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Laboratory Experiment

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Exploring the Stability of Gold Nanoparticles by Experimenting with Adsorption Interactions of Nanomaterials in an Undergraduate Lab

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Ⓢ *Supporting Information*

ABSTRACT: The proposed experiment can help students to understand the factors involved in the stability of gold nanoparticles (Au NPs) by exploring the adsorption interaction between Au NPs and various substances. The students in this study found that the surface plasmon resonance band of Au NP solutions underwent a red shift (i.e., from 520 to 650 nm) because of NaCl-induced aggregation caused by the elimination of the repulsive electrostatic force. In addition, a sufficient amount of bovine serum albumin molecules (29.4 nM) adsorbed on the surface of Au NPs (1.8 nM) through electrostatic interactions provides steric barriers that hinder electrolyte-induced aggregation. This experiment was performed in the fall 2014 semester to improve the recognition of nanoscale science and engineering concepts of undergraduates.

KEYWORDS: *First-Year Undergraduate/General, Laboratory Instruction, Curriculum, Hands-On Learning/Manipulatives, Nanotechnology*

■ INTRODUCTION

Materials in the nanoscale range (1−100 nm) exhibit chemical and physical properties different from those of their bulk counterparts because of quantum effects, higher surface-to-volume ratios, and high activity of surface atoms.[1−3] Various industries, such as electronics, pharmaceuticals, and cosmetics, apply nanotechnology-based solutions for improving their products.[4−6] Thus, not only scientific researchers but also laypeople must have a fundamental understanding of concepts related to nanoscale science and engineering (NSE).[7−11]

Factors such as size, morphology, and surface modifications are strongly influenced by the energy band gap and surface plasmon resonance of nanomaterials. Because biomolecules such as proteins and DNA are nanoscale structures, many modern bioanalytical technologies, including DNA analysis, biomolecule sensing, and cancer therapy, incorporated with nanomaterials are the building blocks of biosensors. The sensing scheme of a biosensor is based on the interaction between nanomaterials and biomolecules for changing the optical properties of the nanomaterials. Because the sensitivity and selectivity of a biosensor are determined according to the adsorption density of proteins on the surface of nanomaterials, ascertaining the stability and saturated adsorption concentration of proteins on the nanomaterials is crucial. Therefore, a coherent curriculum that can help students build meaningful knowledge of the concepts of NSE that can enable them to understand various nanoscience-related phenomena must be developed.[12−16]

Among the nine main concepts of NSE, "forces and interactions" is essential for chemical and physical applications.[3,17] Nanotechnology exploits interactions inherent within nanomaterials and biomolecules to create biosensors. Creating such biosensors requires understanding their fabrication process and how the individual parts interact.[18,19] In addition, the distinct interactions of nanomaterials make them excellent scaffolds for fabricating novel chemical and biological sensors.[3,20] Therefore, it is essential to understand and control the forces that occur between two material structures, not only in designing a nanoscale structure but also in determining the usefulness of a biosensor. Forces and interactions must be carefully considered in controlling nanomaterials from all aspects of the sensing process: design, fabrication, characterization, processing, and manipulation.

Metal nanomaterials are generally synthesized using a chemical reduction method.[21−25] Organic solvents, sodium borohydride as reducing agents, and surfactants and polymers as stabilizer agents are used for synthesizing gold and other metal nanomaterials.[21,23,24] Nanomaterials are typically prepared using aqua regia cleaned glassware. Unfortunately, the organic solvents, surfactants, polymers, and aqua regia substances are harmful to human health and detrimental to the environment. In addition, most of the current synthetic procedures have been designed at a large scale (50−100 mL) with a reflux setup, rather than at a small scale (<10 mL).[22]

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Although several undergraduate experiments on the synthesis of gold nanoparticles (Au NPs) and investigation into their optical properties have been performed, experiments in which students can use a straightforward method for synthesizing Au NPs and explore the forces and interactions between Au NPs and substances (such as electrolytes, nonelectrolytes, and biomolecules) remain limited.

Based on hands-on nanotechnology experiments, we propose the following experimental design as an instructional material for facilitating the understanding of forces and interactions by students. The proposed experiment could be implemented in physical, analytical, materials, or surface chemistry courses. In the fall 2014 semester, chemistry-, biology-, and physiology-major undergraduates, in groups of two or three, performed this experiment for a 3 h general chemistry laboratory session. In the first session, the students were introduced to the experimental process, content background of the synthesis and characterization of Au NPs, and concepts of NSE. In the second session, the students followed a well-defined, simple, and cost-effective synthetic procedure for preparing a colloidal suspension of Au NPs (diameter 13.3 nm) by applying the citrate-mediated reduction of Au^{3+} ions. The students then investigated the stability properties of Au NP colloidal suspensions in various substances. In the third session, the students performed data analysis and discussed the interactions between Au NPs and various substances. This experiment covered concepts on the synthesis, characterization, and stabilization of Au NPs. Moreover, three analytical techniques were used to explore the interactions between Au NPs and other substances: ultraviolet−visible (UV−vis) spectroscopy, transmission electron microscopy (TEM), and zeta-potential measurement. These techniques are selected depending on available instruments. For example, a UV−vis spectroscope, generally available in all teaching laboratories, can be used to study the size-dependent optical properties of Au NPs. TEM analysis and zeta potential measurements were performed for additional verification by graduates who had spent considerable time in an analytical lab. To ensure that the students satisfactorily understood NSE concepts, we administered a multiple-choice question examination on the concepts before and after the curriculum sessions. Additional details are provided in the instructor information section.

■ EXPERIMENTAL PROCEDURES

Chemicals

Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, NaCl, glucose, sucrose, bovine serum albumin (BSA), disodium phosphate, and monosodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q ultrapure water was used in each experiment.

Preparation of Solutions

Sodium citrate, $HAuCl_4$, glucose, sucrose, NaCl, and BSA stock solutions must be prepared. Additional details are provided in the Instructor Information in the Supporting Information.

Synthesis of 13 nm Au NPs

We prepared Au NPs by slightly modifying the citrate-mediated reduction of Au^{3+} ions.[22,23,25] The concentration of Au NPs (approximately 15 nM) was measured using a UV−vis spectrophotometer according to Beer's law by applying an extinction coefficient of 10^8 M^{-1} cm^{-1} at 520 nm for Au NPs with a 13.3 nm diameter.[26] Additional details are provided in the Student Activity and Instructor Information in the Supporting Information. The Au NP colloidal suspensions can be stored for several months in a brown bottle.

General Procedures for the Colorimetric Assay

For evaluating the stability of Au NPs, place 300 μL of Au NP colloidal suspensions into each of four vials. Add 1.5 mL of distilled water to each vial. With a disposable pipet, add 4 drops (200 μL) of distilled water, 1 M glucose solution, 1 M sucrose solution, or 1 M NaCl solution to each of the four vials. Then, record the observations.

To evaluate the saturated adsorption concentration of BSA by using a salt screening process, place 200 μL of Au NP colloidal suspensions into each of 10 vials. Add 1.4 mL of phosphate buffer (PB) solution (pH 9, 8.0 mM) containing different concentrations of BSA solutions (0−0.04 μM) to each vial according to the amounts listed in Table S1 in the Student Activity in the Supporting Information. Close the cap tightly, shake the mixture for 10 min, allow the mixture to stand for 20 min, add two drops (100 μL) of 2 M NaCl solution, and again shake the mixture uniformly. Record the observations.

■ HAZARDS

During all experiments, wear safety glasses and disposable gloves. Boiling water baths must be handled with care to avoid burns. $HAuCl_4$ is corrosive and hygroscopic; it can cause eye and skin burns on contact. Other chemicals used in the experiments are not dangerous; however, contact with these chemicals should be avoided.

■ RESULTS

Stability of Au NPs

Bulk gold has a familiar golden color, which is attributed to the reduction of the reflectivity of light at the end of the spectrum in the visible region. The color of a sufficiently small gold particle is ruby red because of the strong absorption of green light at approximately 520 nm, which corresponds to the frequency at which the plasmon resonance of gold occurs. Plasmon resonance occurs when the radius of an atom is longer than the wavelength of light.[21] Free mobile electrons are trapped in a metal box and show the characteristic collective oscillation frequency of plasma resonance, giving rise to the so-called surface plasmon resonance (SPR) band, which is observed near 520−530 nm for Au NPs ranging from 5 to 20 nm in diameter. This intrinsic property of Au NPs is mainly governed by their size, shape, crystallinity, and structure. For example, the SPR band of spherical Au NPs undergoes red shifts when their size increases. By applying such distinctive optical characteristics, many sensing systems have been developed, based on colorimetric changes and the alternation in the absorption spectra on aggregation.

Figure 1 displays photographic images and UV−vis absorbance spectra of solutions containing Au NPs (a) without any electrolyte and in the presence of (b) 0.1 M glucose, (c) 0.1 M sucrose, and (d) 0.1 M NaCl. The Au NP colloidal suspensions without any electrolyte or with nonelectrolytes (glucose or sucrose) exhibited an SPR band centered at approximately 520 nm; therefore, as shown in the inset of the UV−vis absorbance spectra, these solutions are rose red, which corresponds to the frequency at which the plasmon resonance of gold occurs. After the addition of an electrolyte, NaCl, the rose-red color of the Au NP colloidal suspensions changed to dark gray, and the corresponding SPR band underwent a red

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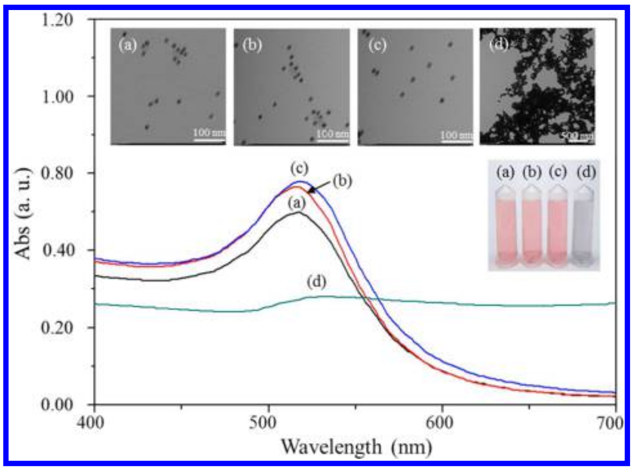

Figure 1. UV−vis absorbance spectra along with the TEM images and photographic image (inset) of Au NP colloidal suspensions (a) without any other solute and with (b) 0.1 M glucose, (c) 0.1 M sucrose, and (d) 0.1 M NaCl substances.

shift with a decreased intensity at 520 nm, whereas the intensity of the band at 650 nm increased. The TEM images in Figure 1 show that Au NPs without additional substances and those in the presence of nonelectrolytes nearly retained their initial sizes and shapes; however, after NaCl was added, Au NPs aggregated. Thus, the absorbance and wavelength at 520 and 650 nm are characteristic of dispersed and aggregated Au NPs, respectively.

The negatively charged citrate anions employed in the preparation of Au NPs by using the citrate-mediated reduction of Au^{3+} ions act as capping agents by adsorbing on the surface of each Au NP, thus producing negative charges that separate NPs through electrostatic repulsion. When NaCl was added to the Au NP colloidal suspensions, the Na^+ ions were attracted by the negatively charged citrate anions adsorbed to the surface of Au NPs, and the ions tended to eliminate the repulsive electrostatic force (i.e., salt screening). Therefore, Au NPs aggregated and the average distance among them decreased to less than their average diameter, and the solution began absorbing light at approximately 650 nm (i.e., red shift in the absorbance spectrum).

Saturated Adsorption Concentration of BSA on the Surface of Au NPs

Figure 2A shows photographic images and UV−vis absorbance spectra of solutions containing Au NPs with different concentrations of BSA in the presence of 0.1 M NaCl. The color of the Au NP colloidal suspensions, with increasing BSA concentration, gradually changed from dark gray to purple and then to rose red. The TEM images at the top of Figure 2 show that Au NPs changed from aggregated particles (for BSA < 25.7 nM) to nonaggregated particles (for BSA > 29.4 nM) in the presence of NaCl.

Proteins constitute by amino acids that can be adsorbed to the surface Au NPs through self-assembly interactions. The types of self-assembly involve ionic, hydrophobic, and electrodynamic (induced-dipole) interactions. Consequently, attached proteins provide steric barriers near the surface of Au NPs, thus hindering electrolyte-induced aggregation.

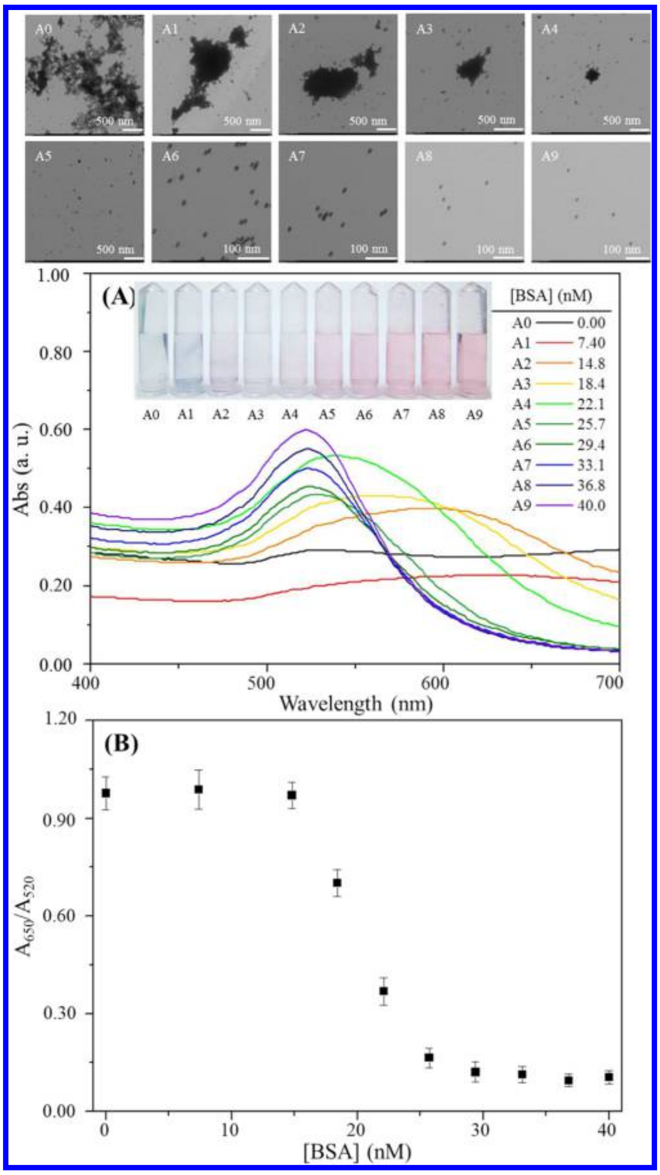

Figure 2. (A) UV−vis absorbance spectra, photographic image (inset), and TEM images of Au NP colloidal suspensions with different concentrations of BSA in the presence of 0.1 M NaCl. (B) Plot of A_{650}/A_{520} of Au NPs as a function of BSA concentration.

Moreover, the saturated adsorption concentration of BSA on the surface of Au NPs can be determined by using a salt screening process. As shown in Figure 2B, the absorbance ratio (A_{650}/A_{520}) of Au NPs decreases with increasing BSA concentration. The point at which the color of the Au NP colloidal suspensions changes from purple to rose red is called the flocculation point (vial A6 in the inset of Figure 2A). The concentration of BSA at the flocculation point is the saturated adsorption concentration of BSA on the surface of Au NPs; this concentration was approximately 29.4 nM. In the experiment, the concentration of Au NPs was 1.8 nM. Assuming that BSA binding to Au NPs occurs in the Langmuir isotherm mode, the average number of BSA molecules adsorbed on each Au NP can be calculated using the following formula: number of BSA on each Au NP = (saturated adsorption concentration of BSA $\times$ solution volume $\times 6.02 \times 10^{23}$)/(concentration of Au NPs $\times$ solution volume $\times 6.02 \times 10^{23}$), in which the volume of the experimental solution was 1.7 mL. Therefore, by using the aforementioned formula, we demonstrated that an average of 17 BSA molecules adsorbed on each Au NP. The number of protein molecules adsorbed on each Au NP (i.e., 19) obtained using a quartz crystal microbalance with dissipation (QCM-D) was higher than that measured using the proposed optical method because the frequency change in liquid QCM-D is more sensitive to both protein and bound water molecules.[27]

In general, when citrate is present, protein molecules are adsorbed on the surface of Au NPs through electrostatic interactions.[27] The degree of adsorption is independent of the isoelectric point, but typically depends on the protein molecular weight.[28] At a constant size of Au NPs, the interaction sites between protein molecules and Au NPs are inversely proportional to two-thirds of the power of the protein molecular weight. In addition, the binding constant for a protein molecule and an Au NP increases with increasing protein molecular weight. The maximum amount of protein adsorption on the surface of an Au NP is inversely proportional to the projection area of the protein.[27]

■ DISCUSSION

To ensure that undergraduates satisfactorily understood the NSE concepts, we conducted an examination based on multiple-choice questions on NSE concepts before and after curriculum sessions. The test included 20 questions [size and scale (2), structure of matter (4), size-dependent properties (4), quantum effects (2), forces and interactions (6), and tools and instrumentation (2)]; the total score was 100. The scores are provided in the Instructor Information (Table S3 in the Supporting Information).

By undergoing this test, regarding size and scale, the students understood that the surface-to-volume ratio of an object depends on its size and shape. Regarding structure of matter, they understood that atoms are the fundamental building blocks of matter to form organized assemblies and structures (e.g., NPs).

Furthermore, regarding forces and interactions, they learned that small objects can interact in various ways. Electric force describes all interactions within matter. The interaction characteristics play a role in assembly formation and functioning. Several factors, including the interacting objects and the environment, play a role in the formation of any interaction. Electrostatic forces are required for explaining various microscopic phenomena. Regarding size-dependent properties, the students understood that some of the characteristic properties of matter change with size, particularly as the length scale of the sample decreases and approaches the atomic scale. Thus, the present experiment demonstrates the size dependence of the optical properties of Au NPs.

In addition, regarding quantum effects, the students understood that as a material becomes small in size at the nanoscale level, the importance of the wavelike character increases, and quantum mechanics is required to predict and explain the behavior of the material. Only discrete energy can interact with nanoscale systems. Regarding tools and instrumentation, the students learned that specialized tools are required to detect, measure, and investigate nanomaterials because nanomaterials are too small to be visualized using optical microscopes.

■ CONCLUSION

The presented experiment highlights the current major concepts of NSE, including (a) size and scale, (b) structure of matter, (c) tools and instrumentation, (d) quantum effects, (e) size-dependent properties, and (f) forces and interactions. In addition, environmentally friendly reagents and solvents were used, thus exhibiting high efficiency regarding both materials and time. Therefore, the experimental procedure partly fulfills the criteria for a green chemistry experiment. After the students performed this experiment, they improved their understanding of certain NSE concepts (Table S3 in the Instructor Information in the Supporting Information). Therefore, the present experiment can help undergraduates to understand NSE concepts.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Typical experimental process and results (the sequential color changes observed during the process of Au NP synthesis, TEM images, Tyndall effect, and dynamic light scattering analysis of the Au NPs colloidal suspensions); Analogous the Programme for International Student Assessment (PISA) test; student activity. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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