Chapter 4: Synthesis and Characterization of Metal Nanoparticles

4.1 Background information for Gold Nanoparticles

Gold nanoparticles (GNPs) have been synthesized dating back to ancient times when they were used for staining glass; due to their optical properties. In 1857, Faraday’s work attracted much attention. He synthesized colloidal gold by reducing gold salt with phosphorus in carbon disulfide which gave a ruby colored solution. Significant progress in the synthesis of non-spherical gold nanoparticles has been achieved in the past century.\textsuperscript{1-3} The advantages of using GNPs in biological applications over other metal nanoparticles include biocompatibility, the chemical stability of the gold surface, the simplicity of bioconjugate formation, no need for premodification of the surface, ease of preparation, and ease of investigation by spectroscopic methods.\textsuperscript{4}

In order to understand the optical properties of gold nanoparticles, the interaction of light with metal nanoparticles and the concept of surface plasmon will be discussed in the following sections.

4.1.1 Interaction of light with matter

Light is electromagnetic radiation consisting of electric and magnetic fields which are perpendicular to each other. The electromagnetic spectrum starts at $10^{-12}$ m (gamma rays) and extends to $10^3$ m (radio waves). When light interacts with matter, the resultant
radiation depends on the type of material as well as the wavelength of incident light. If the light is scattered without loss of energy it is termed “elastic scattering”. If the light is scattered with a change in energy it is termed “inelastic scattering”. There are couples of ways radiation can follow. The radiation can be absorbed, transferred to a different form, reflected, refracted or diffracted by the material.\(^5\) This explains the attenuation of the light intensity when light passes through a medium. This attenuation is described by an exponential decay of the light intensity as follows\(^6\)–\(^9\):

\[ I = I_0 e^{-\sigma h} \]  

(4.1)

The above equation explains the attenuation of a beam of light from \(I_0\) to \(I\) in moving a distance \(h\) through a medium where \(\sigma\) is the attenuation coefficient and can be related to molar extinction coefficient, \(\sigma_m\), as:

\[ \sigma = 2.303\sigma_m c \]  

(4.2)

where \(c\) is the concentration. Extinction is the combination of absorption and scattering:

\[ \sigma = N(C_{\text{abs}} + C_{\text{sca}}) \]  

(4.3)

where \(N\) is the number of particles per unit volume, and \(C_{\text{abs}}\) and \(C_{\text{sca}}\) are the absorption and scattering cross sections, respectively and they have the dimensions of area. For 40 nm gold nanoparticles, typical values of molar extinction coefficient, absorption and scattering cross sections are \(8.1\times10^9\) M\(^{-1}\) cm\(^{-1}\), \(2.93\times10^{-15}\) m\(^2\) and \(1.75\times10^{-14}\) m\(^2\), respectively.\(^10\) In general, cross sections are dependent on the orientation of the particle and the polarization of incident light.\(^7\)

Although scattering and absorption can happen at the same time, there are instances that one or the other mechanism dominates. For example, the scattering mechanism will be dominant when visible light passes through a container that is filled
with milk whereas the absorption mechanism will be dominant when light passes through a container that is filled with ink. Milk is a suspension of weakly absorbing particles; therefore the light is attenuated primarily by scattering. Ink, on the other hand, consists of very small carbon particles which attenuate light primarily by absorption. Also, if the particle is in a homogeneous medium, the absorption will be attenuated more than the scattering.6

Extinction cross section can be expressed in a number of ways for a spherical particle. Physicists prefer using the extinction cross sections with the following formula6:

$$C_{\text{ext}} = C_{\text{abs}} + C_{\text{sca}}$$

(4.4)

If the particle is non-absorbing it can be written7:

$$C_{\text{ext}} = C_{\text{sca}}$$

(4.5)

Typical value of extinction cross section for 40 nm gold nanoparticles is $C_{\text{ext}} = 3.10 \times 10^{-15}$ m$^2$.10

On the other hand, astronomers use the extinction efficiency, $Q_{\text{ext}}$:

$$Q_{\text{ext}} = \frac{C_{\text{ext}}}{\text{Cross sectional Area}} = \frac{C_{\text{ext}}}{\pi a^2}$$

(4.6)

where $a$ is the radius of the particle. Therefore

$$C_{\text{ext}} = Q_{\text{ext}} \pi a^2$$

(4.7)

Typical value of extinction efficiency for 40 nm gold nanoparticles is $Q_{\text{ext}} = 2.5$.10

4.1.2 Surface Plasmon

When light hits a metal surface, the oscillating electric field of the incident light interacts with the free electrons of the metal. These free electrons start to oscillate collectively in resonance with the incident light. These oscillations are called surface plasmons. This means, there are certain wavelengths for metals where the photons are not
reflected, instead they are absorbed and converted into electron cloud vibrations. For metal nanoparticles surface plasmon resonance lies in the visible region of the spectrum.

Surface plasmons can be thought of as a giant electronic dipole whose strength and energy depend on the size and shape of the metal nanoparticle. Since a sphere is symmetric in any perspective, spherical particles will only be characterized by transverse plasmon mode, whereas rod shaped particles will be characterized by longitudinal as well as transverse plasmon mode due to being antisymmetric. Transverse plasmon mode corresponds to electron oscillations perpendicular to the major axis, whereas longitudinal plasmon mode corresponds to electron oscillations along the major axis. The effect of difference in size can be seen colorimetrically as well as a change in location of a transverse plasmon mode.11

Absorption of light by the electrons of the metal creates the surface plasmon resonance. This interaction is enhanced within the bandwidth of surface plasmon mode. The surface plasmon frequency, which depends on the dielectric function of the metal and shape of the metal, can be calculated using the Drude model. This model assumes that atom cores are immobile, and the conduction electrons are treated as a gas inside the metal sphere.12 The Drude model characterizes the frequency dependence as;

\[
\epsilon(\omega) = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma_0 \omega}
\]

(4.8)

where \(\gamma_0\) is the bulk electron collision frequency and \(\omega_p\) is the bulk plasmon frequency of the metal given as:

\[
\omega_p = \sqrt{\frac{Ne^2}{\varepsilon_0 m_{\text{eff}}}}
\]

(4.9)
where $N$ is the electron density of the metal, $e$ is the electron charge, $\varepsilon_0$ is the vacuum permittivity, $\varepsilon_m$ is the medium dielectric constant and $m_{\text{eff}}$ is the effective electron mass. Bulk plasmon frequency of gold metal is $1.35 \times 10^{16}$ sec$^{-1}$. Since $\gamma \ll \omega$ in the visible region, the surface plasmon resonance wavelength maximum ($\lambda_{\text{sp}}$) is:

$$\omega_{\text{sp}} = \sqrt{\frac{Ne^2}{\varepsilon_0 m_{\text{eff}}(1 + \varepsilon_m)}} \quad \text{OR} \quad \lambda_{\text{sp}} = 2\pi c \sqrt{\frac{\varepsilon_0 m_{\text{eff}}(1 + \varepsilon_m)}{Ne^2}}$$

(4.10)

The above equation tells the dependence of the surface plasmon frequency on the medium. When the medium dielectric constant increases, the surface plasmon frequency decreases. This is because an increase in the medium dielectric constant will result in a decrease in Coulombic force that acts on the electronic cloud therefore a decrease in surface plasmon resonance energy. Table 4.1 gives the typical values of the parameters mentioned above for the bulk gold and gold nanoparticles.
Table 4.1: Typical values for Gold metal

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption cross section*</td>
<td>$C_{abs}$</td>
<td>m$^2$</td>
<td>2.93×10$^{-15}$</td>
</tr>
<tr>
<td>Scattering cross section*</td>
<td>$C_{sca}$</td>
<td>m$^2$</td>
<td>1.75×10$^{-16}$</td>
</tr>
<tr>
<td>Extinction cross section*</td>
<td>$C_{ext}$</td>
<td>m$^2$</td>
<td>3.10×10$^{-15}$</td>
</tr>
<tr>
<td>Molar extinction coefficient</td>
<td>$\sigma_m$</td>
<td>M$^{-1}$ cm$^{-1}$</td>
<td>8.10×10$^9$</td>
</tr>
<tr>
<td>Bulk plasmon frequency</td>
<td>$\omega_p$</td>
<td>sec$^{-1}$</td>
<td>1.35×10$^{16}$</td>
</tr>
<tr>
<td>Surface plasmon wavelength*</td>
<td>$\lambda_{sp}$</td>
<td>nm</td>
<td>528</td>
</tr>
<tr>
<td>Extinction efficiency*</td>
<td>$Q_{ext}$</td>
<td>dimensionless</td>
<td>2.5</td>
</tr>
<tr>
<td>Electron density</td>
<td>N</td>
<td>cm$^{-3}$</td>
<td>1.00×10$^{22}$</td>
</tr>
</tbody>
</table>

*For gold nanoparticle having a diameter of 40 nm.
The difference between metals, insulators and semiconductors can be explained by band structures which rely on conduction and valence bands (Figure 4.1). The valence band contains valence electrons and the conduction band contains unoccupied orbitals. Conduction occurs when electrons move from the valence band to the conduction band, where they can move throughout the material and conduct electricity. The difference between the bottom of the conduction band and the top of the valence band is called the *Fermi energy (energy gap)* and is equal to the highest energy achieved by all the fermions at absolute zero. For metals, there is no energy gap so electrons can move throughout the solid and conduct electricity easily which changes their optical properties. For semiconductors, the energy gap is small but only a small number of electrons are in the conduction band so these materials conduct less than metals. For insulators, the energy gap is big, and so no electrons are able to be promoted to the conduction band from thermal energy which is why they cannot conduct electricity.\textsuperscript{11}
Figure 4.1: Energy band diagrams for a) conductor b) Insulator and c) Semiconductor.
4.2 Synthesis of Gold Nanoparticles

Gold Nanoparticles (GNPs) are synthesized by traditional citrate reduction method. This method involves the reduction of gold metals using sodium citrate (Figure 4.2). Experimental details of the synthesis are as follows: 15 mL of picopure water is brought to a boil and then 1% gold (III) chloride solution is added to the boiling water. The solution turns yellow after the addition of gold (III) chloride. Then, 1% sodium citrate solution is added to the mixture. The color of the solution changes from pale yellow to red within a few minutes. The mixture is heated until the color stays constant. The solution is then removed from the hot plate and allowed to cool to room temperature. Sodium citrate first acts as a reducing agent and later adsorption of citrate ions on the metal surface creates an electric double layer which causes coulombic repulsion and prevent aggregation. This is called electrostatic stabilization of the GNPs.

The ratio of gold (III) chloride to sodium citrate determines the size of the GNPs. By decreasing the amount of citrate added, larger particles can be produced. The reduction in the amount of sodium citrate will reduce the amount of citrate ions accessible for stabilizing the particles; therefore small particles will aggregate into larger ones until all the surface of the GNPs are covered completely by the citrate ions available in solution.

GNP synthesis occurs in 3 steps: nucleation, growth and termination. Nucleation is the step where gold ions are reduced to neutral form of gold by the reducing agent, which is sodium citrate in this experiment. After nucleation, aggregation of the GNPs takes place where small particles join together to form larger GNPs. This process is called growth. Finally, adsorption of the citrate ions onto the gold surface stabilizes the
particles which terminates the growth of particles.\textsuperscript{4, 15, 16} Therefore, the structure of a gold nanoparticle contains an inner core (Au\textsuperscript{0}) and a surface of positively charged gold atoms (Au\textsuperscript{+}). Since gold nanoparticles are prepared by the citrate reduction method, there are a large number of citrate ions electrostatically attracted to the positively charged gold atoms. Therefore, gold nanoparticles that are prepared by the citrate reduction method are negatively charged overall. Figure 4.3 shows the gold surface and the x, y and z coordinates of the atoms. Figure 4.4 shows how citrate sits on the gold surface. Each citrate appears in a triangular position on the gold surface, where each corner of the triangle is a carboxyl group of the citrate.
4.2.1 Number Density calculation of Gold Nanoparticles

The number density of gold nanoparticles can be calculated according to the following equations. First, the volume of one gold nanoparticle is calculated. Assuming that gold nanoparticles have diameters of 40 nm (r = 20 nm = 20 \times 10^{-9} \text{ m}), then volume of one gold nanoparticle will be:

\[ V = \frac{4}{3} \cdot \pi \cdot r^3 = 3.4 \times 10^{-23} \text{m}^3 \text{ per particle} \]  

(4.11)

Second, the number of gold atoms in one cubic meter is determined. Gold has a bulk density of 19.3 \text{ g/cm}^3 or 19,300 \text{ kg/m}^3, and molecular weight of 196.96 \text{ g/mole}, and the number of gold atoms per cubic meter can be calculated as:

\[ 19,300 \frac{\text{kg}}{\text{m}^3} \cdot 6.02 \times 10^{23} \frac{\text{atoms}}{\text{mole}} \cdot \frac{\text{mole}}{196.96 \text{ g}} \cdot \frac{1000 \text{ g}}{1 \text{ kg}} = 5.9 \times 10^{28} \frac{\text{atoms}}{\text{m}^3} \]  

(4.12)

and the number of atoms present in one gold nanoparticle can be obtained:

\[ 3.4 \times 10^{-23} \frac{\text{m}^3}{\text{particle}} \cdot 5.9 \times 10^{28} \frac{\text{atoms}}{\text{m}^3} = 1.9 \times 10^6 \frac{\text{atoms}}{\text{particle}} \]  

(4.13)

In the synthesis of gold nanoparticles, $6 \times 10^{-4} \text{ mol/L}$ of gold is used. Therefore, the number density of gold nanoparticles will be:

\[ 6 \times 10^{-4} \frac{\text{mole}}{\text{L}} \cdot 6.02 \times 10^{23} \frac{\text{atoms}}{\text{mole}} \cdot \frac{\text{particle}}{1.9 \times 10^6 \text{ atoms}} = 1.8 \times 10^{14} \frac{\text{particle}}{\text{L}} \]  

(4.14)
Figure 4.2: Sketch of gold nanoparticle synthesis.
**Figure 4.3**: Gold surface and the x, y, z coordinates of the numbered atoms.

<table>
<thead>
<tr>
<th>Coordinates</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.170628</td>
<td>3.02288</td>
<td>-5.09825</td>
</tr>
<tr>
<td>2</td>
<td>3.05895</td>
<td>3.05895</td>
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<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>3.05895</td>
<td>-1.01965</td>
<td>-5.09825</td>
</tr>
<tr>
<td>5</td>
<td>0.170628</td>
<td>-0.983581</td>
<td>-5.09825</td>
</tr>
<tr>
<td>6</td>
<td>-1.90474</td>
<td>1.01965</td>
<td>-5.09825</td>
</tr>
</tbody>
</table>

**Figure 4.4**: Cartoon showing how citrate sits on gold surface, Top view (left), side view (right).
4.3 Characterization of Gold Nanoparticles

Two approaches were used to determine the size of gold nanoparticles. In the first approach, particle sizes were obtained using various methods cited in the literature and the results were averaged. The second approach uses DynaPro Nanostar Dynamic light scattering equipment to determine the size of the nanoparticles.

4.3.1 Literature Research

Haiss et al.\textsuperscript{17} studied gold nanoparticles and determined their size using UV-Vis spectroscopy and TEM. The particles were 5-100 nm in size. These particles were assumed to be spherical since the elongation of the particles was found to be 1.05 and 1.25. Theoretical calculations were performed to calculate the extinction efficiency, \( Q_{\text{ext}} \), using a FORTRAN code written by Bohren and Huffman:

\[
Q_{\text{ext}} = \frac{c_{\text{ext}}}{\pi a^2}
\]  

(4.15)

where \( a \) is the particle radius and \( c_{\text{ext}} \) is the extinction cross section described by:

\[
c_{\text{ext}} = \frac{2\pi}{|q|^2} \sum (2L + 1) \text{Re}(a_L + b_L)
\]  

(4.16)

where \( q = 2\pi \sqrt{\varepsilon_m/\lambda} \) is the wave vector and \( a_L \) and \( b_L \) are scattering coefficients in terms of Ricatti-Bessel functions. The calculated extinction efficiency \( Q_{\text{ext}} \) can be related to absorption (A) by the following equation:

\[
A = \frac{\pi a^2 Q_{\text{ext}} b \mathcal{N}}{2.303}
\]  

(4.17)

where \( b \) is path length of the spectrometer and \( \mathcal{N} \) is the density of particles per unit volume. For particle diameters of 2.5 to 100 nm, the dependence of the extinction efficiency \( Q_{\text{ext}} \) on the wavelength was plotted and a visible surface plasmon peak around 520 nm was observed. Also, for a particle diameter of 20.4 nm, dependence on the
absorption to wavelength was plotted and compared to experimental data where there was a good agreement between theoretical and experimental results. The surface plasmon peak ($\lambda_{spr}$) was plotted versus the particle diameter for particle sizes greater than 25 nm, and a simple exponential function was obtained. Therefore, the particle size could be easily related to the surface plasmon peak by the following equation:

$$a = \frac{\ln\left(\frac{\lambda_{spr} - \lambda_0}{L_1}\right)}{L_2}$$

where $L_1=6.53$ and $L_2=0.0216$. This equation is valid only for particle sizes greater than 25 nm because for smaller particles observed peak position was lower than predicted which may be due to an increase of the ratio of surface atoms to bulk atoms. For this reason, another method was used. A better agreement between theory and experiment was found when the ratio of the absorbance at the surface plasmon resonance peak ($A_{spr}$) to the absorbance at 450 nm ($A_{450}$) was taken. Therefore, for particles smaller than 35 nm, the following equation was used to relate particle size to the surface plasmon resonance peak:

$$a = \exp\left(B_1 \frac{A_{spr}}{A_{450}} - B_2\right)$$

where $B_1$ is the inverse of the slope of the linear fit of the graph ($A_{spr}/A_{450}$ vs $\ln(a)$) and $B_2=B_0/m$ where $B_0$ is the intercept. In summary, this paper demonstrated that the size of the gold nanoparticle can be directly calculated from UV-Vis spectra according to Equations 4.18 and 4.19.

In another study, Jain et al.\textsuperscript{10} studied gold nanospheres, gold nanorods and silica-gold nanospheres and their theoretical optical properties. For the case of gold nanospheres, the absorption and scattering efficiencies ($Q_{abs}$ and $Q_{sca}$) were calculated...
using Mie theory for homogenous spheres. For the case of silica-gold nanoshells, a computer code using Mie scattering for concentric sphere geometry was used. For the case of gold nanorods, the discrete dipole approximation was used. Absorption and scattering efficiencies were converted to cross sections and plotted against the nanoparticle radius. It was observed that for all three types of nanoparticles, the extinction cross section as well as the relative contribution of scattering increased as size of the nanoparticle increases. Furthermore, the surface plasmon resonance wavelength maximum (λ\text{max}) was plotted against the particle size and a small red-shift was observed which might be due to the effect of electromagnetic retardation in larger nanoparticles.

Liu et al.\textsuperscript{18} studied gold nanoparticles with core sizes ranging from ~ 4 to 40 nm and with different capping ligands. The capping ligands used were citrate, oleylamine and decanethiol. First, the average number of gold atoms per nanoparticle (N_{\text{ave}}) was calculated using the following equation:

\[ N_{\text{ave}} = \frac{\pi \rho a^3}{6 M} = 30.89602 \text{ a}^3 \]  

(4.20)

where \( \rho \) is the density for face centered cubic (fcc) gold (19.3 g/cm\(^3\)), \( a \) is radius of particle, and \( M \) is the molar mass of gold (197 g/mol). Secondly, molar concentration (c) was calculated by the following equation:

\[ c = \frac{N_{\text{Total}}}{N_{\text{ave}} VN_A} \]  

(4.21)

where \( N_{\text{total}} \) is total number of gold atoms, \( V \) is volume of reaction vessel in liter, and \( N_A \) is Avogadro’s constant. The molar extinction coefficient, \( \sigma_m \), can be obtained using the Lambert-Beer law where you insert absorption (A), concentration (c) and path length (b) and obtain the molar extinction coefficient as follows:
Finally, the extinction coefficient was plotted against the particle diameter and a linear fitting of this data gave the following equation:

\[ A = \sigma_m b c \]  \hspace{1cm} (4.22)

From the above equation, the molar extinction coefficient can be related to the particle size, so one can use the extinction coefficient to easily determine the particle size.

Hea \textit{et al.} \textsuperscript{19} prepared gold nanoparticles sizes ranging from 12 to 41 nm by the citrate reduction method. The absorption peak of these particles shifted to red as the particle size increased. Also, there was a gradual color change from orange-red to red and red-purple as diameters increased. However, if the difference of the absorption peak was less than 10 nm, the color change was not very obvious. The average sizes of the particles were measured by TEM. The radiiuses of the particles (a) were plotted against \( \lambda_{\text{max}} \) and the following relation was obtained:

\[ \lambda_{\text{max}} = 515.04 + 0.7294 a \]  \hspace{1cm} (4.24)

Since the above equation gave a good correlation, the sizes of the gold nanoparticles can be estimated using the absorption spectrum.

In another study, Link and El-Sayed\textsuperscript{20} observed the dependence of plasmon absorption of gold nanoparticles on size and temperature. Sizes of the gold nanoparticles ranging from 9 to 99 nm were determined from TEM images which revealed that the particles were nearly spherical. The absorption spectrum was obtained for all sizes and the effect of temperature was measured. The temperature effect was very small, and there was a little decrease in intensity of the maximum but there was no broadening of the plasmon peak. To be able to observe a significant effect from temperature, it has to be
increased by several hundred degrees, which is not be possible without changing from water to an inert matrix. Increasing temperature will change the refractive index of the water which will decrease the surface plasmon intensity. Additional broadening of the surface plasmon was observed for all size ranges which was due to the citrate ions adsorbed at the nanoparticle surface. This additional broadening was in the same order for all size ranges because citrate ions were present in excess amounts and dynamic equilibrium was achieved between adsorbed and free citrate ions.

Jana et al.\textsuperscript{21} explained the synthesis of different sized gold nanoparticles using the seeding growth technique. Size ranges between 5-40 nm can be obtained using this method by just changing the ratio of seed to metal salt. This paper was used to compare the absorption maximum with the corresponding particle sizes from previous literature data.

Murillo et al.\textsuperscript{22} plotted the absorption spectrum of different sized gold nanoparticles. This graph was also used to compare to results from the literature search.

The size of the gold nanoparticles was determined by averaging the results of the above mentioned seven techniques. All of these techniques required taking the absorption spectrum of the gold nanoparticles which is shown in Figure 4.5. Light absorption by gold nanoparticles takes place within a narrow range of wavelengths. A plasmon resonance shows the presence of a strong band in the visible region, arising from the collective oscillation of conduction electrons in response to the external electromagnetic field. The particles sizes determined were 3.5, 5.7, 15.4, 20.6, and 22.3 nm. The absorption peaks corresponding to the sizes are located at 510, 518, 523, 529, and 531,
respectively. The absorption peaks shifted to higher wavelengths due to the presence of bigger particles and aggregation.

The sizes determined using these methods plotted against the absorption maximum ($\lambda_{\text{max}}$) as shown in Figure 4.6. The second degree polynomial fit gave the following equation:

$$\text{Radius (nm)} = 2.73 \times 10^{-2} (\lambda_{\text{max}})^2 - 2.74 \times 10^1 (\lambda_{\text{max}}) + 6.903393 \times 10^3 \quad (4.25)$$

and the standard deviation calculated was 3.18.
Figure 4.5: UV-Vis absorption spectrum of gold nanoparticles, sizes 3.5 (black), 5.7 (green), 15.4 (blue), 20.6 (pink), and 22.3 (red).
Figure 4.6: Graph showing the relation between radius of the particles and the absorption maximum ($\lambda_{\text{max}}$) using literature search.
4.3.2awnaPro Nanostar Dynamic Light Scattering Equipment

The sizes of gold nanoparticles were determined using DynaPro Nanostar dynamic light scattering equipment. The working principle of this equipment was explained in Chapter 3. The sizes determined using DynaPro Nanostar equipment are 3.6, 8.8, 10.0, 19.7, 21.2 nm and the absorption peaks corresponding to the sizes are located at 513, 522, 523, 530, and 533 nm, respectively.

The sizes obtained using this method were plotted against the absorption maximum (\( \lambda_{\text{max}} \)) as shown in Figure 4.7. The second degree polynomial fit gave the following equation:

\[
\text{Radius (nm)} = 2.50 \times 10^{-2}(\lambda_{\text{max}})^2 - 2.52 \times 10^1(\lambda_{\text{max}}) + 6.363791 \times 10^3 \\
(4.26)
\]

and the standard deviation calculated was 3.33.

4.3.3 Results of Transmission Electron Microscope

To ensure the accuracy of these methods, Transmission electron microscopy (TEM) was also used to determine the size of nanoparticles. Figure 4.8 shows the TEM image of gold nanoparticles which have an absorption maximum at 522 nm. TEM images of 100 gold nanoparticles were taken into account and the average size was found to be 10 nm (Figure 4.9). Inserting \( \lambda_{\text{max}} = 522 \) into Equation 4.25 results 11.5 nm for the radius and inserting \( \lambda_{\text{max}} = 522 \) into Equation 4.26 results 9.4 nm for the radius. Both of these results are consistent with TEM measurements. To sum up, both of these approaches gave accurate results and can be used to find out the size of gold nanoparticles.
Figure 4.7: Graph showing the relation between radius of nanoparticles and absorption maximum ($\lambda_{\text{max}}$) using DynaPro Nanostar dynamic light scattering equipment.
Figure 4.8: TEM image of gold nanoparticles of radius 10 nm.
Figure 4.9: Size Distribution of gold nanoparticles of average size 10 nm.
4.4 Synthesis of Silver Nanoparticles

There are two different methods used in the literature to synthesize silver nanoparticles. One involves using sodium borohydride (NaBH₄)²³ and the other uses sodium citrate (C₆H₅O₇Na₃) as a reducing agent.²⁴⁻²⁸

Citrate reduction method: In a typical experiment, 0.068 grams of AgNO₃ is dissolved in 100 mL of picopure water and is heated to a boiling solution. 10 ml of 1 % C₆H₅O₇Na₃ is added to this solution drop wise in 2 minutes while stirring vigorously. The solution turns greenish-yellow after couple of minutes indicating the formation of silver nanoparticles. The solution is left to boil continually for about 10 minutes or until the color change is evident. The resulting suspension is cooled to room temperature. The solution prepared with this method should have an absorption maximum at 426 nm. The reaction occurring in this method can be seen:

\[
4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2 \quad (4.27)
\]

Borohydride reduction method: This method is used to make relatively smaller particles than the citrate reduction method. 30 mL of 2 mM NaBH₄ is chilled in an ice bath and added drop wise to 10 mL of 1mM AgNO₃ solution. The reaction mixture is stirred vigorously. After a few minutes the solution turns bright yellow. The silver nanoparticles prepared using this method has an absorption maximum at 398 nm. The reaction occurring in this method is:

\[
AgNO_3 + NaBH_4 \rightarrow Ag + \frac{1}{2}H_2 + \frac{1}{2}B_2H_6 + NaNO_3 \quad (4.28)
\]

Various sizes of silver nanoparticles have been synthesized by changing the ratio of silver nitrate to sodium citrate and analyzed using the same method for gold.
nanoparticles. Figure 4.10 shows the UV-Vis spectra of different sized silver nanoparticles and the change of the solution color as the particle size changes.
Figure 4.10: UV-Vis spectrum of silver nanoparticles and the change of solution color. The ratio of silver nitrate to sodium citrate is $0.5 \times 10^{-3}$ (pink), $1 \times 10^{-3}$ (green), $2 \times 10^{-3}$ (red), $4 \times 10^{-3}$ (blue). Largest on left, smallest on right.
4.5 Synthesis of silica coated silver nanoparticle composites

Under heating and vigorous stirring, 10 mL of the silver nanoparticles (prepared by the citrate reduction method), is mixed with 100 mL of isopropyl alcohol. The heating is continued until the temperature of the solution reaches 40°C. At this point, the heating is stopped and 18 mL of picopure water is added. Immediately after the addition of 5.25 mL of 30 % ammonium hydroxide (NH₄OH), 20 μL tetraethyl orthosilicate (TEOS) is added. The reaction is stirred at room temperature for 20 minutes. The solution of silica-coated silver nanoparticles is washed and centrifuged two times with water at 3500 rpm for 30 minutes. The thickness of the silica layer is around 8-9 nm which is determined using TEM. The absorption maximum of these particles is around 442 nm. Different mixing times will create different silica thickness as shown in Figure 4.11.

4.5.1 Mechanism of Reaction

The most important step in silica coating is the hydrolysis and condensation of TEOS (Si(OC₂H₅)₄) on silver surface.

The hydrolysis reaction occurs as follows:

\[ \text{Si(OC}_2\text{H}_5)_4 + 4 \text{H}_2\text{O} \rightarrow \text{Si(OH)}_4 + 4 \text{C}_2\text{H}_5\text{O} \]  \hspace{1cm} (4.29)

which is followed by condensation reaction as:

\[ \text{Si(OH)}_4 \rightarrow \text{SiO}_2 + 2 \text{H}_2\text{O} \] \hspace{1cm} (4.30)

The hydrolysis reaction needs to happen fast because this increases the monodispersity of the resulting silica-coated silver nanoparticles and prevent competing reactions. However, the hydrolysis of TEOS is very slow in water, so acid or base should be used to catalyze the reaction. Using acid as a catalyst promotes the formation of a gel, where as using a base as a catalyst forms a stable solution. Therefore, ammonium hydroxide is
used as a catalyst. Base catalyzed hydrolysis has the advantage of preventing particles from aggregating due to a negative surface charge at high PH, as well as producing spherical particles. Under basic conditions, hydrolysis reaction is first order, but if the concentration of TEOS is increased, the reaction order deviates from first order and becomes second order. Higher ammonium hydroxide speeds up the reaction rate, but also leads to larger particles. The hydrolysis rate speeds up because the hydroxide particles will attack the central silicon atom and displace a molecule of alcohol, however smaller particle sizes would be expected because there would be more nucleation early in the reaction. The fact that larger particle sizes result from higher ammonia concentration implies that ammonia also catalyzes the rate of silica condensation on existing particles. Higher water concentration will lead to a faster reaction rate and smaller particles, which implies that high water concentrations speed up hydrolysis, as would be expected. Alcohols are good solvents for the growth reaction because they dissolve both TEOS and water well. It is possible for the hydrolysis to run backwards, so the reaction rate can be speed up by selecting an alcohol solvent that will not easily add to the silica monomers.

On the other hand, faster reaction rates may lead to formation of core-free silica particles which is not favorable in this experiment. Therefore, the reagents should be carefully adjusted to obtain a fast reaction rate without allowing the nucleation of core-free silica particles.
**Figure 4.11:** Silica coated silver nanoparticles with different thickness a) 10 minute mixing b) 20 minute mixing c) 30 minute mixing.
4.5.2 Attachment of fluorophore to silica coated silver nanoparticle composites

First approach involves the doping of the fluorophore into the pores of the silica shell. The details are as follows: under stirring, 500 μL of 0.088 mg/ml Rhodamine 101 is added into 500 μL of silica coated silver nanoparticles suspended solution and stirring continued overnight. The mixture is then centrifuged twice at 3300 rpm and the precipitate is washed with water. The supernatant solution is also kept for future applications. To clarify, the precipitate contains the fluorophore doped silica coated silver nanoparticles and the supernatant solution contains the unbound fluorophore mixture. Rhodamine 101 fluorescent dye has an excitation wavelength of 560 nm and emission wavelength of 589 nm in methanol.\textsuperscript{30}

Second approach involves the covalent attachment of the fluorophore to the silica coated particles.\textsuperscript{27} In this regard, 1 mg of silica coated silver nanoparticles is washed with ethanol. Then 20 mL ethanol and 1.65 mL of APS (3-aminopropyl triethoxysilane) is added. The resulting solution is stirred continuously for approximately 4 hours at room temperature. At the end of this reaction, amino-derived nanoparticles are obtained. Then the nanoparticles are centrifuged and redissolved in 1 mL 0.1 M NaHCO\textsubscript{3} (ph 9.0). Finally 600 μL of amino-derived nanoparticles is added to 10 μL of amino-active fluorophore (IRDye 800 CW NHS ESTER). After being stirred at room temperature for 10 minutes, the resulting solution is centrifuged and the precipitate is redissolved in water.
4.5.3 Etching away silver core from fluorophore doped silica coated silver nanoparticles

To be able to see the metal effect on enhancement of the fluorescent signal, the silver core should be etched away (making nanobubbles\textsuperscript{27}) and the fluorescent intensity of the particles with and without silver should be compared. The recipe to etch away the silica core is as follows\textsuperscript{27}: 800 μL of silica coated particles are mixed with 2 mL of 10 mM NaCN and stirred for 2 minutes. The solution is centrifuged and redissolved in picopure water. The resulting UV-vis spectra of silica coated silver nanoparticles and the silica coated bubbles are shown in Figure 4.12. To these two different particles fluorophore is doped and the fluorescent intensity is compared as shown in Figure 4.13. It is clear from the figure that the presence of a silver core increased the signal.
Figure 4.12: UV-vis spectrum of silica coated silver nanoparticles and silica coated nanobubbles.
Figure 4.13: Confocal microscope images of Nanobubbles (left) and fluorophore doped silica coated silver nanoparticles (right)
4.6 Light Scattering Experiments

Another method to characterize nanoparticles is using light scattering methods. As discussed in Chapter 3, dynamic and angle dependent light scattering apparatus have been built to measure the size of the nanoparticles that are of interest. In this section, the preliminary results and some future directions to obtain better results will be discussed.

4.6.1 Results of Dynamic Light Scattering (DLS)

In DLS, the aim is to determine the size of nanoparticles. To achieve this, the steps to follow is as follows: First, scattering intensity from the nanoparticles is obtained, second, autocorrelation function is applied to the scattering intensity, third, exponential curve is fit to the autocorrelation function to obtain a decay rate (Γ) and finally Stokes-Einstein relation is used to determine the size of particles. A Labview program was written to collect scattering signals from particles that are analyzed. The Labview program collected the signals from the oscilloscope and converted the signal into a text file. This text file was fed into another Labview program, which was obtained from Dr. Robert Marlowe at The University of Tennessee. This program applied an autocorrelation function to the scattering signals using a formula node. The equations that were used in the formula node were as follows:

```plaintext
float64 output[100];
int sizeofArray= sizeofDim(inputArray, 0);
int maxB = sizeofArray - numChannels;
int b,k;
for(b = 0; b < numChannels; b++)
    { output[b] = 0;
```
for (k = 0; k < maxB - 1; k++)
    output[b] += (inputArray[k] * inputArray[k+b]);
    output[b] /= (k );}

There were two inputs to this formula node. One input was the signals collected from the oscilloscope (input array) and the other was the number of channels (numChannels). In this specific example shown above, the size of the output array is given to be 100 (which is the autocorrelation function). sizeOfarray has the same dimension as the size of the input array. The output array was calculated using the formula:

\[
output\ array[b] = \frac{input\ array[k] \times input\ array[k+b]}{k}
\] (4.31)

After the output array was calculated, the program wrote the data into a text file. A Delphi Pascal program was written to obtain an exponential fit to the autocorrelation function to obtain the decay rate (\(\Gamma\)).

Scattering signals and autocorrelation functions from both silver and gold nanoparticles were obtained using the above programs. However, the autocorrelation functions did not show exponential decay, so an exponential fit could not be performed and the sizes could not be obtained for gold and silver nanoparticles that are small in size (<100 nm). Therefore, milk fat globule particles were used to test the accuracy of the data analysis. First, 0.2 mL of whole milk was diluted with 100 mL of water. Then, 1 mL of 35 mM EDTA/NaOH (ph=7) was added to the above solution and the solution was stirred for 10 minutes. The scattered light intensities from the milk fat globule particles were collected with an oscilloscope which had a sample rate of 10 Msamples/second as shown in Figure 4.14. These signals were then put into the autocorrelation program, and the autocorrelation function was obtained as shown in Figure 4.15. The last step was to fit
an exponential curve using either Excel or Delphi-Pascal both of which gave similar results as shown in Figure 4.16. The decay rate obtained using Excel and Delphi-Pascal was -0.0209 and -0.0208, respectively. The fitted exponential equation was:

$$g^{(2)}(t) = \beta e^{-\Gamma t}$$  \hspace{1cm} (4.32)

where $\Gamma = 0.0208 \text{ s}^{-1}$. Since:

$$\Gamma = Dq^2 \quad \text{and} \quad q = \frac{4\pi n\sin(\theta/2)}{\lambda}$$

then:

$$q = \frac{4 \times 3.14 \times 1.33 \times \sin(45/2)}{532 \times 10^{-9} \text{m}} = 1.2 \times 10^7 \text{m}^{-1}$$  \hspace{1cm} (4.33)

so:

$$0.0208 \text{ s}^{-1} = D \times (1.2 \times 10^7 \text{m}^{-1})^2 \quad \therefore \quad D = 1.4 \times 10^{-16} \text{ m}^2/\text{s}$$  \hspace{1cm} (4.34)

The radii of the particles can be calculated using the Stokes-Einstein relation:

$$R_h = \frac{kT}{6\pi\eta D}$$

$$R_h = \frac{1.38 \times 10^{-23} \left(\frac{\text{m}^2 \text{kg}}{\text{s}^2 \text{K}}\right) \times 298 \text{ (K)}}{6 \times 3.14 \times 2 \times 10^{-3} \left(\frac{\text{kg}}{\text{m}^2 \text{s}}\right) \times 1.4 \times 10^{-16} \left(\frac{\text{m}^2}{\text{s}}\right)} = 0.00077 \text{ m}$$

$$= 770 \text{ microns}$$  \hspace{1cm} (4.36)

The desired radii for milk fat globule particles are supposed to be between 0.2 - 0.5 $\mu\text{m}^{31}$, but these results could not be achieved due to laser instability. There was some instability with the Nd:YVO$_4$ laser being used for DLS measurements. The laser was connected to the oscilloscope and the signal was observed to be a repeating pattern most likely due to the laser instability. The oscilloscope was not line triggered so it was not picking up the room light. By zooming in to the signal data, it was found that there was a
repeating square wave with a frequency of $10^8$ Hz. The amplitude of this square wave was increasing as the voltage supply to the detector was increased. For example, if 400 V is applied, the amplitude of the square wave was 0.000312 V and the maximum signal was 0.034 V, so the signal to noise ratio is 108.4. Alternatively, if 700 V is applied, the amplitude of the square wave was 0.0203 V and the maximum signal was 2.83 V, so the signal to noise ratio was 123.

Moreover, due to the fact that the Labview program for autocorrelation resulted an exponential decay for only milk fat globule particles but not for any other nanoparticles, this was probably a random occurrence of the program. Therefore, the accuracy of the Labview program is untrustworthy. Better results would have been obtained if a commercial autocorrelator instrument could be obtained which are very sensitive diagnostic tools for measuring ultrafast signals.
Figure 4.14: Scattering light intensities of milk fat globule particles.
**Figure 4.15**: Front panel of the Labview program showing autocorrelation function.
Figure 4.16: The exponential fit results from Delphi Pascal (top) and Excel Worksheet (bottom).
4.6.2 Results of Angle Dependent Light Scattering (ADLS)

The details of the ADLS apparatus are mentioned in Chapter 3. In this section, the preliminary results will be shown and discussed.

The aim is to collect scattering light intensity with respect to scattering angle. By rotating the stage where all the collection optics and the detector were placed, the scattering signal from the particles was collected from $32^\circ$ to $160^\circ$ angles. The signal from the photodiode was fed into current amplifier. The amplified signal was then sent to the lock-in amplifier for signal detection. A custom labview program controlled the data collection and the data analysis. The connection between computer and lock-in amplifier was provided by National Instruments DAQ I/O interface board. A labview program was written to collect and analyze the data.

To characterize the ADLS apparatus, gold and silver nanoparticles were used. Unfortunately, the signals from these small particles were too low to be measured, therefore polystyrene latex particles (Sigma-Aldrich) was used which provides abundant scattering signal when illuminated with a laser light. The particles had an average diameter of $3\mu m$ and 10 weight % dispersion in water. 3 different concentrations of the particles were prepared by diluting the stock solution with distilled water. The stock solution was diluted by 1/250, 1/500, and 1/1000 in the first, second and the third samples, respectively. The scattering signals from each of these samples were measured by rotating the stage by hand.

These results, which are shown in Figure 4.17, were in good agreement with the expected angle dependence of the scattered light where there was a higher intensity for the forward angle (scattering maximum at $32^\circ$). The high angle limit, $160^\circ$, was due to the
geometry of the set up, where collection optics blocked the incoming light at angles higher than $160^0$. These results were compared with a web based Mie scattering calculator$^{32}$ which is shown in Figure 4.18. The model calculation results were in good agreement with experimental measurements having a higher intensity for the forward angle (scattering maximum at $18^0$). In our calculations, the refractive index for the air and water were assumed to be 1.0 and 1.33, respectively. The difference between the scattering maximum for the experiment and theory can be of different reasons. One is that, the rotation stage was being moved by hand which was time consuming and causing low angle resolution, second one is that there was an uncertainty in the polystyrene latex particles size. Therefore, more efficient ADLS set up needs to be assembled to characterize nanoparticles.
Figure 4.17: Relation between scattering intensity and scattering angle for milk fat globule particles, square, sphere, and triangle markers have 0.04 weight %, 0.02 weight % and 0.01 weight % dispersion in water.
Figure 4.18: Relation between scattering intensity and scattering angle for 3 μm particles obtained from web based Mie calculator.
References


(30) [www.sigmaaldrich.com](http://www.sigmaaldrich.com).


(32) omlc.ogi.edu/calc/mie_calc.html.