JOURNAL OF CHEMICAL EDUCATION
Laboratory Experiment
pubs.acs.org/jchemeduc

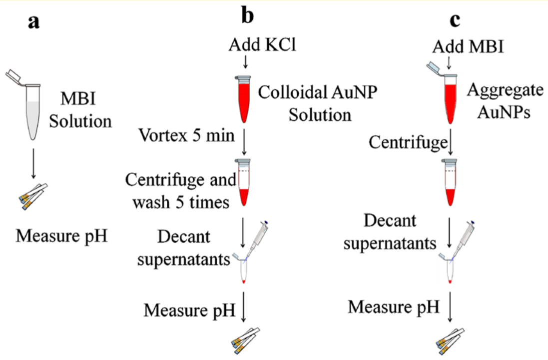
Undergraduate Laboratory Experiment Modules for Probing Gold Nanoparticle Interfacial Phenomena

Akila G. Karunanayake, Sameera R. Gunatilake, Fathima S. Ameer, Manuel Gadogbe, Laura Smith, Deb Mlsna,* and Dongmao Zhang*

Department of Chemistry, Mississippi State University, Mississippi State, Mississippi 39762, United States

Supporting Information

ABSTRACT: Three gold-nanoparticle (AuNP) undergraduate experiment modules that are focused on nanoparticles interfacial phenomena have been developed. Modules 1 and 2 explore the synthesis and characterization of AuNPs of different sizes but with the same total gold mass. These experiments enable students to determine how particle size affects the AuNP optical properties and ligand binding capacities. Module 3 investigates the fundamental mechanism governing organothiol self-assembly onto AuNPs and explores the fate of the sulfur-bounded hydrogen (RS−H) for organothiols on the AuNP surface. A benchtop centrifuge, a UV−vis spectrophotometer, and pH strips are needed. The depth of required chemistry knowledge is appropriate for upper-level chemistry students.



KEYWORDS: *Upper-Division Undergraduate, Laboratory instruction, Nanotechnology, UV-Vis spectroscopy, Hands-On Learning/Manipulatives*

INTRODUCTION

Nanoscience is the study of matter at the nanoscale. Nanoparticles, which are sized below 100 nm in one dimension, show properties different from their bulk counterparts. Generally, bulk materials display constant physical and chemical properties regardless of their sizes, but size-dependent properties are often observed at the nanoscale. Because nanomaterials have large surface to volume ratios, the relative contribution of the surface atoms to the overall physiochemical properties is drastically higher than that in bulk material. Therefore, characterization of nanoparticle surface area, morphology, and reactivity is fundamentally important in nanoscience research and technological development.

The National Science Foundation's estimated U.S. market value of products using nanotechnology is a trillion U.S. dollars or 5% of the GDP by 2020.[1] There is thus an urgent need for nanoscience education at undergraduate and high school levels to train a new generation of skilled workers. Unfortunately, only a very few undergraduate and high school level nanoscience laboratories can be found in literature.[2−7] Moreover, most of the published laboratory experiments focus on specific skills such as synthesizing nanoparticles,[5,7] application of nanoparticles such as synthesizing ZnO based sunscreen,[2] uptake and impact of silver nanoparticles on *Brassica rapa*,[6] and estimating the enhanced factors in surface enhanced Raman spectroscopy (SERS).[3,4] Nanochemistry undergraduate laboratory experiments dedicated to nanoparticle interfacial interaction is, to our knowledge, currently lacking.

Reported herein are three gold-nanoparticle (AuNP) based upper-division undergraduate experiment modules derived from our recent research on AuNP interfacial phenomena.[8−11] Underlying theoretical principles and the measurement techniques behind these experiments are accessible to undergraduate students, so the students can appreciate relevancy of their learning in classroom with cutting-edge research. Students will also recognize how simple techniques can be used to solve challenging scientific problems.

Modules 1 and 2 explore the synthesis and characterization of AuNPs of three different sizes while the total mass of AuNP remains the same. These experiments enable students to explore how the particle size affects the AuNP optical properties and ligand binding capacities. Module 3 aims at the investigation of fundamental mechanisms governing the organothiol self-assembly onto AuNPs. Organothiol (RS−H) self-assembly is the most commonly used strategy in nanoscience for surface modifications of gold.[10] The exact mechanism of the organothiol binding to gold has remained a topic of debate for years.[12−14] Recent research by Ansar et al. confirmed that RS−H hydrogens are released as protons.[10] In this experimental module, students will uses simple pH strips to determine the fate of the RS−H hydrogen in the organothiols adsorbed onto AuNP. This experimental design takes advantage of the fact that AuNP offers large surface-to-volume ratio.

ACS Publications

DOI: 10.1021/acs.jchemed.5b00535
J. Chem. Educ. XXXX, XXX, XXX−XXX

MODULE 1: SYNTHESIS OF AuNPs WITH THREE DIFFERENT SIZES

The AuNPs were synthesized with a citrate reduction method in which citrate serves as both the reductant and the stabilizing agent.[15] The latter prevents AuNP from aggregation. The citrate technique is well-suited for a hands-on, upper-division undergraduate laboratory exercise because of its simplicity and intriguing color changes associated with the synthesis process. In addition, the size of the synthesized AuNPs can be controlled by changing the sodium citrate concentration.[16] More concentrated citrate produces smaller AuNPs and vice versa. This is because the more citrate in the reaction solution, the more nuclei are formed at the beginning of the AuNP formation process. Since the amount of Au^{3+} precursor is constant, the average AuNP size decreases with increasing number of nuclei.

Full experiment details for module 1 including safety information is given in the Supporting Information. Briefly, gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$) stock solution is prepared by dissolving 0.1160 g of gold(III) chloride in 250 mL of nanopure water. Students are asked to calculate gold chloride concentrations on the basis of its measured mass and compare the result with the concentration calculated on the basis of UV−vis spectrum of the stock solution.[11] Because of the hygroscopic nature of $HAuCl_4{\cdot}3H_2O$, the simple mass calculation is likely erroneous. This is a good experience for students to learn how to properly characterize solutions of hygroscopic reagents.

AuNPs of three different sizes but with the identical total gold mass are prepared by adding different amount of sodium citrate into the same amount of gold chloride solutions. For each synthesis, a 250 mL flask that contains 50 mL of 1.18 mM gold chloride solution (quantified based on UV−vis absorbance) was heated to boil on a hot plate. Subsequently, 5.0 mL of sodium citrate solution was added into the gold chloride solution. The concentration of the sodium citrate solutions are 15.5, 38.8, and 77.6 mM, for the preparation of the large, medium, and small AuNPs, respectively. The final reaction solution was kept refluxed for 20 min under rapid magnetic stirring before cooled to room temperature. Conversion of Au^{3+} to AuNPs in these solutions can be confirmed by monitoring UV−vis absorbance of Au^{3+}. There is no detectable gold remaining in the colloidal AuNPs using UV−vis absorption spectrophotometry after centrifugation removal of the AuNPs.

Gold(III) chloride solution is initially yellow in color. Upon addition of sodium citrate, the solution suddenly becomes colorless, then blue within a fraction of a second, and after that slowly turns to wine red nanoparticles. The UV−vis spectra shown in Figure 1 shows that the AuNPs synthesized with lower concentrations of citrate exhibit longer peak UV−vis wavelength, thereby larger particle size. It has been previously established that the peak AuNP UV−vis wavelength increases with increasing AuNP size.[11,17] This UV−vis absorption is due to the AuNP localized surface plasmon resonance that occurs due to collective oscillation of the mobile electrons in AuNPs. Detailed information on how AuNP size, shape, and aggregation state affect the AuNP UV−vis peak intensity and wavelength can be found elsewhere.[18−20] For the sake of convenience, the AuNPs synthesized with different amounts of citrate will be referred to hereafter as $AuNP_S$ (small), $AuNP_M$ (medium), and $AuNP_L$ (large), respectively, in this work.

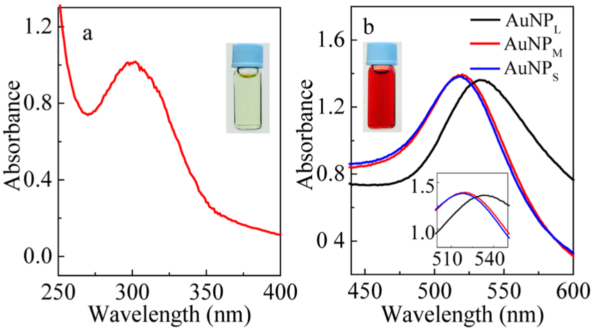


Figure 1. UV−vis spectra of (a) gold(III) chloride, (b) AuNPs of three different sizes. Insets in (a) and (b) are the photographs of the gold chloride and as-synthesized AuNP solutions, respectively.

MODULE 2: UV−VIS CHARACTERIZATION OF THE AuNP SIZE THROUGH QUANTITATIVE LIGAND ADSORPTION

The properties of nanomaterials are strongly size-dependent. Therefore, reliable determination of nanoparticle sizes is critically important in nanomaterial research and development. Multiple methods have been developed for AuNP sizing that include electron microscopy, dynamic light scattering, and AuNP UV−vis absorption method. The latter takes advantage of the fact that AuNP UV−vis peak wavelength depends on its particle size. While this UV−vis method is simple, it has limited sensitivity. For example, the AuNP UV−vis peak wavelength increases by less than 5 nm when AuNP increases from 5 to 35 nm in diameter.[17] In contrast, the UV−vis method presented in this module determines the AuNP size on the basis of the amount of the ligand adsorbed as a monolayer.[11] This ligand adsorption method is very sensitive to variations in AuNP size because the nanoparticle surface to volume ratio is inversely proportional to the size of nanoparticles. For example, the amount of ligand that can be adsorbed onto AuNPs with diameter of 5 nm is 7 times higher than that adsorbed onto AuNPs with diameter of 35 nm, assuming the total AuNP mass in these two samples are identical.

The probe ligand used in this module is 2-mercaptobenzimidazole (MBI), chosen for the following reasons: (1) MBI is a highly stable organothiol that has adequate solubility (~5 mM) and is highly stable in water for at least one year. This is in contrast to many other organothiols that have poor solubility and stability in water. (2) MBI adsorption induces AuNP aggregation and settlement, facilitating separation of the surface adsorbed MBI from the excess MBI in solution. (3) MBI is highly UV−vis active (ε = 27 400 cm^{-1} M^{-1} at λ_{max} of 300 nm). Thereby, one can use UV−vis to quantify the amount of MBI adsorbed onto AuNPs. (4) As a relatively small molecule, MBI can be densely packed onto the AuNP surface with a monolayer packing density of 574 pmol/cm^2.[9] This is important for ensuring the sensitivity of this ligand-adsorption based AuNP-sizing method. (5) MBI adsorption onto AuNP is an exceedingly rapid process. Complete ligand adsorption is achieved within 10 min of MBI mixing with AuNPs. Therefore, this ligand adsorption method can be easily completed within a lab period.

Full experiment details for module 2 is given in the Supporting Information. With the use of UV−vis spectra acquired with the supernatants of the ligand binding solutions (Figure 2A(c)), Figure 2B confirms that the smaller AuNPs adsorbed more MBI. If the mass of gold is held constant among

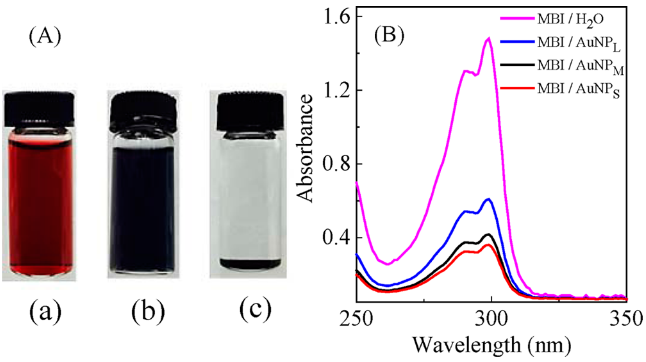


Figure 2. (A) Photographs of AuNP solutions (a) before, (b) right after, and (c) 3 h after MBI addition. (B) UV−vis spectra of (pink) MBI control, MBI mixed with (red) AuNP$_S$, (black) AuNP$_M$, and (blue) AuNP$_L$. Spectra of the samples were taken after AuNPs were completely settled to the bottom of the Eppendorf tube by carefully decanting the supernatant.

the three solutions, then the smaller AuNPs have larger total AuNP surface area, enabling more MBI to be adsorbed onto the AuNP surface.

Comparison of the experimental data obtained with the ligand adsorption method with that from the AuNP UV−vis absorption method is quite revealing. Indeed, reliable determination of the difference between AuNP$_S$ and AuNP$_M$ using AuNP peak UV−vis wavelength is difficult (Figure 1). However, the difference between these two samples in their MBI adsorption is much more prominent (Figure 2), indicating that the MBI adsorption method is more sensitive to particle size change. More importantly, this quantitative ligand experiment allows the student to appreciate how reducing particle sizes increases nanoparticle surface to volume ratio. The latter is one of the most important concepts in nanoscience and will engage students in understanding the unique characteristics inherent to nanoparticle formation.

■ MODULE 3: MECHANISM OF ORGANOTHIOL BINDING TO AuNPs

AuNP surface functionalized with organothiol including thiolated biomolecules have been used extensively in nanoparticle research. However, the exact mechanism of the organothiol binding to AuNP has remained controversial for many years.[17,11] Several possible pathways have been proposed in literature, in which the sulfur-bound hydrogen either remains intact on AuNPs, is released as hydrogen radical that eventually bonds with another hydrogen radical forming hydrogen gas, or is released as a proton (eqs 1−3).[12−14] This experiment module is adopted from a recent publication that shows organothiol binding to AuNP acidifying the ligand binding solution, but not producing hydrogen gas.[10] It provides a definitive evidence confirmation that the pathway depicted with eq 3 is the major reaction scheme when organothiol binds to AuNPs. This experimental module allows students to use a simple pH strip to answer a long-debated important issue regarding the fate of the sulfur-bound hydrogen for the organothiols on gold surface. No sophisticated instruments are needed for the entire experimental module.

$$\mathrm{RS{-}H + AuNP \rightarrow (RS{-}H){-}AuNP} \tag{1}$$

$$\mathrm{2RS{-}H + AuNP \rightarrow (RS)_2{-}AuNP + H_2} \tag{2}$$

$$\mathrm{2RS{-}H + AuNP \rightarrow 2(RS^-){-}AuNP + 2H^+} \tag{3}$$

To ensure the success of the experiment, the electrolytes in the as-synthesized colloidal AuNPs need to be removed. Otherwise, the excess electrolytes such as citrate can act as buffers, making it difficult to detect the pH change. The effect of citrate can be adequately removed by first adding KCl into the as-synthesized AuNPs, followed with washing the AuNPs with water. Experiment details for this module are given in the Supporting Information. Images shown in Figure 3 indicates that MBI adsorption onto AuNP acidified the ligand binding solution. The centrifugation removal of AuNPs in AuNP-containing samples is important to avoid AuNP deposition onto the pH strips. These data showed that the pH of the AuNP and MBI control is between 5 and 6, but that for the AuNP and MBI mixture is between 4 and 5.

No hydrogen bubbles were observed during the entire ligand adsorption process. This confirms that eq 3 is the one main reaction pathway when organothiol is adsorbed onto AuNP surfaces.

■ HAZARDS

Student should wear goggles and gloves throughout the experiment and prevent skin contact with all chemicals. Aqua regia (nitric acid and hydrochloric acid) is caustic and should only be used in a fume hood. Gold(III) chloride and potassium chloride are corrosive chemicals that can cause burns on contact with the skin and eyes. Any waste that contains gold or AuNPs must be disposed of in a hazardous waste container.

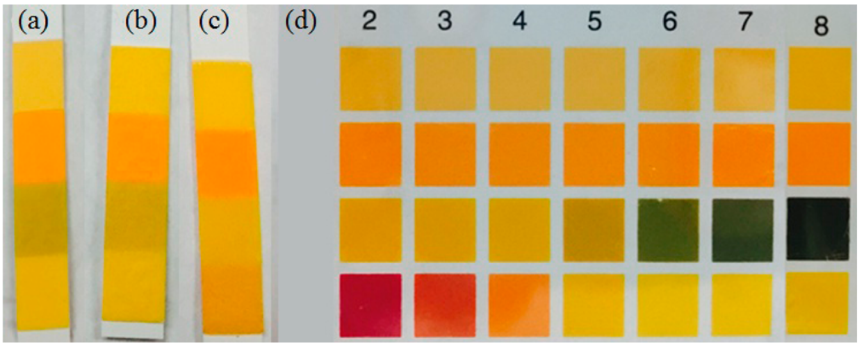


Figure 3. Photograph of the pH strips after dipping them into (a) centrifuge supernatant of AuNP solutions, (b) MBI solution, and (c) centrifuge supernatant of AuNP and MBI mixture, respectively. (d) The scale bars showing the correlation between the colors of the pH strip and solution pHs.

DOI: 10.1021/acs.jchemed.5b00535
J. Chem. Educ. XXXX, XXX, XXX−XXX

STUDENT LEARNING OBJECTIVES AND OUTCOMES

This upper-division undergraduate experiment was performed with three different groups of undergraduate students (24 students total) including upper-level analytical students in an instrumentation analysis course. Student evaluations of the laboratory modules indicated that they felt the nanoparticle topic "engaged their interest" (average of 4.0 "agree" on a Likert scale of 1−5); they found the "experiment interesting to perform" (3.8 out of 5) and they would "recommend others to do this lab" (4.2 out of 5). The rapidly changing colors of the AuNP solutions elicited the most positive comments from students. Ninety-two percent of the students trialed successfully completed the suggested calculations to obtain suitable AuNP sizes. The experience of synthesizing and characterizing these gold nanoparticles is important in exposing students to novel areas of chemistry.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00535.

> Additional information for instructors, detailed procedures, and student worksheets (PDF, DOCX)

AUTHOR INFORMATION

Corresponding Authors

*E-mail:Dongmao@chemistry.msstate.edu (Dr. Dongmao Zhang).
*E-mail: dmlsna@chemistry.msstate.edu (Dr. Deb Mlsna).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by an NSF CAREER Award (CHE 1151057) and a NSF fund (EPS-0903787) provided to D.Z.

REFERENCES

(1) The National Nanotechnology Infrastructure Network website. http://www.nnin.org (accessed April 2014).

(2) Guedens, W. J.; Reynders, M.; Van den Rul, H.; Elen, K.; Hardy, A.; Van Bael, M. K. ZnO-Based Sunscreen: The Perfect Example To Introduce Nanoparticles in an Undergraduate or High School Chemistry Lab. *J. Chem. Educ.* **2014**, *91* (2), 259−263.

(3) Weaver, G. C.; Norrod, K. Surface-enhanced Raman spectroscopy: a novel physical chemistry experiment for the undergraduate laboratory. *J. Chem. Educ.* **1998**, *75* (5), 621−624.

(4) Pavel, I. E.; Alnajjar, K. S.; Monahan, J. L.; Stahler, A.; Hunter, N. E.; Weaver, K. M.; Baker, J. D.; Meyerhoefer, A. J.; Dolson, D. A. Estimating the Analytical and Surface Enhancement Factors in Surface-Enhanced Raman Scattering (SERS): A Novel Physical Chemistry and Nanotechnology Laboratory Experiment. *J. Chem. Educ.* **2012**, *89* (2), 286−290.

(5) McFarland, A. D.; Haynes, C. L.; Mirkin, C. A.; Van Duyne, R. P.; Godwin, H. A. Color my nanoworld. *J. Chem. Educ.* **2004**, *81* (4), 544A−544B.

(6) Metz, K. M.; Sanders, S. E.; Miller, A. K.; French, K. R. Uptake and Impact of Silver Nanoparticles on Brassica rapa: An Environmental Nanoscience Laboratory Sequence for a Nonmajors Course. *J. Chem. Educ.* **2014**, *91* (2), 264−268.

(7) Mulfinger, L.; Solomon, S. D.; Bahadory, M.; Jeyarajasingam, A. V.; Rutkowsky, S. A.; Boritz, C. Synthesis and study of silver nanoparticles. *J. Chem. Educ.* **2007**, *84* (2), 322−325.

(8) Zhang, W.; Govorov, A. O.; Bryant, G. W. Semiconductor-Metal Nanoparticle Molecules: Hybrid Excitons and the Nonlinear Fano Effect. *Phys. Rev. Lett.* **2006**, *97* (14), 146804/1−146804/4.

(9) Ansar, S. M.; Haputhanthri, R.; Edmonds, B.; Liu, D.; Yu, L.; Sygula, A.; Zhang, D. Determination of the Binding Affinity, Packing, and Conformation of Thiolate and Thione Ligands on Gold Nanoparticles. *J. Phys. Chem. C* **2011**, *115* (3), 653−660.

(10) Ansar, S. M.; Perera, G. S.; Jiang, D.; Holler, R. A.; Zhang, D. Organothiols Self-Assembled onto Gold: Evidence for Deprotonation of the Sulfur-Bound Hydrogen and Charge Transfer from Thiolate. *J. Phys. Chem. C* **2013**, *117* (17), 8793−8798.

(11) Gadogbe, M.; Ansar, S. M.; He, G.; Collier, W. E.; Rodriguez, J.; Liu, D.; Chu, I. W.; Zhang, D. Determination of colloidal gold nanoparticle surface areas, concentrations, and sizes through quantitative ligand adsorption. *Anal. Bioanal. Chem.* **2013**, *405* (1), 413−422.

(12) Kankate, L.; Turchanin, A.; Goelzhauser, A. On the Release of Hydrogen from the S-H groups in the Formation of Self-Assembled Monolayers of Thiols. *Langmuir* **2009**, *25* (18), 10435−10438.

(13) Matthiesen, J. E.; Jose, D.; Sorensen, C. M.; Klabunde, K. J. Loss of Hydrogen upon Exposure of Thiol to Gold Clusters at Low Temperature. *J. Am. Chem. Soc.* **2012**, *134* (22), 9376−9379.

(14) Freeman, R. G.; Hommer, M. B.; Grabar, K. C.; Jackson, M. A.; Natan, M. J. Ag-Clad Au Nanoparticles: Novel Aggregation, Optical, and Surface-Enhanced Raman Scattering Properties. *J. Phys. Chem.* **1996**, *100* (2), 718−724.

(15) Turkevich, J.; Stevenson, P. C.; Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, *11* (0), 55−75.

(16) Frens, G. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature, Phys. Sci.* **1973**, *241* (105), 20−2.

(17) Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV−Vis Spectra. *Anal. Chem.* **2007**, *79* (11), 4215−4221.

(18) Masson, J.-F.; Yockell-Lelièvre, H. Spectroscopic and Physical Characterization of Functionalized Au Nanoparticles: A Multiweek Experimental Project. *J. Chem. Educ.* **2014**, *91* (10), 1557−1562.

(19) Lee, C.-F.; You, P.-Y.; Lin, Y.-C.; Hsu, T.-L.; Cheng, P.-Y.; Wu, Y.-X.; Tseng, C.-S.; Chen, S.-W.; Chang, H.-P.; Lin, Y.-W. Exploring the Stability of Gold Nanoparticles by Experimenting with Adsorption Interactions of Nanomaterials in an Undergraduate Lab. *J. Chem. Educ.* **2015**, *92* (6), 1066−1070.

(20) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *J. Phys. Chem. B* **2003**, *107* (3), 668−677.