Supplemental Information

Synthesis of Hollow Gold-Silver Alloyed Nanoparticles: A "Galvanic Replacement"

Experiment for Chemistry and Engineering Students

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I. Materials and Methods

Chemicals

Silver trifluoroacetate (AgTFA), sodium hydrogen sulfide (NaSH), hydrochloric acid (HCl, 99.999%), and tetrachloroauric acid trihydrate (HAuCl$_4$·3H$_2$O), were purchased from Alfa Aesar. Copper sulfate pentahydrate (CuSO$_4$·5H$_2$O), poly(vinylpyrrolidone) (PVP, M.W.=55,000), and 4-mercaptobenzoic acid (4-MBA, 99%) were purchased from Sigma Aldrich. Ethylene glycol (EG) was purchased from J.T. Baker. Acetone was purchased from EMD. Experiments were performed using 18 MΩ H$_2$O to achieve best results. Deionized water can also be used with reasonable success. All chemicals were used as received. The Ag and Au precursor solutions should be freshly prepared prior to the experiments and protected from light using aluminum foil.

Experimental Methods

Synthesis of Ag nanocubes (by instructors prior to class). Ag nanocubes (AgNCs) were synthesized by the polyol method. Briefly, an oil bath was heated to 150 °C. Note that the oil bath needs sufficient stirring to maintain an even temperature, which dramatically improves particle uniformity. 10 mL EG was added to an 8 dr vial with a stir bar and placed in the oil bath while reagent solutions were prepared. The following solutions were prepared separately: 75 mg PVP was dissolved in 3 mL EG, 1 mg NaSH was dissolved in 5.95 mL EG, and 1.5 µL HCl was added to 6 mL EG. Once the oil bath had reached 150 °C, 60 mg AgTFA was dissolved in 0.84 mL EG. Sequentially, 0.12 mL NaSH, 1 mL HCl, and 2.5 mL PVP stock solutions were added to the reaction vial, which was then allowed to equilibrate for 2 min. Next 0.8 mL of the AgTFA solution was added and the vial closed but not purged. Periodically a small sample (~10 µL) was extracted from the reaction vial with a capillary tube and the extinction spectrum acquired. Once

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the LSPR peak reached ~440 nm (~ 35 min after addition of Ag), the reaction was quenched in an ice bath. Upon cooling, the reaction solution was divided into two 50 mL centrifuge tubes that were then filled with acetone and mixed. The tubes were centrifuged at 7,000 RPM for 10 min. The supernatant was removed and the pellets were redispersed by sonication in 6 mL H_2O (total), transferred to 1.5 mL centrifuge tubes, and centrifuged for 10 min at 15000 RPM. The supernatant was again removed and the pellets redispersed in H_2O and centrifuged a second time. The supernatant was once again removed and the sample dispersed in a total volume of 3 mL for use as a stock solution in class. A 1000× dilution of this stock solution had an extinction of ~0.9 a.u. at the LSPR maximum.

Synthesis of hAuAgNPs. To synthesize hAuAgNPs, 10 mL of 18 MΩ H_2O was heated to boiling in a 50-mL round-bottom flask. To the boiling liquid, 0.25 mL of 50 mM PVP (in H_2O) and 0.5 mL of the AgNC suspension (redispersed by sonication prior to use) were added. Next 1 mM $HAuCl_4$ was added dropwise at approximately 0.5 mL/min. The amount of $HAuCl_4$ was varied among student groups, from 3 to 6 mL, allowing students to acquire different, but comparable results. Once the specific amount of $HAuCl_4$ was added, the reaction was allowed to proceed for another 5 min. The reaction flask was then removed from heat and allowed to cool to room temperature.

Sample Characterization. Samples were characterized using transmission electron microscopy (TEM) and UV-vis spectroscopy. TEM samples were prepared by casting a 3 μL of the sample suspension onto a carbon-coated copper grid and drying in air, and images were acquired by the teaching assistant. The AgNC sample was prepared directly from the stock solution while the hAuAgNP sample was concentrated by centrifuging 1 mL of reaction solution and redispersing in 100 μL H_2O for TEM preparation. TEM images were acquired by the teaching assistant

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outside of class. Students were provided with several images of their respective samples and access to images by other groups. UV-vis spectra can be acquired using 4-way acrylic cuvettes. The hAuAgNP sample was measured directly from the reaction solution following a 10× dilution. The AgNC sample was diluted 1000 fold in 2 mL H_2O before measurement, and had an extinction of ~0.9 a.u. Qualitative analysis of the absorption and scattering is performed using the UV-vis samples of nanoparticles a cuvette filled with H_2O and a cuvette containing 100 mM $CuSO_4$. A red laser pointer was shined through the samples in various directions and the shape of the beam is described and/or photographed.

Photothermal Measurement. To demonstrate the photothermal effect, a photodiode laser centered at 808 nm (Power Technologies) and infrared temperature probe (Omega) were used. The UV-vis samples from previous experiments, a solution of 100 mM $CuSO_4$, and a blank of 18 MΩ H_2O were used. The laser was allowed to warm up for 5 min prior to use, and then was positioned to shine downward into the open cuvette. Samples were irradiated for 5 min at 0.9 W/cm^2, then removed from irradiation for 5 min, then irradiated again for 5 min. The temperature was recorded every 15s.

Raman Spectroscopy. SERS was demonstrated using a Raman spectrometer (Ocean Optics), consisting of a laser source (785 nm diode laser), a Raman detector (QE65PRO-RAMAN), a Raman coupled fiber probe, and a Raman sample holder. The SERS samples were prepared according to the following procedure: each sample containing 1 nM hAuAgNPs was incubated with 0.010 M MBA in 2 mL of 0.1 M NaOH aqueous solution for 24 h; and the hAuAgNPs were then collected by centrifugation and redispersed in 2 mL of 0.1 M NaOH aqueous solution. Each SERS sample contained a total surface area of ~0.9 m^2/L. The reference sample was 0.010 M 4-MBA in 0.1 M NaOH aqueous solution. For each sample, the Raman spectra were collected

using laser power of 100 mW at 785 nm and integration time of 30s in one acquisition. A 0.1 M

NaOH aqueous solution was used as a blank for baseline correction.

II. Instructor Notes

General Notes

The authors strongly recommend preparation of stock solutions prior to the class. Stock solutions reduce waste by the student groups and increase the comparability of results across groups. Additionally, the authors recommend a practice run of the experiments be performed prior to class. The resultant samples are useful as a backup in case of experimental failure. The practice run also helps ensure that the stock solutions will work in the classroom.

To achieve the best synthetic results, glassware needs to be extremely clean and free of scratches. Trace impurities, particularly metals, can significantly affect particle morphology. Scratches in glassware provide additional nucleation sites that affect seed formation. If glassware is promptly cleaned with acid following experiments, intense scrubbing is typically not necessary, which reduces the risk of scratching the glass. Stir bars should be cleaned in acid and rinsed with glassware.

Glassware from the AgNC synthesis should be cleaned with concentrated HNO_3 to dissolve the Ag. HNO_3 is highly corrosive and should be used <u>with extreme caution</u> in a fume hood. It is advisable to scrub the glassware with soap and water as well, to remove any adsorbates from the glass. Reaction flasks for the galvanic replacement reaction should be cleaned using *aqua regia* (3:1 HCl:HNO_3) to dissolve Au. *Aqua regia* is best prepared fresh, and **<u>cannot be stored in a closed container</u>**, as it generates gas, which can cause the container to violently explode. If a white film persists in the Au reaction flask, use HNO_3 to dissolve the AgCl more effectively. After acid washing, glassware and stir bars need to be rinsed at least 5 times with tap water, 5 times with DI water, and 5 times with 18 MΩ H_2O to ensure complete removal of the acid. After

use, a*qua regia* or other acid waste should be diluted and carefully neutralized with $NaHCO_3$ in a container.

Synthesis of AgNCs. The reaction can be easily scaled up or down to accommodate different classroom sizes. The authors recommend use of a round-bottom flask if the reaction is to be scaled up any further than described. The oil bath is strongly recommended as a heat source as it will provide more uniform heating of the sample, providing for more uniform particles. Stirring in the oil bath needs to be fast enough that the bath is homogeneous. The bath will be extremely hot, do not touch it. When the reaction is completed, remove the reaction vessel and allow it to drip off excess oil, and then quickly wipe it down before quenching in the ice bath to reduce mess. The PVP and HCl solutions are stable for several days, but it is recommended that the NaSH and AgTFA solutions be prepared fresh prior to the reaction. Molecular oxygen is critical for the etching of the twinned seed particles, so the reaction is not degassed, and is left open to air. High humidity can affect the quality of the cubes; on hot and humid days, allowing the EG to sit in the hot oil bath for an hour before addition of reagents can mitigate this problem. Fast injection of the Ag precursor is crucial to produce highly uniform particles from the synthesis.

The synthetic protocol described yields AgNCs with a particle concentration of ~8 nM, based on the extinction coefficient of 1.2×10^{11} $M^{-1}cm^{-1}$, and the concentration of the stock solution can be ascertained. There will likely be a small number of rods and wires in any AgNC sample (can be <0.1 %); if these particles represent less than 5% of the population, they will not significantly affect the following steps. The acetone is used to precipitate the AgNCs and remove as much EG as possible, but the AgNCs need to be washed twice with H_2O to remove residual acetone, which will affect the galvanic replacement reaction. Carefully following the procedure described above, one can reproducibly synthesize Ag nanocubes with 90% success rate. The unsuccessful

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synthesis usually results in Ag nanocubes with mixed sizes and/or the presence of round particles and rods with increased percentages. This will broaden the LSPR spectra of AgNCs and the resulting hAuAgNPs from the galvanic reactions using these AgNCs

Synthesis of hAuAgNPs. The concentration and size of the AgNCs affect the amount of $HAuCl_4$ that needs to be titrated into the reaction to obtain hAuAgNPs with desired optical properties. For example, larger particles and higher concentrations require more $HAuCl_4$ to achieve the same LSPR position. It is advisable to test the reaction before class to determine the appropriate range of $HAuCl_4$ titration. In our studies, 6 mL of 1 mM $HAuCl_4$ could shift the LSPR beyond 900 nm. During titration, the color of the reaction transitions from yellow to purple to deep, royal blue after addition of ~3 mL of 1 mM $HAuCl_4$. At this point, the LSPR is at ~650 nm indicative of the alloying process of Ag and Au. If this transition occurs at significantly lower Au volumes, it is recommended to proportionally adjust the amount of $HAuCl_4$. If this transition occurs after addition of greater amounts of $HAuCl_4$, the lab can proceed normally, but final spectra can be expected to be blue-shifted. It is recommended that the AgNCs be sonicated well prior to use so students start with identical AgNC samples for the galvanic replacement reaction, which facilitates comparison of results following titration of different $HAuCl_4$ volumes.

The temperature of galvanic reaction is recommended to be close to the boiling point of water to increase the solubility of the byproduct AgCl. After addition of AgNCs, the reaction requires one minute to equilibrate, however, if this period is too long, the AgNCs may begin to grow and agglomerate. In contrast, if $HAuCl_4$ is added at a low temperature, the reaction may not fully proceed to an alloyed shell and the optical properties may be compromised. Vigorous stirring is recommended, as it allows the Au precursor to more homogeneously react with the AgNCs. Results were significantly improved using a high rate of stirring with a creation of

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visible vortex. The rate of titration is recommended to be 0.5 mL/min; however, the range of 0.1 to 1 mL/min is acceptable. Too rapid addition of $HAuCl_4$ (especially at low stir rates) can result in cage breaking and inhomogeneity amongst particles. On the other hand, too slow addition can result in reconstruction of the nanoparticle morphology due to the high temperature.

Acquisition of UV-vis Spectra. Four-way cuvettes are preferable to 2-way cuvettes as they allow for more options in the laser pointer experiment. It is recommended that the samples be diluted to yield extinction maxima of 0.8-1 as described in the experimental methods.

Comparison of Absorption and Scattering. Four-way cuvettes allow the beam to be passed horizontally through multiple samples. The light absorption will likely be greatest for the LSPR of hAuAgNPs that best overlap with the 660 nm wavelength of the red laser beam. If photographing the beam in the dark, it may be necessary to partially block the beam, as there will be extremely high scattering intensity which will cause glare and compromise image quality. Samples that are too dilute will not show significant tapering.

Photothermal Experiment. Care should be exercised that the laser does not directly interact with the temperature probe as it can falsely elevate the temperature. For best results, the laser spot size is required to be less than the width of the cuvette. The photothermal effect is an absorption based process, therefore, the closer the optical density of the $CuSO_4$ solution and the hAuAgNPs at the laser wavelength, the more closely these temperature profiles will match. If sample quantities are limited, the UV-vis samples can be used for this experiment.

SERS Experiment. The concentration of the nanoparticles can be roughly estimated from the concentration of the AgNCs used for the galvanic replacement reaction and dilution factors during the titration. The compound, 4-MBA, is not soluble in pure water, but can be dissolved in 0.1 M NaOH aqueous solution or pure ethanol. The intensity of $v_s(COO^-)$ increases with the pH

increase. Comparing the ordinary spectrum of the pure 4-MBA with the SERS spectrum, the slight shift of some of the peaks can be attributed to the change of the orientation and selection rules of the molecules as they are adsorbed on the surface. To avoid particle aggregation, the samples were prepared using 0.1 M NaOH solutions instead of ethanol and were sonicated before the SERS spectra were taken. Aggregation can significantly increase the value of the enhancement factor.

III. Lab Manual

Synthesis of Hollow Gold-Silver Nanoparticles and Investigation of Their Optical Properties

Objectives
1. Introduce students to the control of nanoparticle composition/morphology to tune their physical and chemical properties.
2. Introduce the solution-phase synthesis of nanoparticles with the emphasis on galvanic replacement reaction.
3. Demonstrate the influence of composition and morphology on nanoparticle optical properties.
4. Demonstrate the unique phenomena that occur due to these optical properties.
5. The end goal is to give students an introductory knowledge of chemical synthesis of nanoparticles and how the properties change with composition and morphology of the nanoparticles.

Safety Rules
1. Safety information must be reviewed prior to experiments. This includes lab safety training, safety data sheets (e.g. MSDS) for chemicals used, and lab procedures.
2. Personal protective equipment is required at all times. These include lab coats, safety glasses, gloves, close-toed shoes, and long pants.
3. Used chemicals and materials should be captured in appropriate waste receptacles and not sent to the sewer.
4. Caution should be taken around heating mantles and reaction flasks to minimize risk of accidental burns.
5. Lasers should never be pointed at individuals and reflective objects should not be near the laser path.

Introduction

Nanomaterials composed of coinage metals (Cu, Ag, and Au) have many attractive biological applications due to their unique optical properties.[1-3] When their size is comparable or smaller to the wavelength of light, their free electrons in the conduction band collectively oscillate with the electric field generated by incident light and give rise to an extinction maximum at the resonant wavelength. This phenomenon is called localized surface plasmon resonance (LSPR). Extinction is the sum of absorption and scattering. In 1908, Gustav Mie developed a theory to describe the light extinction and scattering of spherical metal nanoparticles. The Mie theory is the exact solution to Maxwell's electromagnetic field equations which describe a plane wave interacts with a metal sphere of radius R having the same dielectric functions as its bulk counterpart. If the boundary conditions are specified, the extinction cross-section (σ_{ext}) of a sphere can be obtained, and thus the extinction efficiency factor (Q_{ext}) which is defined as ratio of the extinction cross section to the physical cross-sectional area (πR^2). For example, when $R \ll \lambda$, one can neglect both phase retardation and effects from higher multipoles and consider only the dipole oscillation contribution. The extinction cross-section is then derived as **Eq. 1**.

$$\sigma_{ext}(\lambda) = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon_i(\lambda)}{[\varepsilon_r(\lambda) + 2\varepsilon_m]^2 + \varepsilon_i(\lambda)^2} \tag{1}$$

where ε_m is the dielectric constant of the embedding medium and the ε_r and ε_i are the real and imaginary components of the metal dielectric function, respectively. A resonance peak occurs when the condition of $\varepsilon_r(\lambda) = -2\varepsilon_m$ is satisfied. In the case of Cu, Ag, or Au, this condition is met in the visible region of the electromagnetic spectrum. For a given material, the position, height, and width of resonance peak depend on the size and shape of the nanoparticle.

In addition to size and shape, the dielectric constant (ε_m) of the embedding medium (or refractive index, $n; \varepsilon_m = n^2$) also influences the wavelength maximum of the resonance (λ_m). The shift of this wavelength is dependent on the change in the local environment such as the solvent and the surface coating. For example, the relationship between the shift and the surface adsorbate is shown in **Eq. 2.**[2]

$$\Delta\lambda_{max} = m\Delta n[1 - \exp(-\frac{2d}{l_d})] \qquad (2)$$

where m is the bulk refractive-index of the nanoparticle; Δn is the change in the refractive index induced by the adsorbate; d is the effective thickness of adsorbate layer; and l_d is the characteristic electromagnetic field decay length. The sensitivity of the LSPR to the local environment provides a useful tool for chemical and biological sensing and indirect validation of the success of the surface modification.

Gold nanoparticles are well-studied materials due to their chemical and biological inertness. The LSPR of spherical nanoparticles is in the visible region and varies slightly with the size of the nanoparticles. For biomedical applications, the LSPR of the nanoparticles needs to be shifted to the near-infrared (NIR) region (650-900 nm), which is the "transparent" window for the light, enabling deeper tissue penetration. There are several ways to shift the LSPR of the nanoparticles to NIR: 1) aggregation of individual particles to nanoparticle clusters; 2) coating of SiO_2 particles with metal shells; 3) elongation of the spherical particles into rod-shaped particles; and 4) hollowing out the interior of the metal nanoparticles. The color change in the first scenario is often used for biological sensing.[4] The other three types of nanostructures can be used as contrast agents for optical imaging, therapeutic agents for photothermal therapy, and controlled-release drug delivery.[5] In this module, we will synthesize hollow Au-Ag nanoparticles (hAuAgNPs) and characterize their optical properties.

The hAuAgNPs are synthesized using the galvanic replacement reaction between the Ag nanocubes (AgNCs) and chloroauric acid ($HAuCl_4$).[6] Cubic AgNPs are bounded by {100} facets which are considered to be more reactive than the close-packed {111} facets in absence of other effects such as surface capping ligands. Initially, AgNCs are prepared *via* polyol method (**Fig. 1**), in which ethylene glycol (EG) serves as a reducing agent and a solvent while poly(vinyl pyrrolidone) (PVP) is the capping agent.[7]
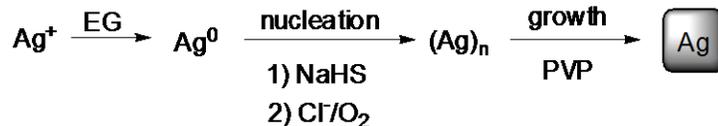

Figure 1. Schematic illustration of the polyol synthesis of AgNCs.

After the Ag reduces, two major processes will occur in the reaction: nucleation and growth.[8,9] The Ag^+ ions were reduced by glycolaldehyde, an oxidation product of EG, to form Ag^0 atoms in solution.[10] Once the atomic concentration of Ag has reached a critical, supersaturated threshold, the atoms independently self-nucleate to form seeds including single-crystalline and twinned seeds. Addition of NaHS can accelerate the nucleation process while the presence of Cl^- ion and O_2 can etch and dissolve the twinned seeds.[11] Nucleation creates small clusters of atoms and lowers the atomic concentration below the critical nucleation threshold. The Ag atoms still persist at a concentration sufficient to grow onto the seeds during the growth phase. At the growth stage, the capping agent PVP strongly interacts with {100} facet of the single-crystalline seeds, and thus the addition of the Ag atoms on the {111} facets lead to the formation of AgNCs.

In the second step, the AgNCs serve as sacrificial templates for the generation of hAuAgNPs via the galvanic replacement reaction (**Eq. 3**).[12,13]

$$3Ag \text{ (s)} + AuCl_4^- \text{ (aq)} \xrightarrow{\text{heat}} Au \text{ (s)} + 3Ag^+ \text{ (aq)} + 4Cl^- \text{ (aq)} \tag{3}$$
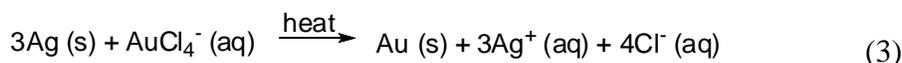
The driving force of the reaction is the reduction potential difference between the redox pairs. The reduction potential for the $AuCl_4^-$/Au pair (0.99 V vs SHE) is higher than the Ag^+/Ag pair (0.80 V vs SHE), resulting in the spontaneous reaction of **Eq. 3** (Gibbs energy change: $\Delta G = -nFE < 0$). The reaction is carried out by titrating different volumes of $HAuCl_4$ solution to the Ag nanoparticle suspension. The morphological change of the nanoparticles is illustrated in **Figure 2**. The replacement reaction is initiated stochastically from the surface of the nanoparticles through a pitting process (A). As the reaction proceeds, nanoboxes are formed through a combination of replacement and alloying between Ag and Au (B-C). By the end of the reaction, Au-Ag nanocages with hollow interiors and porous walls have evolved as a result of dealloying of Ag from the Au-Ag alloyed walls

Figure 2. Schematic illustration of morphological evolution of galvanic replacement reaction. (Reproduced from ref. 12).

(D-F). The formation of the hollow structures is attributed to difference in the diffusion rates between the Au and Ag, known as the Kirkendall effect.[14,15] The 3 to 1 stoichiometry of Ag to Au in the reaction leads to fewer atoms in the final nanoparticles, resulting in porous walls.
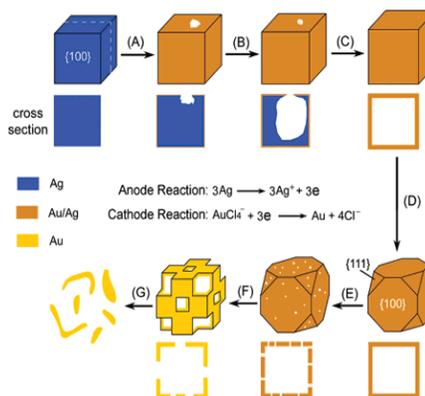
During this replacement process, the extinction spectrum changes with the morphological evolution. The extinction efficiency factor of each nanostructure can be quantified based on the extinction spectra using the Beer's Law (**Eq. 4**) using a UV-vis spectrometer:

$$A = \varepsilon cl \tag{4}$$

where A is the measured absorbance at wavelength maximum of the LSPR; ε is the molar extinction coefficient at this wavelength ($N_A \sigma_{ext}$ with N_A is the Avogadro constant); c is the molar concentration of the sample; and l is the path length of the beam travelling through the sample.

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At a fixed path length usually determined by the cuvette that is used, the absorbance is proportional to the molar concentration of the nanoparticles. By plotting the absorbance as a function of nanoparticle molar concentration, the slope gives the molar extinction coefficient (slope = εl) and the extinction cross-section of individual nanoparticles can be derived. The accuracy of the experimental data highly depends on uniformity of the sample and accurate concentration measurement. In this module, we will learn the synthesis of the metal nanoparticles and study their optical properties.

Materials, Supplies and Instrumentation
Chemicals:
- Poly(vinylpyrrolidone), M.W. 55000 (PVP)
- Tetrachlororauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$)
- Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)
- AgNCs (stock concentration to be provided by the teaching assistant)
- 18 MΩ water

Supplies:
- A 50 mL 3-neck round bottom flasks
- Magnetic stir bars
- A reflux condenser and glass stoppers
- A magnetic stir plate and heating mantle
- A centrifuge and appropriate centrifuge tubes
- A bath sonicator
- A timer
- UV-vis acrylic cuvettes
- Pasteur pipettes and bulbs
- Calibrated micropipette and appropriate tips

Instrumentation:
- UV-Vis spectrophotometer
- Transmission electron microscope (TEM)
- Laser pointer

Experimental Procedures
Solution Preparation:
- AgNCs (prepared in advance)
- 50 mM PVP (5.6 mg/mL), 5 mL
- 1 mM $HAuCl_4$ (0.39 mg/mL), 10 mL
- 100 mM $CuSO_4$ (25 mg/mL), 5 mL

Note: Prepare the following aqueous solutions using 18MΩ water for best results. Use the wide end of a glass Pasteur pipette as a spatula to eliminate any trace metal contamination. Modify volumes as necessary to accommodate accurate concentrations using **Eq. 5**.

$$V_1 = V_2 \times M_2 / M_1 \qquad\qquad (5)$$

where V_1 is the volume of solution that needs to be made, V_2 is 1 mL, M_2 is the mass per mL specified in parentheses above and M_1 is the mass of sample that is weighed out. Stock solutions may be prepared beforehand and diluted, but should be protected from light.

Synthesis of hAuAgNPs:

    Add 10 mL H_2O to the 50 mL flask. Place on a heating mantle with magnetic stirring and allow to heat for 10 minutes, so that it starts to boil. Next add 0.5 mL of PVP solution and 1.0 mL of the silver nanoparticle suspension. Wait 2 min for the solution to equilibrate. Begin adding 1 mM $HAuCl_4$ dropwise to the reaction flask. Add the $HAuCl_4$ solution no faster than 0.5 mL/min (roughly 20 drops/min). Groups will be using different amounts of Au^{3+} solution, so that the hollow particles can be tuned to different wavelengths. Instructors will provide specific quantities of Au to be added for each group. It will likely take between 3 and 6 mL to reach the desired wavelength (e.g., 650 nm); however this value may vary depending on the AgNC size and concentration. Make note of any observed color changes during this process.

Acquisition of UV-vis Spectra:

    Add 2.0 mL H_2O to a cuvette and acquire a blank spectrum. To this add 2 µL of the remaining AgNC solution to yield a 1000× dilution. If needed, dilute the solution further to achieve a clean spectrum. Record the wavelength of maximum absorbance and the value of this absorbance. If this spectrum has a maximum value greater than one, then dilute the sample so that the maximum is ~1 a.u. In a separate cuvette, directly add 1.8 mL H_2O and 0.2 mL of the Au-Ag reaction solution to obtain a 10x dilution. Record the maximum wavelength and similarly dilute the sample to a maximum absorbance of ~1 a.u.

Investigation of Absorption and Scattering:

    Acquire a standard red laser pointer and the samples, in cuvettes, used for the prior UV-vis study. In clean cuvettes, prepare 2 mL of H_2O and 2 mL of 100 mM $CuSO_4$. Shine the beam downward through each sample. *Record any pertinent observations particularly relating to the shape and intensity of the beam.* The beam can be more easily visualized with the room lights off, to minimize ambient light.

TEM Imaging:

    The TEM samples are prepared by putting a drop of the nanoparticle suspension on the TEM grid and allowing it to dry at room temperature. The TEM image will be recorded by the teaching assistant. Identify the size and shape of the nanoparticles. Specifically, measure the diameter of a statistically significant sample of nanoparticles using ImageJ.

Post Experiment Cleaning:

    Clean all glassware following use. Condensers and stoppers need to be thoroughly rinsed with deionized water. Rinse flasks into the liquid waste then scrub with soap and water in the sink. Be careful not to scratch the glass. Used centrifuge tubes, pipette tips, and cuvettes should be discarded in solid waste, and all unusable glassware should be disposed of in glass waste.

Lab Report:

Lab reports will contain the sections described below, and are expected to be written as though it were to be published in a scientific journal. The content described in each section is expected to be present in the report, but additional information is expected.

Abstract – Briefly describe the purposes and broad methodology of the experiment. Include a summary of the major results from YOUR experiments.

Introduction – The background of the experiments is outlined. Specifically, the origin and nature of the optical properties should be considered with regard to the experiments performed and the synthetic methods utilized should be thoroughly explained.

Experimental Methods – All experimental procedure is described in such a manner that the reader could easily repeat the experiment. Important information includes (but is not limited to) equipment used, quantities of solutions, reagents, reaction times, and dilutions should all be included. Methods are expected to describe the procedure followed by the author of the report.

Results and Discussion – All results should be presented, described, and the implications of these data discussed. Include a sample calculation for all unique calculations. Clearly labeled figures should be included which show the relevant data, and any significant observations during the reaction of deviations from protocol should be discussed.

Additionally, the following discussion questions are recommended to be integrated into the text:
1) For AgNCs, the LSPR maximum (λ_{max}, nm) slightly shifts to the red as the particle size increases. This relationship was empirically described by Xia and co-workers using **Eq. 6**,[6]

$$\lambda_{max} = 1.3927(l) + 378.25 \qquad\qquad (6)$$

   where λ_{max} is the LSPR maximum and l (nm) is the edge length of the AgNC.[17] Use this equation to calculate the edge length based on the UV-vis spectrum and the LSPR maximum based on TEM measurements for the AgNCs. Remark on the origin of any discrepancies.
2) Provide a rationalization as to why the LSPR redshifts as (A) AgNP diameter increases and (B) hAuAgNP wall thickness decreases. Consider what happens to the photon energy as the wavelength increases.
3) Briefly compare your results for hAuAgNP synthesis to those of other groups. What trends are observed as the quantity of Au increases?

References – Full citations for references utilized to write the report should be included. A good reference section will include the lab handout, possibly a textbook, and a handful of peer-reviewed papers to support arguments made and background information utilized.

References

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APPENDIX I. Photothermal Conversion

Background
Absorption is one of the two primary components of extinction; it is the process by which the energy of an incident photon is introduced into the molecular energy of a system. Following absorption, the energy must be released to return the system to its ground state. Plasmonic materials are particularly well-suited to convert the absorbed photonic energy into phonon energy (lattice vibrations) that dissipates in the form of heat. As a result, irradiation of these nanomaterials can be used to generate localized heating. The temperature profile can be finely controlled by adjusting the particle concentration, absorption cross-section, and optical dose (power and duration). Removing the irradiation source will cause the system to cool back to room temperature, which can be modeled with **Eq. 7**:[16]

$$T(t) = T_0 + (T_{max} - T_0) * e^{-Bt} \quad\quad\quad (7)$$

where *T(t)* is the temperature at a given time, T_0 is room temperature, T_{max} is the temperature when irradiation was removed, and *t* is time. *B* is the rate constant (in s^{-1}) associated with the heat dissipation from the sample. *B* is influenced by the heat capacity, mass, and surface area of the system. The rise in temperature during laser irradiation can be modelled to fit **Eq. 8**:

$$T(t) = T_0 + {A}/{B} (1 - e^{-Bt}) \quad\quad\quad (8)$$

which uses the same *B* value from **Eq. 7**. *A* is a constant associated with energy absorption (in °C/s) and is influenced heavily by the absorption cross section and heat transfer coefficients of the sample, as well as extrinsic factors like the sample concentration and optical path length, flux, and dose.

Experimental Procedures
An NIR photodiode laser (808 nm), temperature probe, 2 mL of the samples of AgNCs and hAuAgNCs with extinction ~0.1 a.u., a solution of 100 mM $CuSO_4$, and 18 MΩ H_2O are used for this experiment. The laser is warmed up for 5 min, is then positioned to shine downward into the open cuvette. Irradiate a sample for 5 min, then remove from irradiation for 5 min, then irradiate again for 5 min. Record the temperature every 15 s.

Discussion question: Using these data, determine the values for A and B for your hAuAgNP sample. Rationalize the results observed for all four samples measured.

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APPENDIX II. Surface Enhanced Raman Spectroscopy (SERS)

Background
Raman spectroscopy is a commonly-used vibrational spectroscopic technique which is based on the inelastic Raman scattering of higher-energy visible photons by a molecule. The energy shifts in the scattered radiation corresponds to transitions between vibrational energy levels of the molecule. Raman cross sections are very small, and therefore difficult to detect. When molecules are adsorbed or in close proximity to a metal surface, the Raman intensity enhances, known as SERS. A recent review has detailed the concepts and applications of SERS.[17] At the resonance condition with incident radiation, charge separation confined within the nanoparticle surface enhances the electromagnetic (EM) field strength near the surface. The EM enhancement serves as a two-way antenna that increases the light intensity on the molecule and increases the scattered Raman signal, resulting in quartic signal enhancement (E^4). SERS enhancement can be compared quantitatively by enhancement factor (EF).[18] From the SERS substrate point of view, the average EF defined in **Eq. 9** is the most widely-used.

$$EF = \frac{I_{SERS}/N_{Surf}}{I_{RS}/N_{Vol}} \qquad (9)$$

where I_{SERS} is the intensity of a Raman active mode observed in the SERS sample, I_{RS} is the intensity of the same mode detected in the reference sample, N_{SERS} is the average number of adsorbed molecules in the scattering volume for the SERS experiments, and $N_{Vol} = c_{RS}V$ is the average number concentration of molecules in the scattering volume (V). Although it is out of scope of this experiment, this EF definition needs more vigorous considerations on two aspects: 1) the number of molecules should be counted in N_{Surf}; and 2) the scattering volume is usually not uniform. From the analytical chemistry point of view, the analytical enhancement factor (AEF) is defined in **Eq. 10**.

$$AEF = \frac{I_{SERS}/C_{SERS}}{I_{RS}/C_{RS}} \qquad (10)$$

where C_{SERS} is the concentration of molecules adsorbed on the SERS substrate, and C_{RS} is the concentration of molecules dissolved in solution. In **Eq. 10**, the C_{SERS} counts the molecules adsorbed both chemically and physically. In some cases, the adsorbed molecules are multilayered. If monolayer coverage on the surface is ensured, the AEF is a simplified form of the EF in **Eq. 9** and frequently used for colloidal solution.

Experimental procedures
SERS are demonstrated using a Raman spectrometer (Ocean Optics), consisting of a laser source (785 nm diode laser), a Raman detector (QE65PRO-RAMAN), a Raman coupled fiber probe, and a Raman sample holder. SERS samples are prepared by incubating 1 nM hAuAgNPs in a 2 mL solution of 0.010 M 4-mercaptobenzoic acid and 0.1 M NaOH aqueous solution for 24 h; and the hAuAgNPs are then collected by centrifugation and redispersed in 2 mL of 0.1 M NaOH aqueous solution. The reference sample is 0.010 M 4-MBA in 0.1 M NaOH aqueous solution. For each sample, the Raman spectra are collected using laser power of 100 mW at 785 nm and integration time of 30s in one acquisition. A 0.1 M NaOH aqueous solution was used as a blank for baseline correction.

Discussion question: Identify the vibrational peaks. Based on the most intense peak in the spectrum, calculate the values of AEF for hAuAgNPs. In your lab report, provide at least three

rationalizations for why the AEF is not the same and discuss any significant discrepancies between the spectra.

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Appendix III – Sample Calculation for AgNCs

A) Determination of AgNC Stock Solution concentration

A total of 2 µL of the AgNCs prepared for use by the undergraduates was dissolved with HNO_3 and diluted to 2 mL (dilution factor = 1000) with 2 % HNO_3 to prepare the flame atomic absorbance spectroscopy (FAAS) sample. The sample was determined to have a Ag concentration of 4.6 ppm.

Based on the data from FAAS, the mass concentration was converted to atom concentration as follows.
4.6 ppm Ag = 4.6 µg/mL Ag = 4.6 mg/L Ag
(4.6 mg/L Ag)/(107.9 g/mol) = (42.6 µM Ag)×($6.02×10^{23}$ atoms/mol) = $2.56×10^{19}$ Ag atoms/L

Based on the data from TEM, the average size of the AgNCs was measured and then used to determine the number of atoms per AgNC according to the lattice constant of Ag (α = 0.418 nm) and the unit cell structure (face-centered cubic, 4 atoms/unit cell).
Volume (V) of AgNCS with an edge length (l) of 46.6 nm: V = l^3 = $1.01×10^5$ nm^3/AgNC;
Atoms per AgNC:
~~($1.01×10^5$ nm^3/AgNC)/[(0.418 nm/unit cell)×(4 atom/unit cell)] = $5.53×10^6$ Ag atoms per AgNC~~

Then, AgNC concentration was determined from the above information.
($2.56×10^{19}$ Ag atoms/L)/(~~5.53~~×10^6 Ag atoms per AgNC) = ~~4.63~~×10^{12} AgNC/L
or
(~~4.63~~×10^{12} AgNC/L) /($6.02×10^{23}$ atoms/mol) = ~~7.69~~x10^{-12} M AgNC

Considering the dilution factor of 1000, the concentration of the AgNC stock solution is ~~7.7~~×10^{-9} M or ~~7.7~~ nM.

B) Calculation of Extinction Coefficient

AgNC stock solution was diluted between 500× (15.5 pM) and 4000× (1.9 pM) and the UV-vis spectra was acquired. The extinction at 438 nm is plotted in Figure IIIA. According to the Beer's Law: A = εcl, where *l* is 1 cm, slope of the graph gives ε = ~~1.2~~ $pM^{-1}cm^{-1}$ = $1.2×10^{11}$ $M^{-1}cm^{-1}$.
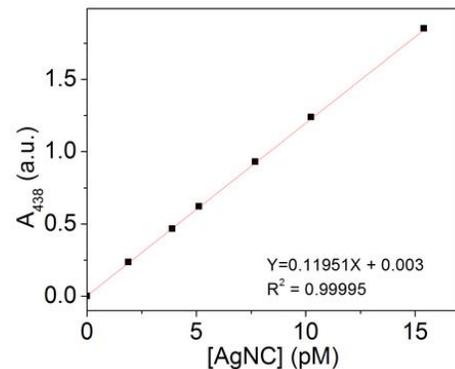

Y=0.11951X + 0.003
R^2 = 0.99995

Figure IIIA. Plot of extinction at 438 nm versus AgNC concentration.

Appendix IV – Sample Quiz

1) What are the functions of ethylene glycol (EG) and poly(vinyl pyrrolidone) PVP during the synthesis of Ag nanocubes?

2) Why does the galvanic replacement reaction between AgNCs and Au^{3+} result in a hollow, porous structure?

3) Why does the galvanic replacement reaction between AgNCs and Au^{3+} proceed spontaneously?

4) What is localized surface plasmon resonance (LSPR)? Give two examples for the applications of metal nanoparticles based on their (LSPR).

5) Briefly describe the physical effect of each component of extinction (*i.e.* what happens to the incident photon)?