INSTRUCTOR MANUAL

Optical properties of gold nanoparticles

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OVERVIEW ON THE USE OF THIS MODULE

This module is designed to guide students to investigate gold nanoparticles, their synthesis and optical properties, with the goal of identifying experimental conditions that lead to the synthesis of nearly monodisperse gold nanoparticles for sensor development applications.

The module is designed as a sequence of class activities and provides a set of experimental data (TEM images, size and size distribution analyses, and absorbance vs. concentration data) that can be downloaded from the ASDL site. Additional TEM images are available upon request from the instructor. Alternatively, if the instructor has access to a transmission electron microscope and a UV-VIS spectrophotometer, the module could be used in the context of a lab experiment where students, divided in groups of 2 or 3, can conduct gold nanoparticle syntheses exploring different experimental parameters. The lab outcome would be a comparison of optical properties for gold nanoparticles synthesized under different conditions of reagents ratio and pH.

Whether conducted as class activity or actual experimental lab, the instructor should ensure that students receive instructions for chemical safety and hazardous material handling (refer to SDS for each chemical). Safety goggles, lab coats and gloves should be worn when performing the activity in a lab setting.

The materials are designed to be modular in their format and used relatively independently. They can also be implemented at different levels of guidance to students. For example, instructors in a general chemistry course could provide students with more detailed instructions on the synthesis and characterization of gold nanoparticles while students in an upper division course may be asked to explore the literature, develop the experimental procedure for the synthesis and fine tune experimental conditions and instrumental parameters if a TEM and/or a UV-VIS spectrophotometer are used.

Students are first introduced to the nano dimension and relevance of nanoparticles in the area of optical sensors development. They are then guided to explore fundamental relationships in spectrophotometry which will be used later in the module to determine the molar extinction coefficient of different sizes of nanoparticles. Depending on the level of preparation, the instructor may choose to provide additional information on gold nanoparticle structure and properties and familiarize students with UV-VIS spectroscopy and use of instrumentation, if available.

ANSWERS TO QUESTIONS

The following section provides answers to the questions inter dispersed in sub sections of the module. A brief introduction to the nano dimension and relevance of nanoparticles in the area of optical sensors development is provided. Depending on the level of preparation, the instructor may choose to provide additional information on gold nanoparticle structure and properties.

Exploring fundamental relationships in spectrophotometry

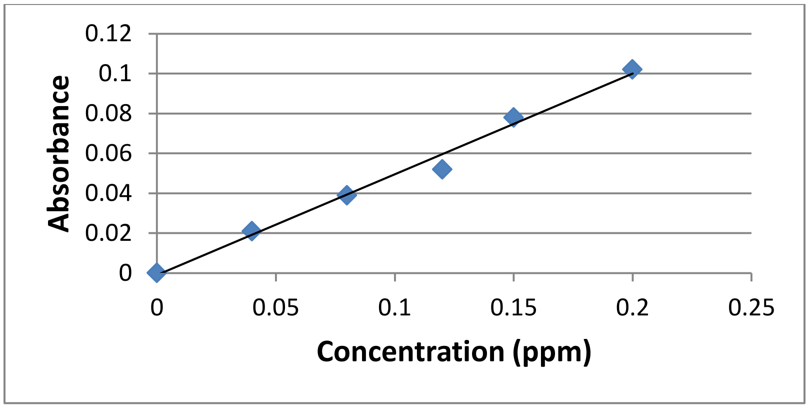
This section of the module is designed to provide a review of basic concepts of spectrophotometry. If students have never been introduced to spectrophotometry, a more in depth coverage may be necessary to bring them up to speed, particularly if the module is being used as a lab experiment.

Q1*. What wavelength range constitutes the visible region in the electromagnetic spectrum?*

The visible region corresponds to wavelengths between 400 and 700 nm.

Q2. *Draw a representative plot of A versus c.*

Students should draw a typical plot reporting the absorbance on the y-axis and the concentration on the x-axis. A generic plot is presented below.



Q3. *How could you use this plot to determine the molar extinction coefficient of the analyte being investigated?*

According to Beer's law, $A = \varepsilon bc$, where A is the absorbance, ε is the molar extinction coefficient, b is the path length of the cuvette and c is the concentration. Thus, the molar extinction coefficient can be obtained by calculating the slope of the absorbance vs. concentration plot. Since in most instances the path length b of the cuvette is equal to 1 cm, the slope is the same value as ε.

Q4. *What color would a particle that absorbs in the blue-green region of the electromagnetic spectrum appear?*

The particle would appear red. As showed in Figure 2, gold nanoparticles can appear anywhere from red to purple/blue depending on their size.

Q5. *What trend can be identified in the molar extinction coefficient value as particle size increases? How may this trend affect the choice of particle size in a colorimetric sensor?*

To answer these questions students should be given time to analyze data provided in Table 1. The table shows that the molar extinction coefficient increases by four orders of magnitude as particle size increases from 5 to 100 nm. Answering the second part of the question may be more challenging. First, the instructor may want to clarify what a colorimetric sensor is. A pregnancy test is a good example. To further guide students, the instructor may want to discuss the significance of the molar extinction coefficient in terms of color intensity. The larger the extinction coefficient, the larger the absorbance for the same concentration of sample. Thus, when developing a colorimetric sensor, particles with larger molar extinction coefficient will produce larger changes in absorbance for the same concentration change, resulting in a more sensitive sensor.

Synthesis of gold nanoparticles

This section of the module first provides some background information about the most common method used for the synthesis of gold nanoparticles which involves the reduction of tetrachloroauric acid ($HAuCl_4$) by small amounts of citric acid. This method was introduced by Turkevich et al. in 1951 and further refined by Frens in 1973. In this method, hot tetrachloroauric acid reacts with a small amount of sodium citrate solution. The citrate ions act as both a reducing agent, and a capping agent. Reduction occurs according to the reactions:

$$Na_3C_6H_5O_7 \text{ (sodium citrate)} + 3H^+ \rightarrow C_6H_8O_7 \text{ (citric acid)} + 3Na^+$$

$$2HAuCl_4 + 3C_6H_8O_7 \text{ (citric acid)} \rightarrow 2Au + 3C_5H_6O_5 \text{ (3-ketoglutaric acid)} + 8HCl + 3CO_2$$

In the traditional Frens' method, small citrate to tetrachloroauric acid ratios are used without particular attention to pH and the typical trend observed is that particle size decreases as the ratio of citrate to tetrachloroauric acid increases. This is consistent with the mechanism where part of the sodium citrate is used to reduce Au^{3+} to Au and the remaining sodium citrate ions are available for stabilizing the particles (capping agent). Nanoparticles continue to aggregate until the total surface area of all particles becomes small enough to be covered by the existing citrate ions. Therefore, higher concentrations of sodium citrate result in less particle aggregation and the final size of the particles decrease. However, Ji et al. (reference 7) observed that if the ratio is increased beyond 3.5:1, the particle size actually increases and eventually levels off for very large ratios. This can be attributed to the effect the citrate plays on the final pH of the solution. Upon addition of larger amounts of Na_3Ct to $HAuCl_4$, the pH increases and shifts the gold complex equilibrium toward more hydrolyzed forms. In addition, oxidation products of citrate are formed. All these processes create a continuously changing Turkevich reaction system with a great complexity and a variety of possible pathways for the reduction of Au^{3+}. pH also affects the ionization of citrate depending where the pH falls with respects to three pKa values of 3.2, 4.8 and 6.4. As more citric acid is in the form of citrate (with a -3 charge), the ionic strength of the solution increases and the colloidal stability of the seed particles decreases. As consequence, the seed particle size increases leading to less particles and larger final sizes.

Students are directed to investigate both the effect of changing the Na_3Ct to $HAuCl_4$ ratio as well as the pH of the solution at three different values slightly above each respective pKa. These values were chosen because of the larger particle size observed which yield larger extinction coefficients and are more suitable for sensor application. However, to thoroughly investigate the role that pH plays in the synthesis of gold nanoparticles, the instructor will have to widen the pH range from 1 to 8 (see reference 7).

Q6. *What is the oxidation number of gold in $HAuCl_4$?*

The oxidation number is +3.

Q7. *Write the half reaction for the reduction of $HAuCl_4$ to Au.*

$HAuCl_4 + 3e^- \rightarrow Au + H^+ + 4Cl^-$

Q8. *Above what pH value will citric acid ($C_6H_8O_7$) be completely dissociated to citrate ($C_6H_5O_7^{3-}$)?*

Since citric acid is a triprotic acid with pKa values of 3.2, 4.8 and 6.4, the acid will be completely dissociated to citrate at pH values above 6.4.

Developing an experimental design to investigate gold nanoparticle synthesis

In this part of the module students are asked to research and read scholarly articles pertaining to the synthesis of gold nanoparticles and come up with an experimental procedure. They should be referred to the basic approach developed by Turkevich (reference 8) as well as more recent papers (reference 6 and 7). Most articles do not address pH as a variable, so the instructor should make sure that students are aware of these two references.

Q9. *Which experimental parameters can be varied that could potentially affect the growth of nanoparticles?*

The most obvious parameters are the relative concentrations of citrate to auric acid. Students can explore any ratio but data are available for 2:1, 4:1, and 7:1 ratio of citrate to auric acid. pH is also an important variable and gold nanoparticle synthesis could be explored at pHs slightly above each pKa value of citric acid. Data are included for pH values of 4.2, 5.4 and 7.0. Other possible variables to explore include rate of citrate addition, order of reagents, temperature, stirring rate, etc. However, these variables have not been investigated in our study and no data are available to illustrate any potential effects.

Determining nanoparticles size and size distribution

In this part of the module students first visually examine Transmission Electron Microscopy (TEM) images of two different nanoparticle solutions synthesized under different conditions and qualitatively estimate differences in size and size distribution. Next, they can use the provided image files and a free software called **ImageJ** (http://imagej.nih.gov/ij/) to calculate the Feret's diameter and particle size distribution for each synthetic condition. Instructions on how to use ImageJ are provided in the module.

Q10. *Looking at the images on a qualitative basis, what differences do you observe? What can be said about the size of the particles and their size distribution at the two different experimental conditions?*

There are distinct differences between the two high resolution transmission electron microscopy images. The gold nanoparticles synthesized at pH 5.4 with a 2:1 ratio of citrate to tetrachloroauric acid (figure 5a) appear smaller and much more uniform than those synthesized at pH 5.4 with a 7:1 ratio (figure 5b). The larger citrate to auric acid ratio definitely generates particles of larger size although a wide distribution of sizes is apparent.

Q11. *What is the value of the Feret's diameter you obtained from the analysis of the image showed in Figure 5a?*

The average diameter of particles synthesized at pH 5.4 with a 2:1 ratio of citrate to tetrachloroauric acid is 21.73 ± 0.40 (estimated through ImageJ analysis). Note the small standard deviation indicating a homogeneous distribution of sizes.

Q12. *What is the effect of changing the reagents molar ratio on particle size?*

As the ratio of citrate to tetrachloroauric acid increases from 2:1 to 7:1, the average diameter of the gold nanoparticles increases from approximately 21 to 34 nm.

Q13. *What is the effect of varying the pH on particle size?*

Although within the range of pH values explored in this study the pH only slightly affects the particle size, overall pH plays a major role on particle size. Students will observe later that pH also has a dramatic effect on particle size distribution. The traditional conditions of gold nanoparticle synthesis happen to be in a fortuitous pH window (2.7 – 4, see reference 7) that promotes seed-mediated growth mechanism with particles of diameter around 10 nm. However, outside this window, the synthesis outcome is very different. For pH < 1.5, no particles are observed because the protonated citric acid is incapable of reducing Au^{3+} since the reduction mechanism requires one deprotonated carboxy group. For pH > 4, which is the pH range covered in this experiment, the initial amount of reactive $[AuCl_4]^-$ is too low to trigger the fast formation of seed particles. As a consequence of the changed growth mechanism, the reduction of Au^{3+} can occur unselectively leading to nonuniform particles for very acidic or neutral reaction conditions.

Q14. *What statistical tests could you apply to determine whether differences in particle diameter are statistically different?*

Since the data presented in Table 2 are averages of independent measurements from 15 different images collected on each gold nanoparticle preparation, students could apply a t-test comparing two different means.

Q15. *Summarize your findings. Is there a relationship between molar ratios of reagents and particle size?*

The molar ratio of the reagents definitely affects the particle size. As the concentration of citrate increases compared to the concentration of gold, so does the particle size.

Q16. *If you wanted to synthesize particles with a diameter of approximately 20 nm, which experimental conditions would you use?*

A smaller citrate to tetrachloroauric acid ratio yields particles with an approximate diameter of 20 nm.

Q17. *What trends emerge from your analysis? Is there a set of experimental conditions that yield a more uniform particle distribution?*

If students don't have access to their own experimental data, they can download the 'Size-distribution-data' Excel file which provides particles sizes determined on multiple images of the same particle solution. They can then generate a graph showing the percentage of occurrence of each particle of a given diameter.

By comparing particle size distributions for the different sets of experimental conditions, they should observe that lower pH values yield more homogeneous distributions. This can also be observed in Table 2 which shows smaller standard deviations for nanoparticles synthesized using the same citrate to tetrachloroauric acid ratio at lower pH values. As the pH increases the seed-mediated growth mechanism, which is predominant at lower pH values, is not retained and the final particles are rather polydisperse. The initial concentration of $[AuCl_4]^-$ is too low to trigger the fast formation of seed particles. Seed particle formation might still occur but simultaneously uncontrolled growth of existing and formation of further particles can take place, resulting in a larger dispersion of particle sizes.

Q18. *What can be said about the distribution obtained by this specific set of synthetic parameters?*

The distribution is relatively uniform with 87% of all particles falling within approximately 2.5 nm from the average diameter.

Q19. *If you purchased 30 nm gold nanoparticles from NIST, what particle size diameter would you get? Are all the particles of the same size?*

This is a great opportunity for students to discover that gold nanoparticles synthesized as standard material are not truly monodisperse and have their own size distribution. Students can be asked to research the NIST website for RM 8012 gold nanoparticles (nominal 30 nm diameter). These particles have a diameter of 27.6 nm with a standard deviation of 2.1 nm. This deviation is comparable to particles of similar diameter synthesized as part of this experiment using a 7:1 citrate to tetrachloroauric acid ratio at pH 4.2.

Q20. *Is there an effect on the particle size distribution when changing the reagents molar ratio?*

No, the molar ratio only minimally affects the size distribution. The molar ratio plays a major role in the actual size of the particles.

Q21. *Is there an effect on the particle size distribution when varying the pH?*

Yes, pH has a dramatic effect on particle size and size distribution. The traditional conditions of gold nanoparticle synthesis happen to be in a fortuitous pH window (2.7 – 4, see reference 7) that promotes seed-mediated growth mechanism with particles of diameter around 10 nm. However, outside this window, the synthesis outcome is very different. For pH < 1.5, no particles are observed because the protonated citric acid is incapable of reducing Au^{3+} since the reduction mechanism requires one deprotonated carboxy group. For pH > 4, which is the pH range covered in this experiment, the initial amount of reactive $[AuCl_4]^-$ is too low to trigger the fast formation of seed particles. As a consequence of the changed growth mechanism, the reduction of Au^{3+} can occur unselectively leading to nonuniform particles for very acidic or neutral reaction conditions.

Q22. *Summarize your findings. Is molar ratio or pH the controlling factor in ensuring a yield of uniform particles?*

pH is the parameter that more dramatically affects the size distribution. For each citrate to tetrachloroauric acid ratio explored, more monodisperse particles were obtained at lower pH values. This is in agreement with findings by Wuithschick et al. (see reference 7). The paper contains an excellent summary in figure 9 which shows the dependence of polydispersity on pH.

Estimating the concentration of nanoparticles from the particle size data

In this section of the module students calculate the nanoparticle concentration for each synthetic condition. First, they calculate the average number of gold atoms per nanoparticle (N), then the total number of particles based on the moles of gold used in the reaction and from there, the actual nanoparticle concentration expressed in molarity.

Q23. *According to data from Table 2, nanoparticles synthesized with a 2:1 citrate to tetrachloroauric acid ratio and pH 5.4 have a Feret's diameter of 21.7 nm. What is the value of N?*

The relationship between the average number of gold atoms (N) per nanoparticle and the particle diameter (*D*) is provided by equation 1:

$$N = \frac{\pi\left(19.3\frac{g}{cm3}\right)D^3}{6\left(197\frac{g}{mol}\right)} \quad \text{(eq. 1)}$$

This equation assumes a spherical shape and a uniform face-centered cubic (fcc) structure. In equation 1, 19.3 g/cm^3 is the density for fcc gold and 197 g/mol is the gold atomic mass. For D = 21.7 nm,

$$N = \frac{\pi\ (19.3\ g/cm^3)\ D^3}{6\ (197\ g/mol)} = 316{,}946 \text{ atoms/nanoparticle}$$

Q24. *What is the total number of gold atoms (N_{Total}) in 50.0 mL of a 0.25 mM solution of $HAuCl_4$?*

The total number of gold atoms in 50.0 mL of a 0.25nM solution of $HAuCl_4$ can be estimated by calculating the number of moles of gold and multiplying by Avogadro's number:

0.050 L x 0.00025 mol/L x 1 mol Au/1 mol $HAuCl_4$ x 6.02 x 10^{23} atoms/mole = 7.5 x 10^{19} atoms

Q25. *What is the molar concentration of nanoparticles in this solution?*

The molar concentration of a nanoparticle solution can be estimated by dividing the total number of gold atoms (N_{total}) equivalent to the amount of auric acid added to the reaction volume by the average number of gold atoms (N).

$$C = \frac{N_{total}}{NVN_A}$$ This is equivalent to the equation $C = \frac{\text{moles from reaction}}{\text{N x Volume}}$

$$C = \frac{1.25 \times 10^{-5} \text{ mol Au}}{316{,}946 \text{ atoms/NP} \times 0.05\text{L}} = 7.9 \times 10^{-10} \text{ mol/L}$$

Following the example provided above, students should repeat the calculations for all particles and complete all data in table 4.

Determining the molar extinction coefficient of gold nanoparticles

Using the data sets made available in the experimental data, students can first estimate the wavelength of maximum absorbance and then estimate the absorptivity coefficient for each nanoparticle preparation by calculating a linear regression equation based on the absorbance vs. concentration data.

Q26. *At which wavelength does each nanoparticle solution exhibit the maximum absorbance? This wavelength is referred to as* λ_{max}

Students can estimate λ_{max} by examining figure 6 which shows spectra recorded on nanoparticles synthesized under different pH conditions or analyzing the visible spectra provided in the experimental data. As the pH increases, a red shift is observed in agreement with increased particle size. This shift is also portrayed in Table 1 in the module.

Q27. *What wavelength would you choose to quantitatively determine the concentration of the nanoparticles? Why did you choose this wavelength?*

Students should answer this question by reporting λ_{max}. This wavelength is chosen because it provides the maximum sensitivity for the determination of the concentration according to Beer's law.

Q28. *What is the value of the absorbance at* λ_{max} *for each nanoparticle solution? Complete table 5 by reporting the nanoparticle concentration from Table 4 and* λ_{max} *estimated from the spectra found in the link to the experimental data.*

Table 5 can be completed by inserting the nanoparticle concentrations calculated in Table 4 and by reporting the λ_{max} derived by analyzing the visible spectra provided in the experimental data.

Q29. *How could you use a plot of absorbance vs. concentration at the wavelength chosen above to determine the molar extinction coefficient of a gold nanoparticle solution?*

According to Beer's law, $A = \varepsilon bc$, where A is the absorbance, ε is the molar extinction coefficient, b is the path length of the cuvette and c is the concentration. Thus, the molar extinction coefficient can be obtained by calculating the slope of the absorbance vs. concentration plot. Since in most instances the path length b of the cuvette is equal to 1 cm, the slope is the same value as ε.

Preparing gold nanoparticle dilutions for molar extinction coefficient estimation

Q30: *Consider the 2:1 pH 5.4 gold nanoparticle preparation. Given that the concentration of the stock solution is of the order of 3×10^{-9} M, how many milliliters of this solution will you have to pipette to prepare 5.00 mL of the following dilutions?*

Students will use the dilution formula: $M_1 \times V_1 = M_2 \times V_2$ where M_1 and V_1 are the molarity and volume of the stock and M_2 and V_2 are the molarity and volume of the dilute solution.

For example, if we wish to prepare 5.00 mL of a 1.5×10^{-9} M solution from a 3×10^{-9} M stock:

$3 \times 10^{-9}\ \text{M} \times V_x = 1.5 \times 10^{-9}\ \text{M} \times 5.00\ \text{mL}$

Solving for $V_x = \dfrac{1.5 \times 10^{-9}\ \text{M} \times 5.00\ \text{mL}}{3 \times 10^{-9}\ \text{M}} = 2.5\ \text{mL}$

To prepare the dilution, 2.5 mL of the stock solution (3×10^{-9} M) would have to be pipetted using a volumetric pipette into a 5.00 mL volumetric flask and brought to volume with water.
The best approach will be to make a number of serial dilutions as preparation of the more diluted solutions from the stock solution is not feasible in light of very small volumes that would have to be measured. Such small volumes would increase the inaccuracy of the analysis.

Q31. *What is the purpose of preparing a 0.00 M dilution? How is it used in the analysis?*

A solution containing zero concentration of analyte is considered a blank and it is used to zero the instrument to eliminate interferences from any reagents used in the analysis.

Q32. *What relationship do you observe between the absorbance and concentration of nanoparticles?*

There is a very linear relationship between the absorbance and the concentration for any of the nanoparticles preparations.

Q33. *Do any of the data points deviate from the general behavior observed in the plot?*

In general, there is very small deviation in any of the plots. The absorbance of nanoparticles solutions displays a high linearity with changes in concentration.

Q34. *What parameter allows us to determine whether there is a good fit between absorbance and gold nanoparticle concentration?*

The correlation coefficient is a measure of good fit. For most solutions examined in this study the correlation coefficient is 0.999 or better.

Q35. *What is the molar extinction coefficient for this specific gold nanoparticle preparation? How does this value compare to the extinction coefficient reported in Table 1 for a gold nanoparticle of similar size?*

Students can analyze the data provided in the experimental results. Based on the absorbance vs. concentration plot, the slope of the curve is 831284607.8 or 8.3×10^8 $M^{-1}cm^{-1}$. By comparison, the extinction coefficient for a 20 nm particle is approximately 9×10^8 $M^{-1}cm^{-1}$ as reported in Table 1.

Q36. *How can you estimate the uncertainty on the molar extinction coefficient from the regression analysis?*

The Summary Output from the regression analysis is displayed for each set of absorbance vs. concentration data. The Standard Error associated with X Variable 1 is the uncertainty on the slope. In this particular case it is 4289294.315. Thus the extinction coefficient can be reported as $(8.3 \pm 0.4) \times 10^8$ $M^{-1}cm^{-1}$.

Putting it all together

Q37. *Based on the analysis of the data you summarized in Table 6, how does particle size affect the molar extinction coefficient?*

In general, the molar extinction coefficient increases with increasing particle size.

Q38. *How does the citrate to tetrachloroauric acid ratio affect the molar extinction coefficient?*

Since the citrate to tetrachloroauric acid ratio affects the particle size, the ratio does affect the molar extinction coefficient. As the particle size increases, so does the molar extinction coefficient.

Q39. *Does pH play a role in the value of the molar extinction coefficient?*

In the pH range explored in this experiment, pH does not dramatically affect particle size and, therefore, molar extinction coefficient. However, for pH values lower than 4, pH plays a dramatic effect on particle size, thus affecting the molar absorptivity coefficient.

Q40. *If you were to develop a colorimetric sensor, what experimental conditions would you choose to synthesize nanoparticles of consistent size with a large molar extinction coefficient?*

This is an open ended question. Looking at the overall results, particles with an approximate diameter of 20 nm can be synthesized with relatively small polydispersion using a 2:1 citrate to auric acid ratio at pH values between 4 and 5. By increasing the citrate to auric acid ratio to 4:1, the particle size increases along with increased polydispersion. This trend continues for the higher ratio (7:1) where particle size is above 30 nm with wider particle distribution.

For sensor application, probably the 2:1 citrate to auric acid at pH values between 4 and 5 would yield the best conditions.