

Nanoparticle biosensors

January 25, 2007

1 Objectives

- To measure the absorbance of Au nanoparticles
- To calculate the concentration of Au nanoparticles using Beer' Law
- To employ simple surface chemistry to make a rudimentary nanoparticle sensor
- To verify the simple aggregation picture by using dynamic light scattering
- To estimate the equilibrium constant of the process through absorbance measurements

2 Introduction

One of the most exciting areas of current nanoscience and nanotechnology is the nano/bio interface. There exists tremendous interest in applying nanoscale materials to biological problems. One of the more advanced areas in this field currently involves the use of metal and semiconductor nanoparticles (NPs) in biological sensing applications. Such sensing schemes can be achieved using color changes, changes in the fluorescence intensity, changes in the emission color or even changes in the electrical current running through a nanostructure. Motivating much of this work are commercial interests. For example, the anthrax attacks soon after 9-11 have shown the need to develop highly sensitive, specific biosensors. This is of special interest to defense agencies especially considering the intrinsic vulnerability of large urban environments to these attacks. Numerous researchers in the field have therefore taken advantage of the surface functionalization chemistries available to chemically synthesized nanostructures in order to make rudimentary nanoscale sensors that try to address these needs.

In this laboratory we will begin to look at the absorption of Au NPs and see how it might be used for sensing purposes. First, the extinction coefficient of the particles will be introduced and some aspects of it will be discussed. This value will subsequently enable us to quantify the concentration of the stock Au NP solution we have made. Furthermore, this value can be compared to values

derived from a back of the envelope calculation that you will carry out. Next we will employ the flexible surface chemistry of these NPs to make a rudimentary sensor using color changes in the NP absorbance. Not only will you be able to see the color change by eye, but you will also take UV/Visible absorbance measurements to make estimates of the reaction equilibrium constant. You will also read some literature showing how researchers at Northwestern University have used the same sensing scheme to make a colorimetric DNA sensor.[1,2]

3 Nanoparticle “plasmon” resonance

Metals are typically characterized by the presence of “free” electrons. In nanometer sized metal particles (such as the Au sols we are dealing with) there will be a strong absorption of light by the collective excitation of these unbound electrons. This absorption is referred to as a plasmon resonance and in the case of Au NPs will have a spectral position and width that depends on the size, shape and to some extent the size distribution of the ensemble.

Mie was the first to model the extinction (extinction is the sum of both absorption and scattering for objects interacting with light) of such spherical particles. By solving Maxwell’s equations for a polarizable sphere interacting with plane wave electromagnetic radiation he was able to find expressions for the absorption and scattering of light by the particle. In his model, for small enough materials (i.e. particle size much smaller than the wavelength of light) and for dilute solutions scattering contributions can be neglected. As a consequence, the bulk of the contribution to the measured extinction (this is why you have so called Molar extinction coefficients) stems from absorption alone. Mie showed that the basic shape of the predicted plasmon absorption line had a Lorentzian lineshape. (If you are unfamiliar with this look it up and compare it to its complementary Gaussian lineshape.) Mie’s derived absorption is essentially

$$Abs \propto \frac{\epsilon_2}{(\epsilon_1 + 2\epsilon_m)^2 + \epsilon_2^2} \quad (1)$$

where the equation employs the frequency dependent real (ϵ_1) and imaginary (ϵ_2) parts of the material’s complex dielectric constant as well as the dielectric constant of the surrounding medium (ϵ_m).

Now the dielectric constants of a material are very important quantities and there are tables of them out there. (Someone obviously spent a lot of time measuring them and compiling them for the rest of us.) However, there exist simple classical models that can let us derive paper and pencil approximations of these complex values for a given material. The model is called the Drude model or alternatively the Drude-Lorentz model. Although this topic is beyond the scope of this class you should know that such models exist. An analogous model that can be used for semiconductors exists and is called the Lorentz model. In later physics classes you may have the opportunity to study these classic “oscillator” models in more detail. Examples of the absorbance of Au NPs that you will make are shown in Figure 1.

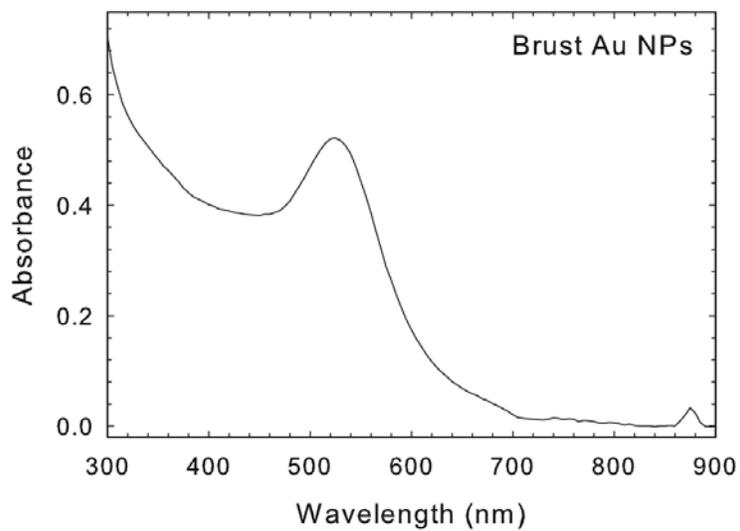
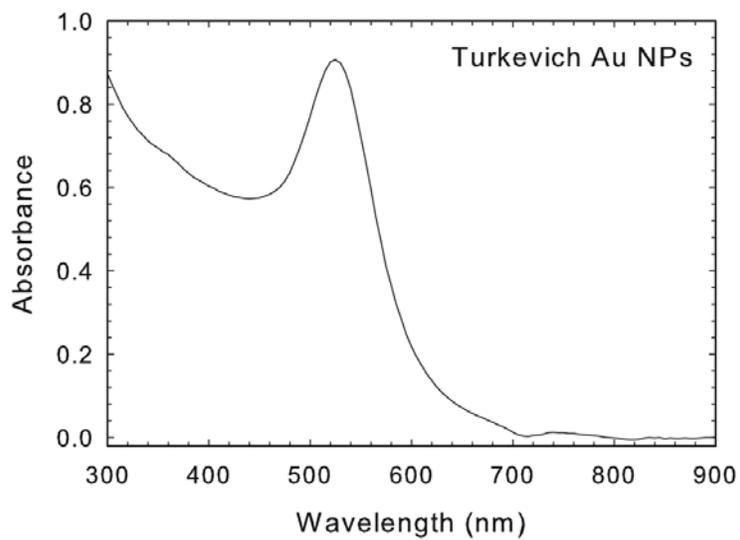


Figure 1: Linear absorption of Turkevich and Brust Au NPs

4 Nanoparticle extinction coefficient

Now that we have crudely seen the origin of the plasmon resonance in Au NPs we begin a discussion on its molar extinction coefficient and a closely related quantity, its absorption cross section. In this respect, how effectively a particular substance absorbs electromagnetic radiation can be characterized by two interrelated optical properties: the absorption cross-section and the molar extinction coefficient. Experimental estimates of these frequency dependent optical constants represent an area of key interest in nanoscience and nanotechnology. Specifically, a direct measure of how well a nanostructure absorbs light holds wide use in fundamental studies as well as in applied interests, both involving materials such as nanoparticles, nanowires and other so called “low dimensional materials”. For example, such values are useful in quantifying properties such as sample concentrations, fluorescence quantum yields, photovoltaic efficiencies, and lasing thresholds.

Despite the recognized usefulness of these values, it has not been straightforward to extract cross-sections and extinction coefficients from chemically synthesized nanostructures. Unlike molecules or other systems with well defined molecular weights, colloidal NPs and other chemically synthesized nanostructures do not possess such exact physical properties. This arises, in part, because current syntheses do not fully control sample size polydispersities (You saw this in an earlier experiment using dynamic light scattering). Even in the best preparations, colloidal NPs show size distributions ranging from 4-5%. The effects of polydispersity have been ameliorated, to some extent, by the development of size selective precipitation or by improved chemical syntheses that yield high quality NPs with narrow (as made) size distributions.

Further hindering the definitive structural characterization of chemically grown nanostructures are organic surfactants used in above-mentioned syntheses. These surfactants control the size, shape, growth kinetics and even crystallographic phase of solution-based nanomaterials. They also electronically passivate nanomaterial surfaces, improve their fluorescence quantum yields, provide solubility in a variety of organic and aqueous solvents, and protect their surfaces from oxidation. However, as an unintended consequence, actual masses of solution-based nanostructures are greatly influenced by these surface bound species. Thus the weight of dried nanomaterial powders is not necessarily representative of the actual number of nanostructures present in a sample.

In spite of these problems, absorption cross-sections and associated molar extinction coefficients have been evaluated using techniques such as thermal gravimetric analysis (TGA) (a separate project one can pursue later on), acid digestion followed by inductively coupled plasma mass spectroscopy (alternatively, inductively coupled plasma atomic emission spectroscopy), atomic absorption spectroscopy, anodic stripping voltammetry or other approaches all in conjunction with absorption spectroscopy. For example, TGA measurements are susceptible to errors from insufficient “washing” of the material, allowing resulting sample masses to be corrupted by surfactant contributions. Furthermore, well-separated temperature dependent weight drops are required to un-

ambiguously differentiate surfactant from core contributions. Potential errors in inductively coupled plasma (ICP)-based measurements originate from the inexact stoichiometry of many nanostructures given that binary NPs (i.e. things like CdSe, InP, PbSe) often have slight excesses of one element over the other. This likely originates from the abrupt termination of nanomaterials at their surfaces. On the other hand, ICP-based measurements are less sensitive to surfactant issues, making the approach intrinsically more reproducible. With all methods, however, robust absorption cross sections and molar extinction coefficients can be found.

In this laboratory, rather than actually measure the molar extinction coefficient of the Au NPs you made in a previous laboratory (an interesting and possible extension of this laboratory) we will use a literature value. Reference [1] has determined that the peak extinction coefficient of $\sim 16 \pm 1$ nm diameter Au NPs (this size is close enough to that of your particles for our purposes) is

$$\boxed{\epsilon = 4.2 \times 10^8 M^{-1} cm^{-1}} \quad (2)$$

If you look up this reference you will see that they are using TEM to size their particles. Then they use ICP to determine the concentration of Au ions in their samples from which the estimated concentration of Au NPs can be determined. Finally, note that the extinction coefficient is actually a frequency dependent number. The present value is good at 520 nm. This is roughly the peak of the characteristic Au NP plasmon resonance. But if you wanted to look at the absorbance at a different wavelength and quantify something you would need the molar extinction coefficient at the new wavelength. In this respect, do you think it is possible to estimate the molar extinction coefficient at a different wavelength using the provided value above? If your answer is yes, how exactly would you go about doing this? Hint: think of Beer's law (below).

5 Beer's Law Redux

Beer's law relates the absorbance of a sample to its concentration through the molar extinction coefficient (ϵ). Presumably you have seen this before but if not here it is again.

$$\boxed{A = \epsilon cl} \quad (3)$$

In the expression A is the sample absorbance (unitless), ϵ is the molar extinction coefficient ($M^{-1}cm^{-1}$), c is the sample concentration (M units) and l is the optical pathlength (typically 1 cm). So by simply plugging in the above ϵ value for Au NPs you can relate the absorbance of a sample to its concentration. You should also know that Beer's law is also connected to something called the exponential attenuation law which states that the intensity of a beam traversing through a dilute absorbing medium will fall off exponentially with distance. The exponential attenuation law can be derived using simple geometric arguments (not done here to save time) and is related to what is known as the absorption

cross section (σ) with units of cm^2 . The cross section as the name implies is an effective area. So if you picture a stream of photons moving towards the molecule or NP of interest in solution, this is the effective area presented by the sample whereupon any photon hitting this area will be absorbed. The exponential attenuation law is written as

$$\boxed{I = I_o e^{-\sigma n l}} \quad (4)$$

where in the expression, I is the transmitted intensity, I_o is the incident light intensity, σ is your molecule's absorption cross section (cm^2), n is the concentration of the sample in units of $\frac{\#}{cm^3}$ and l is the optical pathlength in cm units.

The connection between this expression and Beer's law is through the transmittance T of the sample. Namely,

$$\boxed{A = \log \frac{1}{T}} \quad (5)$$

where $T = \frac{I}{I_o}$. You get the point. So, in fact, there is a direct relation between the absorption cross section of a single Au NP and the molar extinction coefficient provided. Can you come up with the expression which connects the two? What is the cross section of a single Au NP at 520 nm using ϵ provided above?

5.0.1 Back of the envelope calculation

Now in this part of the experiment you will compare the measured NP concentration to the number obtained from a "back of the envelope" estimate that you will carry out. In the calculation you will assume that all of the introduced Au ions are used up to make a NP of a specific size. You will then use the density of bulk gold (ρ_{Au}) and the dynamic light scattering determined diameter to find out how many moles of Au NPs you have made. Then by knowing the volume of the final solution you can determine the predicted concentration of the sample. Does the experimentally determined concentration match the calculated value? We will outline this calculation for you below. In addition we note that there is at least one other way you can do this calculation without knowing the density of gold. Instead this approach uses some information about the crystal structure of Au. Hint: What is the volume of the Au unit cell? Perhaps you could run this calculation on your own.

Outline

- First what is the final size of the Au NPs you have made? Determine the corresponding volume of the particle in cm^3 units.
- Next what is the corresponding weight of a single Au NP in grams? (This uses the bulk density of Au which you will look up)
- How many moles of Au per NP? (This uses the atomic weight of Au)
- How many total moles of Au did you start with in the reaction?

- From the two previous quantities you should be able to determine the total number of NPs produced assuming a 100% yield of the reaction.
- Using Avogadro's number, how many moles of NPs did you make?
- Finally, using the volume of your final solution, what is the concentration of the stock, in M units?

6 Controlled Au NP aggregation

Now that you know something about the concentration of your gold NP solution, we will demonstrate its usefulness in an experiment intended to demonstrate an application of its absorbance to sensing. Here we will perform the controlled aggregation of Au NPs to develop a crude NP-based sensor. It mimics the function of real biosensors made using the same Au NPs.[1,2] We will use the Au NPs that we have made and will add in a second reagent that we wish to sense. This will be thioacetamide (TAA) and is shown in more detail in Figure 2. On exposure to TAA, the surface of the Au NPs will begin to get decorated with these molecules. Empirically it has been found that if another Au NP comes in close proximity, it is possible to have multiple Au NPs aggregate with each other. Now the exact origin of the aggregation appears to be somewhat of an open question. However there are hypotheses for why this aggregation occurs. One of the ideas is that the surface passivation of the Au NPs reduces the citrate electrostatic stabilization.[3] As a consequence, interparticle Van der Waals forces can then cause particles to begin aggregating on being in close proximity. Alternatively, many of the surface passivating agents that have been found to induce aggregation have two functional groups. The first is a thiol termination which often preferentially attaches to the Au NP surface. The other end of the molecule may also have a thiol group or a primary amine group. Both can form dative/covalent bonds to the surface of another NP. This can, in turn, induce two or more Au NPs to begin aggregating together.

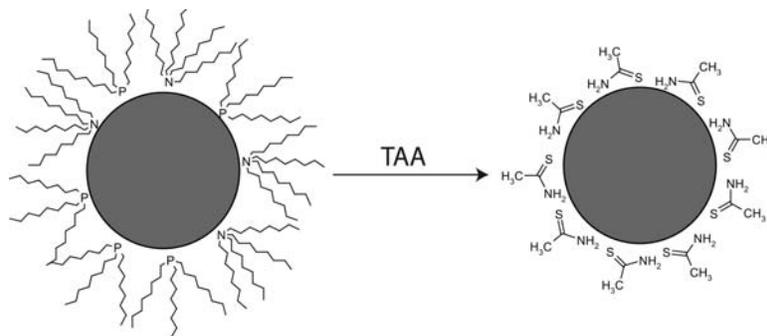


Figure 2: Ligand exchange with TAA

Now what is interesting is that upon being attached to each other the so called plasmon resonance of the Au NPs changes. In effect, there will be an shift of the absorbance to redder wavelengths (a “redshift”). The origin of the shift is the change in the effective shape of the particle to something that is not necessarily spherical. Perhaps the resulting shape is rodlike. This is supported by some experimental measurements as well as by the observation of similar resonances at red wavelengths in gold nanorods. However, irrespective of the actual shape of the resulting Au NP complex, the close proximity of the Au NPs enables electrons in attached particles begin to interact with each other. This causes the original plasmon resonance to change.

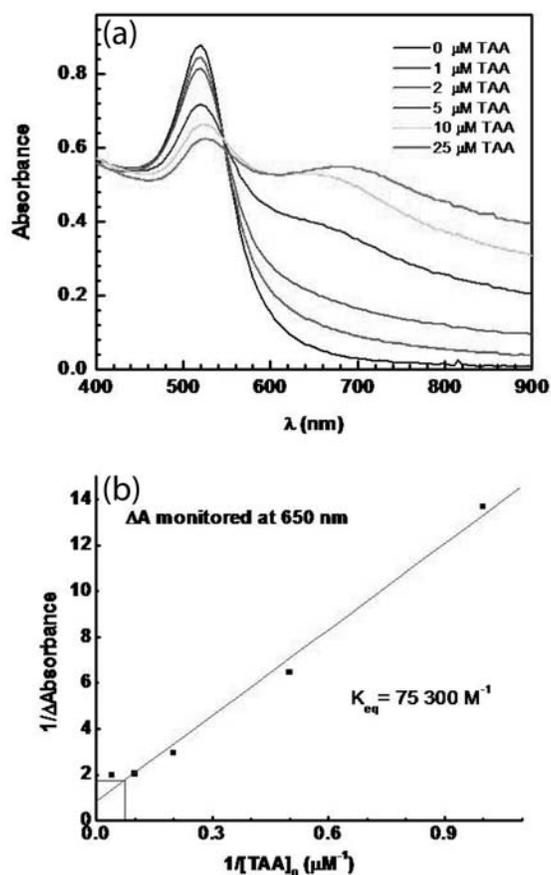


Figure 3: TAA experiment

Now since the absorbance of a material is proportional to its extinction coefficient, it is possible to measure the change in the absorbance of an Au NP solution as the target molecule (TAA) is introduced. Because the Au NP

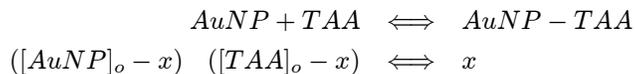
complex occurs at a redder wavelength (i.e. closer to 700 nm) you can monitor the increase of this absorption to learn something about the equilibrium constant of the reaction. Then, with the model derived below, you will also be able to extract a measure of the new complex's extinction coefficient (ϵ_c). So in effect, by doing a sensing experiment you have learned not only the equilibrium constant (K_{eq}) of the process but also something about the optical properties of the newly made material. The following model is taken from Reference [4] and is translated to the case of Au NPs.

Figure 3 shows the effects of adding thioacetamide (TAA) to a solution of citrate stabilized Au NPs (Turkevich NPs). In the top graph you can see the original gold NP absorbance on the left side of the plot. As more TAA is added to the NP solution, it begins to induce the aggregation of the particles. A rise in the absorbance around 700 nm occurs. This can be seen on the right side of the plot. Thus with increasing TAA added, the original Au NP absorption at 520 nm decreases while the absorption at $\simeq 700$ nm increases. Quick question: do you think that the decrease in absorption equals the increase in the absorption at 700 nm? In not why? Hint: it has to do with the extinction coefficient of the gold NPs versus the extinction coefficient of the new complex.

Next using the formula derived below it is possible to extract a linear relationship between $\frac{1}{\Delta A}$ (where ΔA is the new complex absorption) and $\frac{1}{[TAA]_o}$ (where $[TAA]_o$ is the concentration of TAA in solution). Since you control the TAA concentration in the experiment, this will be your dependent variable (i.e. your x axis). Both the slope and the intercept of the line tell you something useful about the reaction and about the new complex. Specifically, from the intercept you will find out an estimate for the extinction coefficient of the new complex. From the slope of the plot you will find the equilibrium constant of the process. The bottom plot of Figure 3 illustrates the sort of data that you will get and the general appearance of your plot. A derivation of these relationships is provided below.

6.0.2 Derivation

A very simplified version of our reaction is



where $[AuNP]_o$ is the initial concentration of Au NPs in solution (you can determine this via Beer's law), $[TAA]_o$ is the initial concentration of TAA in solution (this is a parameter which you control), and x is the concentration of Au NP complex made.

The equilibrium constant of the reaction is defined as

$$\boxed{K_{eq} = \frac{x}{([AuNP]_o - x)([TAA]_o - x)}} \quad (6)$$

where we will assume that at all times $[TAA]_o \gg x$. (The TAA is always in

great excess). We therefore have

$$\begin{aligned}
 K_{eq} &\simeq \frac{x}{([AuNPs]_o - x)([TAA]_o)} \\
 \frac{1}{K_{eq}} &\simeq \frac{[AuNP]_o[TAA]_o}{x} - [TAA]_o \\
 \frac{1}{K_{eq}[TAA]_o} &\simeq \frac{[AuNP]_o}{x} - 1 \\
 \frac{1}{K_{eq}[TAA]_o} + 1 &\simeq \frac{[AuNP]_o}{x} \\
 \frac{[AuNP]_o}{x} &\simeq \frac{1}{K_{eq}[TAA]_o} + 1
 \end{aligned}$$

Now from Beer's law we also know that the concentration of complex x is proportional to its absorbance (ΔA). In particular

$$\Delta A = \epsilon_c x l$$

so that

$$x = \frac{\Delta A}{\epsilon_c l}$$

Plug this into our expression to get

$$\frac{[AuNP]_o \epsilon_c l}{\Delta A} \simeq \frac{1}{K_{eq}[TAA]_o} + 1$$

Our final expression is

$$\boxed{\frac{1}{\Delta A} \simeq \frac{1}{K_{eq}[AuNP]_o \epsilon_c l} \left(\frac{1}{[TAA]_o} \right) + \frac{1}{[AuNP]_o \epsilon_c l}} \quad (7)$$

You will note that the final expression has the form of a line $y = mx + b$. Its intercept is $\frac{1}{[AuNP]_o \epsilon_c l}$. The slope is $\frac{1}{K_{eq}[AuNP]_o \epsilon_c l}$. The dependent variable is $\frac{1}{[TAA]_o}$ which you will control in the actual experiment. Thus to generate the desired linear graph you plot $\frac{1}{\Delta A}$ versus $\frac{1}{[TAA]_o}$.

Now some things to consider.

- Recall that this approximation is only valid when $[TAA]_o \gg x$. (The TAA is always in great excess.) What does this tell you? Well it means that on the above linear plot, points closer to the origin should behave as a straight line whereas points further out along the x axis may show deviations from linear behavior because the approximation begins to break down.
- Now having $[AuNP]_o$ one can obtain an estimate for the extinction coefficient (ϵ_c) of the complex. See the expression for the intercept and recall that $l = 1cm$.

- Is this extinction coefficient big? Alternatively, is it small? What are the magnitude of typical extinction coefficients in dye molecules? What are the typical magnitude of corresponding absorption cross sections? Recall that you derived a formula above that could relate the two.
- Then putting everything together, the slope provides one the desired estimate for the equilibrium constant (K_{eq}).
- Is this equilibrium constant big? Is it small? How good a sensor is this gold NP complex?
- Note that there is a kinetic interpretation of equilibrium. The idea here is that equilibrium is achieved when the forward reaction rate equals the reverse reaction rate. So for our reaction of interest the forward reaction rate is

$$k_f[AuNP][TAA]$$

where k_f is the forward rate constant. Likewise, the reverse reaction rate is

$$k_r[AuNP - TAA]$$

with k_r being the corresponding reverse rate constant. At equilibrium, the forward and reverse rates equal

$$k_f[AuNP][TAA] = k_r[AuNP - TAA]$$

By rearranging this expression you can see that the equilibrium constant K_{eq} is also equivalent to the ratio of the forward and reverse reaction rate constants

$$K_{eq} = \frac{k_f}{k_r} = \frac{[AuNP - TAA]}{[AuNP][TAA]}$$

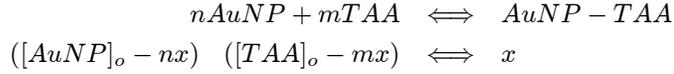
Therefore if in principle you could find one of the rates (either the forward or reverse rates) using a time resolved technique such as transient differential absorption spectroscopy you could get information about the kinetics of this process as well. Obviously this is a little stretch for this laboratory since we don't have a turnkey transient absorption spectrometer available for your use. Nonetheless here is another possible extension of this laboratory.

- In addition, note again that there is more information that one can get from such experiments. Do you remember the relation between the equilibrium constant the the Gibbs free energy change of the reaction? (If you are unfamiliar with the Gibbs free energy consult your instructor.) Well it can be shown that at equilibrium the following relation applies

$$K_{eq} = e^{\frac{-\Delta G}{RT}}$$

where ΔG is the reaction free energy change, R is the ideal gas constant 8.314 J/molK and T is the temperature in K.

- Finally, note that the above model is possibly the simplest reaction model that one can come up with. What is simple about it? If you think about it you realize that the above reaction simply says that one Au NP will react with one TAA molecule to make a complex. If this were really the case then you wouldn't have an increase in the size of the resulting NP aggregate. In fact, what really must be happening is that there are several Au NPs coming together. Furthermore, not one but many TAA molecules must coat the surface of the particles to induce the ligand exchange. As a consequence our reaction scheme should really be written as



where n is the number of Au NPs in a typical aggregate and m is the typical number of TAA molecules that decorate each Au NP. The expression for the equilibrium constant then becomes more complicated.

$$K_{eq} = \frac{x}{([AuNP]_o - nx)^n ([TAA]_o - mx)^m} \quad (8)$$

Note the exponents. This then starts to get intractable to solve analytically. However one can determine n independently using dynamic light scattering. One can also assume that the “free” TAA is always in great excess to what is consumed ($[TAA]_o \gg mx$). This leaves us with

$$\begin{aligned} K_{eq} &\simeq \frac{x}{([AuNP]_o - nx)^n ([TAA]_o)^m} \\ \frac{1}{K_{eq}} &\simeq \frac{([AuNP]_o - nx)^n [TAA]_o^m}{x} \\ &\simeq \frac{[AuNP]^n (1 - f)^n [TAA]_o^m}{x} \end{aligned}$$

where $f = \frac{nx}{[AuNP]_o}$ and is a small unitless number. ($f^2 < 1$) Next using a series expansion

$$(1 - f)^n = 1 - nf + \dots$$

and keeping only terms to first order we get

$$\begin{aligned} \frac{1}{K_{eq}} &\simeq \frac{[AuNP]_o^n (1 - nf) [TAA]_o^m}{x} \\ &\simeq \frac{[AuNP]_o^n [TAA]_o^m}{x} - \frac{nf [TAA]_o^m [AuNP]_o^n}{x} \\ &\simeq \frac{[AuNP]_o^n [TAA]_o^m}{x} - n^2 [TAA]_o^m \end{aligned}$$

Next using Beer's law $x = \frac{\Delta A}{\epsilon c l}$ we get

$$\frac{1}{K_{eq}} \simeq \frac{[AuNP]_o^n [TAA]_o^m \epsilon c l}{\Delta A} - n^2 [TAA]_o^m$$

$$\frac{1}{K_{eq}[TAA]^m} \simeq \frac{[AuNP]_o^n \epsilon_{cl}}{\Delta A} - n^2$$

$$\frac{[AuNP]_o^n \epsilon_{cl}}{\Delta A} \simeq \frac{1}{K_{eq}[TAA]_o^m} + n^2$$

or that

$$\frac{1}{\Delta A} \simeq \left(\frac{1}{K_{eq}[AuNP]_o^n \epsilon_{cl}} \right) \left(\frac{1}{[TAA]_o^m} \right) + \left(\frac{n^2}{[AuNP]_o^n \epsilon_{cl}} \right)$$

Note that this expression reduces to our previous expression in the limit where $n \rightarrow 1$ and $m \rightarrow 1$.

6.0.3 An extension

There are a number of compounds other than TAA which induce the aggregation of citrate stabilized Au NPs. Some examples from the literature are shown in Figure 4.[4] They include thionictinamide (TNA) thiourea (TUA), 1,6 mercaptoethylamine (MEA) and 1,9 nonanedithiol (NDT). Out of curiosity why do you suppose MEA or NDT causes particle aggregation? Do you think it happens via the same Van der Waals mechanism proposed earlier? What about these molecules is different? Other compounds causing particle aggregation are described in Reference [5]. In principle, it is possible extend the above measurements to these other surface binding species. Furthermore, in addition to dynamic light scattering, both TEM and AFM sizing could be done to characterize the resulting NP aggregates. This may be done in future versions of this laboratory.

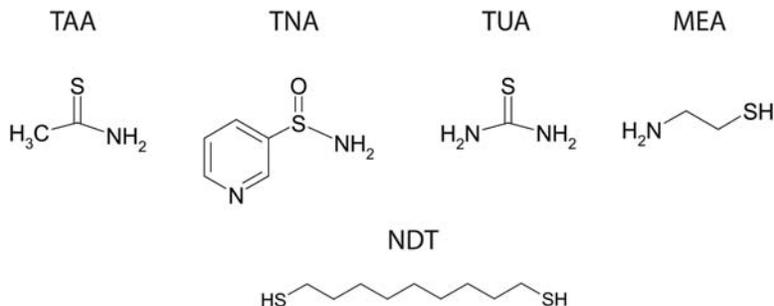


Figure 4: Other surface binding molecules

7 Procedure

7.1 Getting started

This is essentially a repeat of an earlier experiment we did. Prepare the following stock solutions. When working with gold tetrachloroaurate ensure that any

metal spatulas used are covered with teflon tape. Also ensure that you wear gloves and that this chemical does not get on your skin. Clean up after yourself to ensure that no one unexpectedly gets exposed to this chemical. The gold salt will discolor your skin and takes a few weeks before it comes off. Note also that the gold tetrachloroaurate is hygroscopic and will begin to pick up water on exposure to air. Thus the more time spent at the balance the more uncertain the actual weight of the gold salt measured. Finally store any unused gold tetrachloroaurate in the dessicator. Note, thioacetamide is a suspected **carcinogen**. It is prudent to avoid any contact with this chemical.

7.1.1 Gold stock solution, Turkevich procedure

Add 0.155 g HAuCl_4 to 91 mL of deionized water. The gold salt should dissolve readily in water.

7.1.2 Citrate solution, Turkevich procedure

Add 0.25 g sodium citrate to 50 mL deionized water. As before the salt should dissolve readily.

7.1.3 Thioacetamide solution

A 10 mM solution of thioacetamide (TAA, MW 75.13) is prepared by dissolving 0.0188 g in 25 mL of deionized water. Then take 1 mL of this stock solution and dilute it 1 to 10 by adding 10 mL of deionized water. You will use this last 1 mM solution.

7.1.4 Gold nanoparticle synthesis, Turkevich procedure

Take 1 mL of the gold tetrachloroaurate stock and add it to 18 mL of deionized water. Use an appropriate sized earlemeyer flask (~ 50 mL works fine). Now bring this solution to a boil on a hot plate. Also stir the solution with a magnetic stirrer. Wait until the solution is really boiling. Now add 1 mL of the sodium citrate stock while stirring and boiling. Wait for an eventual color change. You will see the solution begin to slowly turn color from clear to a purple-like hue. Wait until the color looks like grape Cool Aid before removing the solution from the hot plate. Let the solution cool to room temperature. Add some more deionized water when cool to bring the total volume up to 20 mL.

7.1.5 Using the UV/Visible spectrometer

Now take the absorbance of this solution and determine the concentration of particles made. Ensure that you know the exact dilution of the sample from the original stock solution. You will need this dilution in order to back calculate the concentration of the stock. Compare the experimental result to the paper and pencil calculation that you did earlier.

Using the Genesis 5 spectrophotometer The spectrometer should be turned on and warmed up for at least 30 minutes before collecting any data. Once on and warmed up, the Genesis 5 spectrometer should be displaying the main menu. Press the number on the keypad that corresponds to the “Survey Scan” program. A screen which displays a graph (and may contain data from the previous run) should appear. Press the “Test types” key on the keypad. This will bring up a listing of available experiments. Press the number corresponding to “SCANINOR” to load this program from the B card in the spectrometer (if necessary, press the “Change Card” softkey to switch to card B). This will load the appropriate parameters into the spectrometer for the lab.

Insert the blank into the first position of the cell changer and your sample into the second position. Close the lid of the spectrometer. The screen may have the message “New Baseline Suggested” displayed on it. This is just a reminder that a new background should be collected for your sample. To begin the background collection, press the softkey (these are the 4 keys just in front of the display screen) labeled “Collect Baseline”. The spectrometer will begin collecting the background. Wait until the spectrometer completes the scan and then you may press the “New Scan” softkey to collect your sample data.

At the end of the run, save your data to the memory card in the spectrometer. This is accomplished by pressing the “Load/Save Data File” softkey followed by the “Save File” softkey on the following screen. Enter your file name (8 characters max) following the on screen instructions (use the softkeys to move up/down/right/left through the alphabet and numbers; press the “Enter” key on the keypad to accept the highlighted letter/number). Press the “Exit” key on the keypad to save your data. Return to the main survey scan screen to prepare for the next run.

When you are done, download the data to the computer. This is accomplished by pressing “Exit” until you are at the main menu of the spectrometer. Press 2 on the keypad to go to the “File Manager” program. Select the number on the keypad corresponding to the survey scan data and press the “Test /Data” softkey to get to the data screen. Press the number on the keypad corresponding to your data file and then press the “Conversion Options” softkey to get to the data transfer table. Press “3” on the keypad to transfer the file in “Lotus” format to the computer (the computer needs to be in the Windows XP operating system to accept the data). Once the computer is in the send mode, find the “Thermo Genesis 5 Spectrometer” folder on the computer. Open the folder and double click on the “GENXFER” icon. The file you selected will be transferred to the Thermo folder. Repeat this process for all files that you wish to transfer. When you are done transferring all of your datafiles, press the “Exit” key on the keypad until you get to the main menu of the spectrometer.

You will need to do some clean-up work on the transferred files before you can use Excel to plot and analyze your data. Open your file in Excel. Select the first column (which is the only column) of data. Under the “DATA” menu, select the “Text to Columns” option. This will start a wizard to assist you. Select “delimited” from the first screen and press next. Select “comma” as the delimiter, deselect all other options, and make sure that “(quotes)” are entered

in the text qualifier box. Select “Finish”. This will parse your data into the appropriate columns. From here, you will be able to plot your data and complete your analysis.

7.1.6 Using the Coulter light scattering apparatus

Determine the size of the initial Au NPs prior to adding any TAA. We will use the DLS technique to determine the size and size distribution of various particles in (aqueous) suspensions across this Lab course. The same instrument, Beckman-Coulter N4-Plus, will be utilized at all stages.

Instrument specifications: Sizing range: 3 nm - 3 μ m; Excitation source: He-Ne (Helium-Neon) Laser (632.8 nm); Detection system: 6 - fiber optics located at 14.9°, 20.6°, 30.4°, 40.2°, 50.4°, and 90°. We will use only the 90° angle and assume spherical particles. The software is self-explanatory and an instructions manual will be available from the instructor.

Before you start the measurement, turn instrument on and after a few minutes turn the computer on. The machine needs to warm-up and the temperature in the cell compartment equilibrated with the sample (approximately 15 minutes) before you can perform the measurements. The computer will report that the machine is ready.

Use your own samples from the synthesis above. Determine by simple dilutions the appropriate concentration for your sample. Dilute the sample until two consecutive dilutions give the same sizes. Once you have the right concentration perform three repetitive measurements and report the average size and the width at half maximum of your size distribution.

7.1.7 Controlled Au NP aggregation

Next 4 mL of the prepared gold NP solution is placed in a cuvette. A background spectrum is acquired. TAA is subsequently added in small increments. See the Table 1 for an example. After each addition, the sample is stirred for approximately 30 s before recording the background corrected absorbance spectrum of the sample. Save these spectra and record both the drop in the absorbance at the peak of the Au NP absorption and corresponding rise of the absorbance at the redder wavelength. You may choose which exact wavelength to monitor but it should be in the spectral region near 700 nm. Plot and analyze your data as illustrated in Figure 3. Also remember to conduct dynamic light scattering experiments on each of these samples to monitor changes in the average size of the particles in the ensemble.

8 Clean-up

Any gold solutions not needed should be discarded in the appropriate waste container. If the particles are in water they should be placed in an aqueous waste container. Likewise, if the particles are in toluene, they should be discarded in the organic waste container.

9 Laboratory report points to consider

You should consider the following points in your report.

- In the above experiment we added a surface binding molecule to induce the aggregation of Au NPs. However since citrate ions electrostatically passivate the NP surfaces how else could you induce aggregation of the particles to occur.
- What do you think would happen if you added some salt to the Au NP solution?
- If there is an effect, why did the effect occur, explain.
- In the aggregation reaction of interest, what was the sign of the Gibbs free energy change? What does that tell you?
- Do you remember Le Chatelier's principle? What do you think would happen to your reaction if you somehow found a way to remove the Au NP complex from your reaction?

10 References

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Table 1: TAA experiment

Volume of Au NP solution (mL)	Volume of 1 mM TAA solution added (μ L)	Total solution volume (mL)	[TAA] (μ M)
4	4	4.004	1.02
4	8	4.008	2.04
4	16	4.016	4.07
4	32	4.032	8.11
4	100	4.100	24.93