ SHG time profiles and corresponding extinction spectra of the seed-mediated gold nanoparticle growth dynamics are consistent with a two-step process. The first step is characterized by a rapid growth resulting in an uneven, bumpy surface and a high surface concentration of plasmonic hot spots. The second step is characterized by the nanoparticle surface becoming more smooth over time resulting in increased monodispersity, narrowing plasmonic spectra, and lower, more stable SHG signals as the nanoparticle surface reaches thermodynamic equilibrium under a characteristic size-dependent exponential growth lifetime. The peak SHG electric field, shown in Figure 6b, is observed to increase as the final nanoparticle diameter increases. The addition of sodium citrate reduces Au\(^{III}\) to Au\(^+\) followed by the selective reduction by HQ of Au\(^+\) adsorbed on the seed surface. Lower seed concentrations here correspond to higher concentrations of Au\(^0\) adsorbed to each nanoparticle surface to produce larger gold nanoparticles. A higher Au\(^0\) to seed ratio has been observed to promote the formation of urchin-like gold nanoparticles with high surface energy facets. These higher surface energy facets produce plasmonic hot spots corresponding to the large SHG signals observed shortly after time zero. These uneven surface energy facets that form under high concentrations of Au\(^0\) in the presence of HQ become less bumpy in morphology over time, reaching a thermodynamic equilibrium corresponding to a relatively smooth gold nanoparticle surface according to a size-dependent exponential time profile. Additional future studies, including different in situ scattering measurements combined with computational simulations, can be done to further characterize the nanoparticle size, shape, and surface morphology distributions during the nanoparticle growth reaction. Using the baseline SHG for each nanoparticle sample after the reaction is complete, the final contribution to the SHG electric field per nanoparticle is also determined, as plotted in Figure 6c. These results demonstrate that the larger gold nanoparticles have a higher SHG signal, in agreement with previous theoretical work on the enhancement of the second-harmonic field from different spherical nanoparticles. Overall, the in situ SHG and extinction spectroscopy measurements provide crucial insight for monitoring the colloidal seed-mediated gold nanoparticle growth reaction under varying synthesis conditions.

CONCLUSION

In summary, we have demonstrated the versatility of in situ SHG and extinction spectroscopy in the investigation of size-dependent seed-mediated gold nanoparticle growth dynamics in water. The in situ SHG and extinction spectroscopy results are consistent with a two-step growth process. During the first step of the nanoparticle growth reaction, rough and uneven surfaces are formed rapidly giving rise to plasmonic hot spots that dramatically enhance the SHG electric field and have corresponding broad, red-shifted plasmonic spectra. In the second step, the nanoparticle surface becomes smoother, reaching a thermodynamic equilibrium over a corresponding size-dependent exponential growth lifetime that results in a final nanoparticle sample with a lower, more stable SHG signal and corresponding narrower, blue-shifted plasmonic spectra that agrees with Mie theory. The seed-mediated nanoparticle growth lifetimes measured using in situ SHG are faster for smaller final gold nanoparticle sizes, varying from 0.45 to 1.7 min for final nanoparticle sizes of 66 and 94 nm, respectively. The combination of in situ SHG and extinction spectroscopy provides complementary information that can be used to monitor colloidal nanoparticle growth dynamics for improving nanomaterial synthesis and characterization.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.8b07176.

TEM images of the gold nanoparticles with their corresponding size distributions, additional Mie theory fitting, and discussion on nanoparticle concentrations; additional in situ SHG and in situ extinction spectra as well as the tabulated fitting values for the growth lifetimes (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail lhaber@lsu.edu (L.H.H.).

ORCID

Tony E. Karam: 0000-0002-1244-5141
Holden T. Smith: 0000-0001-5487-8431
Louis H. Haber: 0000-0001-7706-7789

DOI: 10.1021/acs.jpcc.8b07176


24404
The authors thank Louisiana State University and the Consortium for Innovation and Manufacturing in Materials (CIMM) under Award #01A-1541079 for generous financial support for this work. The authors also thank the LSU Material Characterization Center (LSUMCC) and Ying Xiao for assistance with transmission electron microscopy.

**REFERENCES**


