An Improved Synthesis of Gold Nanorods with Tunable Dimensions and Localized Surface Plasmon Resonance Properties

by

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B.Sc., University of Toronto, 2012

Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science

in the Department of Chemistry Faculty of Science

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SIMON FRASER UNIVERSITY

Spring 2018

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Abstract

Gold nanorods have been pursued due to their unique optoelectronic properties, which have led to potential uses in multiple applications. We sought to prepare gold nanorods that would potentially be used in biomedical applications, such as bio-imaging, photothermal therapies, and drug delivery systems. Typically in biomedical applications, gold nanorods with a localized surface plasmon resonance band that lies in the near infrared window between 650 to 1350 nm is highly desirable to obtain better images and an efficient photothermal effect over a range of depths within biological tissues. In addition, the dimensions of gold nanorods also play an important role in terms of cellular uptake and retention, as well as controlling the ratio between their absorbance and scattering properties. Thus, a primary goal of our study was to regulate dimensions and localized surface plasmon resonance of the gold nanorods to improve their potential utility in applications requiring both cellular uptake and photothermal triggered processes through the use of localized surface plasmon resonance bands in the near infrared “window”. We have modified the seed-mediated method by sequentially varying concentrations of hydrochloric acid and chloroauric acid to tune the dimensions, and thus the properties of the gold nanorods. The average dimensions of the gold nanorods were tuned from 24±4 nm in length and 7±1 nm in width, to 47±10 nm in length and 11±2 nm in width from these adjustments in the concentration of hydrochloric acid and chloroauric acid in the growth solution.

Keywords: gold nanorods, localized surface plasmon resonance, surface energy, photothermal effect, extinction
Acknowledgements

I would like to give special thanks to my supervisor, Dr. Byron D. Gates for the opportunity and valuable experience in research. I would like to acknowledge my committee members, Dr. Bingyun Sun and Dr. Krzysztof Starosta and co-supervisor, Dr. Donald Yapp for their guidance and supervision on my research as well. I really appreciate all the helps from Dr. Gates group members. I really enjoyed working with the members. Lastly, I would like to give thanks to my family for all the support throughout my studies. I sincerely appreciate all the help and support. Thank you.
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<td>AuNPs</td>
<td>Gold nanoparticles</td>
</tr>
<tr>
<td>AuNRs</td>
<td>Gold nanorods</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
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<tr>
<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
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<tr>
<td>CTAC</td>
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</tr>
<tr>
<td>BDAC</td>
<td>Benzyldimethylammonium chloride</td>
</tr>
<tr>
<td>FCC</td>
<td>Face center cubic</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
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Chapter 1.

Introduction to Gold Nanorods

In our study, we were interested in preparing metal nanoparticles that would potentially be used in biomedical applications, such as bio-imaging, photothermal therapies, and drug delivery systems. In general for biomedical applications, non-ionizing radiation that includes visible and infrared (IR) light are highly desired since they are relatively less damaging than ionizing radiation sources, such as ultraviolet (UV) or X-rays. In addition electromagnetic energy in the visible and infrared regions are relatively easy to handle and require less costs for implementation. Electromagnetic waves in the visible and infrared regions can, however, be significantly impeded for transmission by the tissues in the body. For example, an electromagnetic waves in the visible region can be absorbed by hemoglobin and melanin and electromagnetic waves in the infrared region can be absorbed by water molecules (Figure 1.1).

![Figure 1.1](extinction_spectra_of_major_components_of_the_tissues_in_the_body_include_hemoglobin,_melanin,_and_water_which_impede_transmission_ofElectromagnetic_waves_in_the_visible_and_infrared_regions_(Licensed_under_CC-BY)).

Figure 1.1 Extinction spectra of major components of the tissues in the body include hemoglobin, melanin, and water which impede transmission of electromagnetic waves in the visible and infrared regions (Licensed under CC-BY).
Therefore, tissues of the body can significantly limit penetration of electromagnetic energy with wavelengths in the visible and infrared regions. Several studies have shown incident light in the near infrared (NIR) window region, specifically between 650 to 1350 nm has the highest depth of penetration into the tissues of a body.\textsuperscript{1,2,3,5} Although penetration depth depends on the power density of a laser and the type of tissues in the body, generally incident light in the near infrared region can penetrate 1 to 10 cm into the tissues of a body.\textsuperscript{1,5} Metal nanoparticles with a localized surface plasmon resonance (LSPR) band that lies in the near infrared window between 650 to 1350 nm is, therefore, highly desirable for use in biomedical applications to obtain better images and an efficient photothermal effect over a range of depths within tissues of a body.

Conducting materials, typically metals, can exhibit a plasmon. When a metal is exposed to light, the oscillating electromagnetic field of the light induces free electrons in a conduction band of the metal to oscillate with respect to the fixed atoms in the lattice. This collective oscillation of the electron cloud around the metal surfaces causes a charge separation, which construct a dipole oscillation along the direction of the electric field of the light known as surface plasmon. In the case of metal nanoparticles, where their size is much smaller than the wavelength of the incident light used to excite the surface plasmon, this surface plasmon can be confined within the nanoparticles and is referred to as a localized surface plasmon.\textsuperscript{6,7} When the frequency of the electromagnetic wave from the incident light matches the natural frequency of the localized surface plasmon of a nanoparticle, the electromagnetic waves can enhance the localized surface plasmon. This excited electron cloud rapidly get thermalized due to the collisions within the electron cloud and with the atomic lattice of the nanoparticle. Thermalized electron cloud at the
surfaces of a nanoparticle is coupled with the phonons of a crystal lattice where they exchange energy until thermal equilibrium is reached within a metal nanoparticle. The thermalized metal nanoparticle dissipates heat into the surrounding medium through phonon-phonon coupling and the excited electron cloud returns back to its relaxed state until the next cycle of excitation occurs from the light. This transformation of energy from incident light into heat is known as the photothermal effect.\textsuperscript{8,9} Alternatively incident light may also be scattered at the surfaces of the nanoparticles. The sum of the contributions from the plasmon resonance and scattering can be measured by extinction spectroscopy.\textsuperscript{9,10,11}

There are numerous metal nanoparticles that are known to exhibit a localized surface plasmon resonance band that lies in the visible to near infrared region, such as gold, silver, copper, platinum, and palladium. The intensity of the localized surface plasmon resonance of platinum and palladium is, however, relatively weak when compared to gold, silver, and copper. Among gold, silver, and copper, gold is known to be more resistant to oxidation. Hence, gold is a more suitable material for use in biomedical applications, since many biologically induced oxidative reactions are present in biological systems.\textsuperscript{6,12,13}

Gold nanoparticles (AuNPs) have been extensively studied since the first reported colloidal gold by Faraday in 1857.\textsuperscript{14} Gold nanoparticles can take on many different shapes that include spheres, rods, shells, cubes, wires, stars, cages, and triangles. Depending on the dimension, shape, and surface chemistry of the gold nanoparticles, the localized surface plasmon resonance band can shift its peak position and the relative contributions of the absorbance and scattering can change.\textsuperscript{9,10,11,15,16} In addition, the
dimension, shape, and surface chemistry of the gold nanoparticles affect cellular uptake and retention of these nanoparticles which is another important factor to consider for many biomedical applications.\textsuperscript{17,18,19} Size, shape and surface chemistry of the gold nanoparticles are one of the key aspects to control their optoelectronic and biological properties for appropriate use in these types of applications.

Gold nanorods are one of the shapes that can exhibit an intense and well-defined localized surface plasmon resonance within the near infrared region. In addition, the localized surface plasmon resonance of the gold nanorods is relatively easy to tune over a broad range of wavelengths by altering the ratio of their length to width, otherwise referred to as the aspect ratio of these materials (Figure 1.2).\textsuperscript{6,20,21} This thesis will, therefore, focus on preparing gold nanorods with a broad range of dimensions and localized surface plasmon resonance properties with a goal of improving our understanding of their synthetic control to better prepare these materials for use in many biomedical applications.
Figure 1.2  Extinction spectra of gold nanorods and their change in surface plasmon resonance band with respect to the change in length to width (or aspect ratio) of the gold nanorods (each spectra were normalized to the centroid of their longitudinal surface plasmon resonance band).
1.1 Synthesis of Gold Nanorods

There are numerous methods for synthesizing gold nanorods, such as seed-mediated growth methods, templating methods, and electrochemical methods. Cepak and Martin prepared gold nanorods by a templating method for the first time in 1998. In general, templating methods use a porous material as a hard template in which to grow the gold nanorods within these porous confines by electrochemical deposition. This
approach requires preparation of the template followed by a formation of the gold nanorods within these templates, which requires the subsequent removal of the templates (Figure 1.3). This makes synthetic processes by the template method more complicated and time-consuming than some of the other synthetic approaches. The gold nanorods prepared by this technique are highly uniform in their dimension, such that their dimension can be precisely controlled by regulating the dimension of the porous channels. A limitation is that the number of gold nanorods prepared by this method is limited to the number of available channels for the growth.

Wang et al. prepared gold nanorods using an electrochemical method for the first time in 1997. Generally, electrochemical methods utilize at least a two-electrode electrochemical cell where the anode electrode is metallic gold and the cathode electrode is commonly metallic platinum. Once an appropriate potential is applied between these electrodes, the gold metal oxidizes to form dissolved gold ions in the electrolyte solution. These gold ions migrate and are reduced in micelles formed from the cetyltrimethylammonium bromide (CTAB), leading to nucleation of gold nanoparticles on the surfaces of the cathode. These gold nanoparticles grow into gold nanorods through the assistance of rod-inducing CTAB surfactant added to the system. The nanorods can be removed from the surfaces of the cathode through the use of sonication techniques (Figure 1.3). Electrochemical methods can have a complicated set-up, but the gold nanorods prepared by this technique can be obtained in a relatively pure solution, such that the as-prepared gold nanorods do not require multiple purification steps. The dimension of gold nanorods can be controlled by adjusting the reaction conditions such as through tuning the current density, sonication power and duration, and reaction
temperature. The gold nanorods formed using this electrochemical approach are, however, limited to the available surface area on the cathode and these surfaces tend to diminish during the electrodeposition process as the surfaces are progressively covered as a result of gold deposition.

A seed-mediated approach to preparing gold nanorods was first reported by Wiesner and Wokaun in 1989, and this method was further modified by Murphy et al. in 2001 to control the length to width aspect ratio of gold nanorods.\textsuperscript{21,25,26} For the seed-mediated method, gold seeds first need to be prepared by reducing chloroauric acid (HAuCl\textsubscript{4}) with sodium borohydride (NaBH\textsubscript{4}) in the presence of CTAB surfactant. These gold seeds are subsequently introduced into a growth solution containing further CTAB, silver nitrate (AgNO\textsubscript{3}), chloroauric acid, L-ascorbic acid, and hydrochloric acid (HCl). The gold seeds then slowly grow into gold nanorods within 24 hr (Figure 1.3). The dimension of the gold nanorods can be controlled by altering concentrations of each of these reagents as well as the amount of gold seeds added to the solution.\textsuperscript{27} The seed-mediated growth methods require a number of purification steps, which include dialysis, centrifugation, and/or chromatography to remove the excess reagents involved in the synthesis. This seed-mediated growth method is, however, the most popular method due to high yields and its relative simplicity for execution. We have, therefore, adapted the previously published seed-mediated growth method demonstrated by Murphy et al. with modifications to the procedures to extend the tunability of the dimension and localized surface plasmon resonance of these gold nanorods.\textsuperscript{21} Further details of the growth mechanism for preparing gold nanorods by the seed-mediated growth method will be discussed in following sections.
1.2 Growth Mechanism of Gold Nanorods

1.2.1 Geometrical Change with Growth of the Gold Nanoparticles

Figure 1.4  Schematics for the crystalline structures of the gold nanoparticles and their facets associated with each of the different geometries of the gold nanoparticles (Reprinted with permission from Yang P. et al. 2011. Copyright American Institute of Physics; Welinder A. C. et al. 2010. Copyright Royal Society of Chemistry). \(^{28,29}\)

Gold nanoparticles typically have a face center cubic (fcc) crystalline structure. There are a number of different facets in this crystalline structure and each of these facets will have a different surface energy due to differences in its coordination, or lack therein,
with neighboring atoms. In the case of fcc crystalline structures, the (111) facets exhibit
the highest coordination number between the atoms within its surface, the (100) facets
exhibit the 2nd highest coordination number between atoms within its surface, and (110)
facets exhibit the lowest coordination number between atoms within its surface (Figure
1.4). The surface energy of a facet describes the energy required to disrupt the
intermolecular interactions to form a new surface within the material. Surface energy
decreases following this order: (110) > (100) > (111) for an fcc crystalline structure. 30,31
According to the Wulff theory, crystals form with a crystalline structure that has a
minimum Gibb’s free energy. 32 Small gold nanoparticles adapt to multiply twinned
structures, which consist of primarily (111) facets due to its lower surface energy. A
multiply twinned structure, however, have high internal energy as their internal structure
is distorted to create surfaces with triangular (111) facets. Thus, as the size of the gold
nanoparticles increases, this internal strain builds-up and the crystalline structure starts to
change into truncated octahedron to reduce the internal differences in energy. The
truncated octahedron consists of eight hexagonal (111) facets and six square (100) facets.
In the seed-mediated method, a typical size of the gold seeds is approximately 4 nm and
most of these nanoparticles adopt the truncated octahedron crystalline structure. There is
a lower coordination number at the edges and corners of crystalline structure than across
the facets of the crystalline structure. As the nanoparticles continue to grow,
asymmetrical truncations occur between the edges of the (111) facets resulting in the
formation of additional (110) facets to reduce the surface energy at the edges. This
asymmetrical truncation contributes to the ability for gold seeds to grow anisotropically
into gold nanorods (Figure 1.4). 31,33,34,35
1.2.2 The Role of Specific Reagents in Growth of the Gold Nanorods

As mentioned above, the reagents in the growth solution for gold nanorods include cetyltrimethylammonium bromide (CTAB), silver nitrate, chloroauric acid, L-ascorbic acid, hydrochloric acid, and gold seeds. Each of these reagents plays an important role in the growth of the gold nanorods and in their final characteristics, such as dimension and optoelectronic properties.

Figure 1.5  Schematics of the chemical structure of cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), L-ascorbic acid, and L-dehydroascorbic acid and their interaction with the surfaces of a gold.
Silver ions (Ag\(^{+}\)) and gold ions (Au\(^{3+}\)) form a metallomicelle with CTAB. These assemblies can minimize the reduction of Ag\(^{+}\) ions upon addition of the L-ascorbic acid, while also enabling the Au\(^{3+}\) ions to be reduced to Au\(^{+}\) ions in the presence of L-ascorbic acid as shown in Equation 1.1, 1.2, and 1.3.\(^{36}\)

\[
4 \text{CTAB} + \text{AgNO}_3 \rightarrow \text{CTA-AgBr}_2 + 3 \text{CTANO}_3 \quad (\text{Eq. 1.1})
\]

\[
4 \text{CTAB} + \text{HAuCl}_4 \rightarrow \text{CTA-AuBr}_4 + 3 \text{CTAC} + \text{HCl} \quad (\text{Eq. 1.2})
\]

\[
\text{CTA-AuBr}_4 + C_6H_8O_6 \rightarrow \text{CTA-AuBr}_2 + C_6H_6O_6 + 2 \text{HBr} \quad (\text{Eq. 1.3})
\]

The gold seeds added to this solution acts as a catalyst to further reduce the Au\(^{+}\) ions to Au\(^0\) as shown in Equation 1.4.\(^{36}\)

\[
2 \text{CTA-AuBr}_2 + C_6H_8O_6 \rightarrow 2 \text{Au}^0 + C_6H_6O_6 + 2 \text{CTAB} + 2 \text{HBr} \quad (\text{Eq. 1.4})
\]

The double bond in L-ascorbic acid breaks by an oxidative process, and this oxidized species forms a bond with the surfaces of the gold seeds. A metallomicelle containing Au\(^{+}\) ions likely interacts through electrostatic forces with halides that are coordinated to the gold atoms exposed on the surfaces of the gold seeds. These gold ions in proximity to the L-ascorbic acid on the surfaces of a gold seed can be further reduced to Au\(^0\), which results in the deposition of further gold atoms onto the surfaces of the gold seed. The L-dehydroascorbic acid (or the oxidized form of the L-ascorbic acid) can be subsequently removed from the surfaces of the gold particle, and replaced by additional L-ascorbic acid species or other surfactants in the systems.\(^{27,37,38,39}\)
The role of Ag\(^+\) ions in the growth of the gold nanorods is still being debated in the literature.\(^{26,34,40}\) One hypothesis is that the silver ions interact with the bromide species associated with the CTAB surfactant layers to form a CTAB-Ag-CTAB complex, which could reduce the repulsive forces between the quaternary ammonium head groups of the CTAB. In this way, the packing density of the CTAB could be further increased, which also changes the growth kinetics of the gold nanorods.\(^{26}\) A second hypothesis is that Ag\(^+\) ions tend to compete with the Au\(^+\) ions and block catalytic sites on the surfaces of the gold seed. Incorporation of the Ag\(^+\) ions into the surfaces of the gold would be greater along the longitudinal sides of the gold nanorod than at the ends of the gold nanorods due to the surface energies of the associated facets at the sides [e.g., (110) and (100)] and ends [e.g., (111) and (110)] of the gold nanorods. Thus, the presence of Ag\(^+\) ions can assist with the preference towards anisotropic growth of the gold nanoparticles by altering the rates of deposition of gold atoms on the various facets of the nanoparticles. In addition, coordination of the metallomicelles containing Ag\(^+\) ions with the surfaces of the gold seeds can assist in truncation at the edges of the (111) facets, such as those present in truncated octahedral structures, as required during the symmetry breaking step for the transformation of the gold seeds into faceted nanoparticles. Growth of the nanoparticle subsequently follows a process that is dominated by differences in the surface energies between the (100), (110), and (111) facets. Through these, and potentially other processes, the Ag\(^+\) ions assist to increase the number or yield, of gold nanorods produced from a synthesis as opposed to the non-rod shaped gold nanoparticles.\(^{34,40}\)
The CTAB adsorbs with a different packing density and strength of interaction on each of the facets of the gold nanorods, which also assists with the preferential growth into the nanorod shape. The adsorption energy of CTAB on the gold changes in order of the facets from (110) > (100) > (111). A result of these differences is that the CTAB packing density on the longitudinal sides of the gold nanorods, which consist of (110) and (100) facets, tends to be greater than the packing density of CTAB on the transverse ends of the gold nanorods with predominately (111) and (110) facets. This means the catalytic sites for further deposition of gold atoms during the growth process would be more readily available (e.g., covered with less dense layers of CTAB) on the transverse ends than on the longitudinal sides of the gold nanorods. The CTAB assisted growth of gold nanorods would, therefore, be dominated by the kinetic differences of gold deposition onto each of the different facets.\(^41,42\)

The L-ascorbic acid is a relatively weak reducing agent with a reduction potential between -0.283 to -0.066 V at pH values between 2.0 to 7.0, respectively.\(^43\) Hydrochloric acid can be added to alter the pH of the growth solution, which changes the reduction potential of the L-ascorbic acid. Thus, hydrochloric acid plays an important role in the growth solution through controlling growth kinetics of the gold nanorods, such as by altering the reduction potential of the L-ascorbic acid.\(^44\) In addition, the chloride ions from the hydrochloric acid can also influence growth kinetics of the gold nanorods. These chloride ions can exchange with the bromide counter ions associated with the quaternary ammonium head group of CTAB, forming cetyltrimethylammonium chloride (CTAC). The chloride ions have an electronegativity of 2.83 and bromide ions have an electronegativity of 2.74.\(^45\) The difference in their electronegativity results in the chloride
ions having a weaker affinity to the gold surfaces than the bromide ions as their interaction strength gets weaker with the increase in electronegativity. This suggests that the CTAC adsorbed onto the surfaces of the gold nanorods would have a relatively weaker association than the CTAB species. As a result, an increased number of CTAC molecules in solution could result in the surfaces of the gold nanorods becoming more accessible to gold ions in solution and, in turn, could have a correlative influence on the growth kinetics of the gold nanorods.\textsuperscript{46}

Gold seeds are prepared separately from the growth solution by reducing chloroauric acid with sodium borohydride in the presence of CTAB surfactant as shown in Equation 1.5.

\[
8 \text{HAuCl}_4 + 3 \text{NaBH}_4 + 9 \text{H}_2\text{O} \rightleftharpoons 8 \text{Au}^0 + 3 \text{B(OH)}_3 + 29 \text{HCl} + 3 \text{NaCl} \quad (\text{Eq. 1.5})
\]

Once the gold seeds have been prepared, they are introduced into the growth solution. The gold seeds act as nucleation centers for the gold ions in solution to deposit onto, and to grow into gold nanorods. Thus, the initial concentration, size and size distribution of the gold seeds can greatly influence the final dimensions and their uniformity for the product of gold nanorods.\textsuperscript{37,38,47,48}

Likewise, each of the reagents in the growth solution plays an important role in the growth of the gold nanorods. Altering the concentrations of these reagents could greatly influence the growth of the gold nanorod and the properties of the final product. Therefore the concentrations of each of the reagents can be adjusted to tune the dimensions and localized surface plasmon resonance of the gold nanorods.
Chapter 2.

Characterization Techniques

Several different characterization techniques were used to assess the products from a series of syntheses that sought to tune the properties of gold nanorods, and to further understand the limitations and tunability of the seed-mediated approach to their preparation. Synthesized gold nanorods were characterized by extinction spectroscopy to monitor the change in their localized surface plasmon resonance. In addition, the as-prepared gold nanorods were characterized by transmission electron microscopy to determine their dimension and number of gold nanorods produced from each synthesis over the presence of non-rod shaped gold nanoparticles. The details of these characterization techniques are discussed further in the following sections.

2.1 Extinction Spectroscopy

As described above, light can be absorbed or scattered by gold nanoparticles. Extinction spectroscopy can measure attenuation of transmitted light that occurs at each wavelength. Extinction spectroscopy can be divided into four major compartments which include a source of radiation, a diffraction grating, a sample compartment, and a detector (Figure 2.1).
Figure 2.1  Schematic diagram of a diode array extinction spectrophotometer.

The light sources include deuterium arc lamps, which are commonly used to generate radiation from ionized gas with emission in ultraviolet to visible region, and tungsten-halogen lamps, which are generally used to generate radiation spanning the visible to near infrared region of the electromagnetic spectrum. The light from these lamps is collimated by a series of lens, passing through the sample to measure its attenuation of light at each wavelength. The sample, a suspension of gold nanoparticles in this case, is typically held in a cuvette. There are four common types of cuvettes used in these experiments: (1) plastic; (2) glass; (3) quartz; and (4) sapphire. Plastic and glass cuvettes are transparent to the light in the visible to near infrared regions. Plastic and glass cuvettes are relatively less durable against chemicals or scratch in comparison to quartz or sapphire cuvettes but these cuvettes are disposable thereby avoiding cross contamination between samples that can result from the reuse of cuvettes. Quartz cuvettes are transparent to the light from the ultraviolet to near infrared region and
sapphire cuvettes are transparent to the light from the ultraviolet to mid infrared region. Quartz and sapphire cuvettes both cover a broader range of the electromagnetic spectrum, but they are relatively more expensive in comparison to plastic and glass cuvettes. The gold nanorod samples for our experiments were compatible with plastic cuvettes and the region of the electromagnetic spectrum of primary interest for these experiments was from the visible to near infrared region. For these reasons listed above, we used a relatively inexpensive and disposable polystyrene cuvette to measure the extinction spectra of our samples.

As the transmitted light passes through the sample, the intensity of the light decreases due to attenuation by the sample. The ratio between the intensity of the transmitted light and the incident light is expressed as transmittance as shown in Equation 2.1. According to the Beer-Lambert law, the relationship between transmittance and extinction is expressed as in Equation 2.2.

\[
T = \frac{1}{I_0} \quad \text{(Eq. 2.1)}
\]

\[
A = -\log T \quad \text{(Eq. 2.2)}
\]

In Equation 2.1 and 2.2, \( T \) represents transmittance, \( I_0 \) represents intensity of incident light, \( I \) represents the intensity of the transmitted light, and the value \( A \) represents the measured extinction of the sample. Extinction depends on the extinction coefficient of the material, concentration of the absorbing and/or scattering material in the sample, and the path length of light through the sample. The relationship between these values is expressed as in Equation 2.3:

\[
A = \varepsilon cl \quad \text{(Eq. 2.3)}
\]
In Equation 2.3, $\varepsilon$ represents the extinction coefficient, $c$ represents the concentration of the absorbing and/or scattering material in the sample, and $l$ represents the path length of light through the sample. The concentration of the gold nanoparticles can, therefore, be determined from the extinction spectra using the Beer-Lambert law.$^{49}$ The extinction coefficient does, however, depend on the size (volume) of gold nanoparticles, and in general the extinction coefficient can increase as the size (volume) of gold nanoparticles increases.$^{51,52}$ It is important to know the size (volume) of the gold nanoparticles and apply an appropriate extinction coefficient to accurately measure the concentrations of gold nanoparticles in a sample. The extinction coefficient can also be calculated using an independent method of quantification of the material present in the sample. For example, elemental analysis or inductively coupled plasma mass spectrometry can be used to quantify the gold, in combination with electron microscopy to quantify the dimensions of the particles. A reference sample that is identical to the samples, except without the analyte (gold nanoparticles) of the interest must also be prepared for the extinction spectroscopy measurements for each cuvette. This reference sample is measured before the analysis of the sample to account for potential contributions to the extinction from other components of the sample or from the cuvette. For our experiments, the samples of gold nanorods were purified using at least two processes of isolating the particles by centrifugation followed by decanting the supernatant and resuspending the isolated particles before obtaining the extinction measurements. A solution of 0.02 M CTAB was used as the resuspending medium, as well as for the reference sample to accurately measure the extinction spectra of the gold nanorods.
Once light is transmitted through the sample, the light is refocused by lenses onto a diffraction grating where different wavelengths of light are dispersed at different angles. The relationship between the angles of the diffracted light and the wavelength of the light is expressed in Equation 2.4.

\[ dsin\theta = n\lambda \]  
(Eq. 2.4)

For the Equation 2.4, \( d \) represents the spacing between the groves in the diffraction grating, \( \theta \) represents the angle between the diffracted light and a vector normal to the incident light, \( n \) represents the order of the diffraction, and \( \lambda \) represents the wavelength of light. This dispersed light is detected through the use of a photodiode array, which is typically comprised of 100 to 1000 photodiodes that convert the absorbed light energy into a proportional electric current for digital read out. This multichannel detector enables the measurement of multiple wavelengths across a broad spectral range and is, therefore, able to generate a wide spectral range at a single pass. Depending on the system one or multiple diffraction gratings might be used to cover the entire, desired spectral range from the ultraviolet to near infrared regions. We utilized extinction spectroscopy as a regular technique to characterize the localized surface plasmon resonance properties of the gold nanorods.

### 2.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is an instrument that uses a high energy beam of focused electrons to generate images as a function of their projection through a thin sample. Transmission electron microscopes need to be operated under high vacuum conditions, such as under pressures around \( 10^{-9} \) Pa since air can absorb or scatter
the electron beam.\textsuperscript{53,54} Transmission electron microscopy has four major components. These include an electron gun, a series of electromagnetic lenses, a sample compartment, and a detector for visualizing the interactions of the focused electron beam with the sample (Figure 2.2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tem_diagram}
\caption{Schematic diagram of a transmission electron microscopy (TEM) (Reprinted with permission from Mescher A. L. 2009. Copyright ©The McGraw-Hill Companies, Inc.).\textsuperscript{55}}
\end{figure}

A field emission gun is used to generate the electron beam. When strong electrostatic fields that exceed the work function of the cathode tip are applied, electrons can be extracted and accelerate towards the anode with a speed depends on the potential difference between the cathode and anode.\textsuperscript{53} According to the de Broglie relationship,
wavelength of the electron depends on Planck’s constant and the momentum of the electron as shown in Equation 2.5.

\[ \lambda = \frac{\hbar}{p} \]  

(Eq. 2.5)

For the Equation 2.5, \( \lambda \) represents the wavelength of the electron, \( \hbar \) represents Planck’s constant, and \( p \) represents the momentum of the electron. The relationship between the momentum of the electron and the accelerating potential is shown in Equation 2.6.

\[ p = \sqrt{2meV\left(1 + \frac{eV}{c^2}\right)} \]  

(Eq. 2.6)

For the Equation 2.6, \( p \) represents the momentum of the electron, \( m \) represents the mass of an electron, \( e \) represents the charge of an electron, \( V \) represents the accelerating potential, and \( c \) represents the speed of light. The resolution limit of transmission electron microscopy techniques depends on the wavelength of the electron beam, aberrations in the electron beam, and the thickness of the sample. According to the Abbe’s theory of resolution, relation between the resolution limit and the wavelength of the electron beam can be expressed as shown in Equation 2.7.

\[ R = \frac{0.753}{\sin \theta \sqrt{V}} \]  

(Eq. 2.7)

For the Equation 2.7, \( R \) represents the resolution of the electron beam transmitted through the sample, \( V \) represents the accelerating potential, and \( \theta \) represents the nominal collection angle of the aperture. Resolution limit of non-aberration corrected transmission electron microscopy is typically around 0.2 nm depending on the accelerating voltage used to generate the electron beam and the electromagnetic optics used in the system.
Transmission electron microscopy techniques from non-aberration corrected microscopes were sufficient to visualize the gold nanorods with sufficiently high resolution for discerning differences in their shape, size, and sample purity.

An electron beam generated by the electron gun enters into a system of electromagnetic lenses where the resultant fields are used to direct and shape the beam of focused electrons. The electron beam then transmits through the sample and is projected onto a detector for visualization. The sample needs to be prepared on a TEM grid, which is generally composed of a conductive metal frame coated with a thin film of carbon. Copper is generally used as the conductive metal frame, which provides a structural support to the carbon film and minimizes charge build-up from the focused electron beam. The carbon film is conductive and transparent to the incident electron beam; thereby the carbon film has a high stability under the electron beam. Sometimes an additional formvar film (a thermoplastic resin) is incorporated onto the TEM grids to provide further stability to the carbon film. We primarily used copper TEM grids coated with carbon and formvar onto which we cast the samples of gold nanorods for their characterization.

Interactions between the focused electron beam and the sample cause changes in the pathway of an incident electron beam. A decrease in the electron beam signal observed at the detector could result from diffraction of the electron beam off from the sample, in contrast to other regions of the sample that are more transparent to the electron beam. The TEM images are generated based on variations in the intensity of electron beam transmitted through the sample (e.g., brighter regions in the image correspond to more electrons transmitted through the sample than the darker regions). Through
these TEM imaging techniques the dimension of the gold nanorods can be measured for each sample, as well as the number of gold nanorods produced from each synthesis and its ratio to gold nanoparticles with other shapes.

Our TEM system was also equipped with an X-ray detector for use in elemental analysis. The focused electron beam can be used to excite an electron from an inner shell of an atom, which results an electron hole. As an electron from an outer shell relaxes to fill up this hole, the difference in energy between the outer and inner shells is released in the form of an X-ray. The energy of the X-ray is proportional to the specific binding energy of the electron to the element from which it was emitted, which enables the user to determine the composition of a sample. This energy dispersive X-ray spectroscopy (EDS) technique was used in our TEM system to verify the composition of the nanomaterials within our samples.
Chapter 3.

Tuning the Dimensions and Localized Surface Plasmon Resonance of Gold Nanorods

3.1 Introduction

Gold nanorods exhibit two distinct localized surface plasmon resonance bands. One band at ~525 nm associated with the transverse excitation, and one band that can be tuned across the visible and near infrared regions of the electromagnetic spectrum that corresponds to longitudinal excitation of gold nanorods. Although the transverse surface plasmon resonance band is fairly consistent in its position, the position of the longitudinal surface plasmon resonance band can be tuned by controlling the aspect ratio of the gold nanorods.\textsuperscript{6,21,26} Many studies have already demonstrated the ability to fine tune the position of the localized surface plasmon resonance of the gold nanorods, such that their longitudinal surface plasmon resonance band lies within specific regions of the near infrared “window”. Many of these studies adapted the seed mediated growth method demonstrated by Murphy \textit{et al.} and tuned the localized surface plasmon resonance of the gold nanorods by adjusting a couple of variables within the synthesis.\textsuperscript{21}

El-Sayed and co-workers modified the seed mediated method by varying the concentration of silver ions in the growth solution to tune the localized surface plasmon resonance from 600 nm to 850 nm. In addition, this same group further extended the tunability of the localized surface plasmon resonance from 600 nm to 1300 nm through the use of a binary surfactants system.\textsuperscript{26} The incorporation of additional silver ions into
the growth solution exhibited the ability to fine tune the localized surface plasmon resonance band from 600 nm to 850 nm, but further extension of the localized surface plasmon resonance with the additional co-surfactant, benzyldimethylammoniumchloride (BDAC) lacked reproducibility.

Guo and co-workers tuned the localized surface plasmon resonance band of gold nanorods from 630 nm to 1250 nm by controlling duration of the growth period for the gold nanorods. This group demonstrated that the changes in the aspect ratio and dimensions of the gold nanorods progressed over time, and they demonstrated that hydrochloric acid can alter the growth kinetics of the gold nanorods. This method of preparing the gold nanorods did, however, lack reproducibility and the kinetics associated with growth of the gold nanorods as a result of changes to the concentration of the hydrochloric acid were lacking from the study.

Juarez and co-workers tuned the localized surface plasmon resonance of gold nanorods from 740 nm to 840 nm using different ratios of L-ascorbic acid to gold ions. As the ratio of L-ascorbic acid to gold ion increased, the aspect ratio of gold nanorods also increased, but the number of dogbone shaped gold nanoparticles in the product also increased. The yield of gold nanorods was, therefore, decreased in the final product.

Murray and coworkers extended the ability to tune the properties of the gold nanorods with localized surface plasmon resonance bands extending from 630 nm to 1250 nm through the introduction of aromatic compounds into the solutions during the seed mediated syntheses. The addition of aromatic compounds improved the ability to
tune the position of the plasmon band and improved the yield of the gold nanorods, but this process also increased the overall size (volume) of the gold nanorods.

Wang and co-workers further tuned the localized surface plasmon resonance from 720 nm to 830 nm by changing the ratio of gold seeds to gold ions in the growth solution.\textsuperscript{58} By altering the ratio of gold seeds to gold ions in the growth solution, gold nanorods were synthesized at a variety of dimensions and with various localized surface plasmon resonance properties. Although nanorods of various dimensions and localized surface plasmon resonance properties could be synthesized, the ability to maintain relatively small dimensions while also controlling their optoelectronic properties are desirable for photothermal and related processes for use in biological and other systems.

These previous studies demonstrated a range of abilities to tune the localized surface plasmon resonance of the gold nanorods. A range of nanorods with different dimensions were demonstrated in the literature, but there were relatively few studies that fine-tuned the overall size (volume) of the gold nanorods, and fewer yet that maintained relatively small dimensions for the final product with a plasmon band in the near infrared region. As mentioned previously, the size of gold nanorods can play an important role in terms of cellular uptake and retention, as well as for controlling the ratio between their absorbance and scattering properties. The smaller gold nanoparticles exhibit a higher proportion of absorbance over scattering, which are desirable for the use in photothermal based processes as more incident radiation energy is converted into localized heat. On the other hand, gold nanoparticles with a larger volume can exhibit a higher proportion of scattering over absorbance, which can be desirable when seeking to use these materials in optical based imaging within biological systems.\textsuperscript{6} In addition, higher cellular uptake of
gold nanoparticles can be achieved with the smaller gold nanoparticles, but retention of the gold nanoparticles can also decrease with a decrease in their size.\textsuperscript{17,18,19} Fine tuning the size of gold nanorods is highly desirable for optimizing their use in biomedical applications.

We have explored the ability to further tune the properties of the gold nanorods prepared using the seed mediated growth method originally published by Murphy \textit{et al.}\textsuperscript{21} This tunability includes adjusting the dimensions of the gold nanorods along with their localized surface plasmon resonance. More importantly, these properties were individually controlled by adjusting multiple reagents used in the synthesis of the gold nanorods, which would improve further in optimizing properties of gold nanorods for the used in applications requiring both cellular uptake and photothermal triggered processes through the use of localized surface plasmon resonance bands in the near infrared “window”.

\textbf{3.2 Materials and Methods}

\textbf{3.2.1 Materials}

To prepare the desired gold nanorods, chloroauric acid (\textgtr=99.9\%, lot# MKBR7979V), sodium borohydride (\textgtr=99\%, lot# SHBD4770V), L-ascorbic acid (\textgtr=99\%, lot# 031M0164V), silver nitrate (\textgtr=99\%, lot# MKBH5454V), and cetyltrimethylammonium bromide (\textgtr=96\%, lot# BCBH3450V) were purchased from Sigma-Aldrich, Inc. These reagents were used upon receipt without further purification. Hydrochloric acid (37\% w/w, lot# H0815) was purchased from ACP Chemicals, Inc.,
sulphuric acid (97% w/w, lot# 151002) was purchased from Caledon Laboratories Ltd.,
nitric acid (70% w/w, lot#311356) was purchased from Anachemia Science Inc., and
hydrogen peroxide (30% w/w, lot#155067) was purchased from Fisher Scientific Ltd. for
the preparation of the aqua regia and piranha solutions.

3.2.2 Gold Nanorods Synthesis by a Modified Seed-Mediated Method

A day prior to the synthesis, a 50 mL glass round bottom flask and Teflon coated
stir bar were washed using aqua regia. The aqua regia solution was freshly prepared from
a 1:3 (v/v) mixture of 70% nitric acid and 37% hydrochloric acid. CAUTION: Aqua
regia solution is corrosive and it needs to be handled with extreme care. The aqua regia
solution was transferred to the round bottom flask containing the stir bar. These supplies
were kept under ventilation (e.g., in a fume hood) for 15 min while containing the aqua
regia solution. After aqua regia was disposed of in an appropriately vented glass waste
bottle, the supplies were rinsed several times with an excess quantity of 18.2 MΩ·cm
water (produced using a Barnstead NANOpure Diamond Life Science water filtration
system). The rinsed round bottom flask and stir bar were further cleaned by soaking with
a piranha solution. The piranha solution was freshly prepared from a 3:7 (v/v) mixture of
30% hydrogen peroxide and 97% sulphuric acid. CAUTION: Piranha solution is a
strong oxidizing agent and reacts violently with organic compounds and it needs to be
handled with extreme care. The piranha solution was transferred to the round bottom
flask containing the stir bar. These supplies were kept under ventilation for 15 min. Once
the piranha solution was discarded in an appropriately vented glass waste bottle, the
supplies were rinsed several times with 18.2MΩ·cm water. In addition, solutions of 0.5
mM and 10 mM chloroauric acid were prepared a day before the synthesis of the gold nanorods and these solutions were stored in a polystyrene vial (Dynalab Corp., NY, USA.) and covered with aluminum foil to protect the solutions from exposure to ambient light.

### 3.2.2.1 Preparation of Gold Seed

To prepare the gold seeds, a 5.0±0.1 mL solution of 0.2 M cetyltrimethylammonium bromide (CTAB) was transferred to the acid cleaned 50 mL round bottom flask. While stirring this solution at 1,000 rpm, 5.0±0.1 mL of the 0.5 mM HAuCl₄ solution was added all at once to the flask. A 750±8 µL solution of freshly prepared, ice cold 0.01 M sodium borohydride (NaBH₄) was added all at once to this mixture. The final mixture turned light brown upon addition of the NaBH₄ and the reaction was continued for 30 min before introducing a portion of this seed solution into the solution used to grow the gold nanorods.

### 3.2.2.2 Synthesis of Gold Nanorods

To synthesize the gold nanorods (AuNRs), 25.0±0.6 mL of a 0.2 M CTAB solution was prepared in a clean 50 mL polystyrene vial. A 170±2 µL portion of a10 mM solution of silver nitrate (AgNO₃) was added to the CTAB solution, and the mixture was swirled for 10 s. A 1.20±0.01 mL portion of the 10 mM HAuCl₄ solution was added to the mixture and swirled for 10 s. Subsequently, a 168±2 µL portion of a 100 mM L-ascorbic acid solution was added to the vial and the mixture was swirled until it became colorless. At this point, a 210±2 µL portion of a 0.2 M HCl solution was added to the
mixture, which was swirled for 10 s. A 210±2 µL portion of the as-prepared gold seed solution was subsequently added to this mixture. After another 10 s of swirling, the final mixture was held at 30 °C for 24 h using water bath on a hot plate. The resulting solution of AuNRs was purified by a process of centrifugation using a Fisher Scientific Accupin 400 centrifuge. The samples were distributed into a series of 2 mL centrifuge tubes (Ultident Scientific Inc., QC, Canada). After these aliquots were centrifuged at 13,000 rpm for 20 min, the supernatant was carefully removed using a glass pipette. The isolated solids were resuspended in 2.00±0.02 mL of 18.2 MΩ·cm water. The gold nanorods were further purified by centrifugation at 13,000 rpm for 15 min. After the supernatant was removed, the isolated AuNRs were resuspended in 0.02 M CTAB. The different portions of the purified sample were combined together to achieve a total volume of 6.0±0.2 mL for the purified product. To tune the dimension and optoelectronic properties of the gold nanorods, the concentration of both HCl and HAuCl₄ were methodically varied as described below in further detail in Section 3.3.

3.2.2.3 Sample Preparation for Characterization of the Gold Nanorods

Samples of the purified gold nanorods were diluted by ten times with 18.2 MΩ·cm water and transferred to semi-micro polystyrene cuvettes (Starna Cells Inc., CA, USA). Extinction spectra of these gold nanorods were recorded between 300 nm to 900 nm at room temperature using an Agilent 8452 extinction spectrometer to assess their localized surface plasmon resonance properties. Eight microliter aliquots of these diluted samples were also drop cast onto copper transmission electron microscopy (TEM) grids coated with carbon and formvar (200 mesh; Electron Microscopy Sciences, PA, USA),
which were dried under vacuum with a pressure of ~750 mmHg in a desiccator. Transmission electron microscopy images of the gold nanoparticles were obtained using an FEI Tecnai Osiris scanning TEM (STEM) to assess the dimensions and shapes of the gold nanoparticles. Mountain Map program (version 7) from Digital Surf Ltd. was used to analyze these TEM images to determine size distributions of the gold nanorods by generating histograms on the length, width, and aspect ratio of the gold nanorods. The individual particles were identified by image segregation, which enabled isolating and counting the gold nanorods independently from the other shapes of gold nanoparticles. The later were utilized to determine the yield of nanorods from each synthesis relative to the other types of particles.

### 3.3 Results and Discussion

As described above, fine tuning the dimension and localized surface plasmon resonance of the gold nanorods is highly desirable for optimizing their use in biomedical applications. One goal of our study included the ability to regulate these properties of the gold nanorods to improve their potential utility in applications that require both cellular uptake of the nanomaterials and the ability to trigger photothermal processes through the use of localized surface plasmon resonance bands centered in the near infrared region. We have approached this task by sequentially adjusting the multiple variables involved in the seed mediated growth of gold nanorods, extending the work originally published by Murphy et al.\textsuperscript{21}

As mentioned above, silver nitrate, chloroauric acid, L-ascorbic acid, hydrochloric acid, and gold seeds are used to synthesize the suspensions of gold
nanorods. The concentration of chloroauric acid, L-ascorbic acid, and gold seeds, as well as their ratios in solution, will greatly change the growth kinetics of gold nanorods. Gold seeds act as both catalysts and nucleation centers for the deposition of the gold ions. Changes in the concentration of the chloroauric acid and L-ascorbic acid can alter the deposition rate of the gold ions onto the surfaces of a gold seed. The interaction frequency or probability for interaction of the chloroauric acid and L-ascorbic acid at the surfaces of the gold seeds will increase in proportion to the concentration of these reagents in solution. A change in the ratio of the concentrations between the L-ascorbic acid, chloroauric acid, and gold seeds will greatly influence the dimensions of the gold nanorods. As shown in the study from Juarez and co-workers, the aspect ratio of the gold nanorods can increase with an increase in the concentration of L-ascorbic acid, and vice versa, but the overall volume of the gold nanorods remained relatively consistent. This implies that the rate of gold deposition onto the different facets of the gold nanorods can change with alterations to the concentration of L-ascorbic acid in the solution, but the total amount of gold ions deposited per nanorods remains relatively consistent. On the other hand, the aspect ratio of the gold nanorods is expected to decrease with a decrease in the concentration of chloroauric acid solution, and the volume of each of the gold nanorods would also be anticipated to decrease as well. The source of gold ions from the chloroauric acid in solution limits the growth of the gold nanorods. As mentioned above, the gold seeds are prepared by reducing chloroauric acid with sodium borohydride in the presence of the CTAB surfactant. In a study demonstrated by Wang and co-workers, the dimensions of the gold nanorods were altered by adjusting the amount of gold seeds introduced into the solution used to grow the nanorods. Their study also introduced
excess sodium borohydride to this growth solution when adding the gold seeds, which tends to form additional gold seeds. This approach, makes it difficult to regulate the dimensions of the gold nanorods by simply adjusting the amount of gold seeds added to the solution. The size of the additional gold seeds formed in the growth solution may be different from the original gold seeds, which can also affect the overall size distribution of the final product of gold nanorods.

In a study from El-Sayed and co-workers, the concentration of silver nitrate was varied to tune the localized surface plasmon resonance of the gold nanorods. In general, an increase in the concentration of silver nitrate in solution during growth of the gold nanorods will result in a red-shift of the localized surface plasmon resonance of the gold nanorods. The silver nitrate can adjust the packing density of the CTAB as described in Chapter 1, but at high concentrations of silver nitrate these species can screen the charges on the quaternary ammonium head group of the CTAB and reduce the electrostatic interactions between the CTAB and the surfaces of the gold nanoparticles. Changes in the concentration of hydrochloric acid, on the other hand, alters the reduction potential of L-ascorbic acid thereby changing the growth kinetics of the gold nanorods. As demonstrated in a study from Guo and co-workers, a decrease in the pH of the growth solution with an increase in the concentration of hydrochloric acid can result in a decrease in the reduction capacity of the L-ascorbic acid. The rate of gold deposition can, therefore, be controlled by adjusting the hydrochloric acid concentration in the growth solution. In addition, Wang and co-workers showed that the dogbone shaped gold nanoparticles tend to decrease as the gold deposition rate slows down with an increase in the concentration of hydrochloric acid.
As mentioned above, hydrochloric acid alters the growth kinetics of the gold nanorods and limits the source of gold ions from the chloroauric acid species, which can limit the growth of the gold nanorods. Thus, the hydrochloric acid and chloroauric acid were two variables we have adjusted to further tune the dimensions and the localized surface plasmon resonance properties of the gold nanorods. These changes to the properties of the gold nanorods were characterized by transmission electron microscopy and extinction spectroscopy.

3.3.1 Effect of Changes in the Chloroauric Acid Concentration

Gold nanorods were prepared as described in Section 3.2.2.2, but different volumes of the 10 mM chloroauric acid were added to growth solution while keeping all of the other variables constant. Volumes of the chloroauric acid added were adjusted between 0.30 mL (3.0 µmol) and 1.50 mL (15.0 µmol). Each of the aliquots evaluated in this study were different by at least 0.30 mL (3.0 µmol). The upper limit to the volume of chloroauric acid added to the growth solution of nanorods was 1.50 mL because of the concentration of L-ascorbic acid available in solution that was needed to reduce the chloroauric acid. On the contrary, the lower limit to the volume of chloroauric acid added to growth solution of nanorods was 0.30 mL since a further increase in the concentration ratio between the L-ascorbic acid and the chloroauric acid would result in the formation of a significant number of non-rod shaped gold nanoparticles, as observed in our experiments. These non-ideal shapes included dogbone, spherical, and cubic nanoparticles instead of the desired product of gold nanorods. As the volume (or moles) of the chloroauric acid that was introduced into the growth solution gradually decreased
from 1.50 mL (15.0 µmol) to 0.30 mL (3.0 µmol), the central peak position of the localized surface plasmon resonance band for the gold nanorods blue shifted from 800 nm to 725 nm as shown in Figure 3.1.

Figure 3.1  Extinction spectra of gold nanorods and their change in the localized surface plasmon resonance band with respect to the change in the moles of the chloroauric acid in the growth solution of the gold nanorods (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).

The difference in relative intensity of the localized surface plasmon resonance observed in Figure 3.1 is largely due to the difference in the number of the gold nanorods produced, but as described in Section 2.1, it is also due to the decreased extinction
coefficient of the gold nanoparticles as the size (volume) decreased. In addition, a shoulder peak was observed at ~570 nm. This shoulder peak was attributed to the dogbone-like shape of some of the gold nanoparticles. Further details on this shoulder peak are discussed in the following Section 3.3.2.

The samples of purified gold nanorods were characterized by transmission electron microscopy to determine their average dimensions and the relative number of gold nanorods produced from each synthesis in contrast to the non-rod shaped gold nanoparticles as shown in Figure 3.2.

Figure 3.2 Transmission electron microscope (TEM) images of gold nanorods synthesized with different moles of chloroauric acid (HAuCl4) in the growth solution of the gold nanorods: a) 3.0 µmol; b) 6.0 µmol; c) 9.0 µmol; d) 12.0 µmol; and e) 15.0 µmol.
These TEM analyses also confirmed that the blue shifts observed in the position of the localized surface plasmon resonance for these gold nanorods were a result of a change in their overall aspect ratio. The average lengths, widths, centroid of longitudinal surface plasmon resonance bands, yields, and calculated volumes and aspect ratios for the as-prepared, but purified gold nanorods are summarized in Table 3.1.

**Table 3.1 The change in properties of gold nanorods from the adjustment in the moles of chloroauric acid in the growth solution of the gold nanorods.**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Moles of HAuCl₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 µmol</td>
</tr>
<tr>
<td>Length (nm)</td>
<td>24±4</td>
</tr>
<tr>
<td>Width (nm)</td>
<td>7±1</td>
</tr>
<tr>
<td>Volume (nm³)</td>
<td>5±1 x10³</td>
</tr>
<tr>
<td>Aspect Ratio</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>Longitudinal Peak Centroid (nm)</td>
<td>725</td>
</tr>
<tr>
<td>Yield</td>
<td>58 %</td>
</tr>
</tbody>
</table>

As observed in Table 3.1, the average dimensions of the gold nanorods decreased in proportion to the decreased volume of chloroauric acid added to the nanorod growth solution. As expected, this trend is likely due to limited supply of gold ions available for deposition onto the gold seeds, which limits their growth into gold nanorods. In this way, the overall dimensions of the gold nanorods were tuned on average from 37±7 nm
(length) by 10±4 nm (width) to 24±4 nm by 7±1 nm through a process of altering the concentration of chloroauric acid in the growth solution (Figure 3.3). The errors shown in these measurements are one standard deviation from the mean for each of a sample.

Figure 3.3  Histograms representing the change in the average dimension and the size distribution of gold nanorods from the adjustment in the moles of the chloroauric acid in the growth solution of the gold nanorods.

3.3.2 Effect of Change in Hydrochloric Acid Concentration
To tune the localized surface plasmon resonance of the gold nanorods, their dimensions were further adjusted to span the near infrared window. These adjustments were pursued through tuning the concentration of hydrochloric acid added to the nanorod growth solution while holding the other reaction variables constant. In follow-up to the studies described above, a series of syntheses were carried out that also adjusted the amount of chloroauric acid available in the nanorod growth solution. To each of these series of reactions, a 210 µL portion of hydrochloric acid (at different concentrations) was introduced into the growth solutions. The initial reactions, for comparison, contained 0 M HCl, and were adjusted with further addition of HCl to subsequent reactions until observing no further shift in the localized surface plasmon resonance to longer wavelengths (Table 3.2).

Table 3.2 Adjustment in the concentration of hydrochloric acid added to each of growth solutions of gold nanorod with different moles of chloroauric acid.

<table>
<thead>
<tr>
<th>Moles of HAuCl₄</th>
<th>3.0 µmol</th>
<th>6.0 µmol</th>
<th>9.0 µmol</th>
<th>12.0 µmol</th>
<th>15.0 µmol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 M</td>
<td>0.2 M</td>
<td>0.4 M</td>
<td>0.6 M</td>
<td>0.8 M</td>
</tr>
<tr>
<td>3.0 µmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>0 M</td>
<td>0.2 M</td>
<td>0.4 M</td>
<td>0.6 M</td>
<td>0.8 M</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>0 M</td>
<td>0.2 M</td>
<td>0.4 M</td>
<td>0.6 M</td>
<td>0.8 M</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>0 M</td>
<td>0.2 M</td>
<td>0.4 M</td>
<td>0.6 M</td>
<td>-</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>0 M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
For example, the concentration of hydrochloric acid was adjusted from 0 M to 3.0 M and added to the nanorod growth solution, which contained 0.3 mL of 10 mM chloroauric acid. In another example, to the nanorod growth solution containing 1.5 mL of 10 mM chloroauric acid, additional hydrochloric acid was not added and instead a 210µL portion of 18.2 MΩ·cm water was added to the growth solution to maintain the same dilution for each nanorod synthesis. Similarly, selected concentrations of hydrochloric acid were added to the growth solution while also adjusting the amounts of chloroauric acid until observing no further shift in localized surface plasmon resonance towards longer wavelengths. Each of the combinations of varying hydrochloric acid and chloroauric acid resulted in a different boundary condition where localized surface plasmon resonance no longer shifted towards longer wavelengths, but instead these peak positions started to shift back to shorter wavelengths. This phenomenon is likely due, in part, to the high concentrations of chloride ions in solution with contributions from both the chloroauric acid and hydrochloric acid. These anions can screen the positive charge of the quaternary ammonium head group of the CTAB. Initially, the charge screening in solution could assist with improvements to the packing density of the CTAB molecules by reducing the electrostatic repulsion between the quaternary ammonium head groups of CTAB within the layers interacting on the surfaces of the gold nanoparticles. This screening effect also reduces the electrostatic interactions between the CTAB and the surfaces of the gold nanorods. High concentrations of the chloride ions can, therefore, destabilize the interaction strength of the CTAB surfactants with the gold, which would result in a decreased selectivity in the deposition of the gold atoms onto the surfaces of the nanorods. This change is manifested in a blue shift of the localized surface plasmon
resonance band, and a decrease in the number of gold nanorods produced from a synthesis (Figure 3.4). In general, an increased amount of hydrochloric acid added to the growth solution resulted in a red shift of the localized surface plasmon resonance of the gold nanorods until reaching this boundary as shown in Figure 3.5, 3.6, 3.7, 3.8, and 3.9.

Figure 3.4  Extinction spectra of gold nanorods representing boundary to the change in the concentration of the hydrochloric acid in the growth solution containing 9.0 µmol of the chloroauric acid (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).
Figure 3.5  Extinction spectra of gold nanorods synthesized from the growth solution of the gold nanorods containing 120 μL of 0 M hydrochloric acid and 15.0 μmol of the chloroauric acid.
Figure 3.6  Extinction spectra of gold nanorods and their change in the localized surface plasmon resonance band with respect to the change in the concentration of the hydrochloric acid in the nanorods growth solution containing 12.0 µmol of the chloroauric acid (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).
Figure 3.7  Extinction spectra of gold nanorods and their change in the localized surface plasmon resonance band with respect to the change in the concentration of the hydrochloric acid in the nanorods growth solution containing 9.0 µmol of the chloroauric acid (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).
Figure 3.8  Extinction spectra of gold nanorods and their change in the localized surface plasmon resonance band with respect to the change in the concentration of the hydrochloric acid in the nanorods growth solution containing 6.0 µmol of the chloroaauric acid (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).
Figure 3.9  Extinction spectra of gold nanorods and their change in the localized surface plasmon resonance band with respect to the change in the concentration of the hydrochloric acid in the nanorods growth solution containing 3.0 µmol of the chlorauric acid (each spectra were normalized to the centroid of the longitudinal surface plasmon resonance band).
For samples with a low concentration of chloroauric acid added to the growth solution, there was also a shoulder peak observed at ~570 nm. This shoulder was prominent when there was an absence of hydrochloric acid in growth solution. A dogbone-like shape of the gold nanoparticles is responsible for this shoulder peak, as mentioned for example in the study by Wang and co-workers. The dogbone shape of the gold nanoparticles forms as a result of the higher CTAB packing density along the longitudinal surfaces of the gold nanorods than at the ends of the gold nanorods, such as due to the surface energy differences between the side facets and the end facets as discussed in Chapter 1. In addition, the curvature of the gold nanorods at the ends distorts the packing density of the CTAB, resulting in the ends of the nanorods having an enhanced reactivity in contrast to the longitudinal sides of the gold nanorods. In this way, as the concentration ratio between the L-ascorbic acid and chloroauric acid significantly increased, gold atoms deposited at the ends of the nanorods much faster than onto the longitudinal sides of the gold nanorods. This alteration resulted in the formation of the dogbone shaped nanoparticles. The addition of the hydrochloric acid to the growth solution did, however, alter the deposition rate of the gold as a result of the decrease in the reducing capacity of the L-ascorbic acid, while also adjusting the packing density of the CTAB layers on the growing nanoparticles as discussed above. The addition of hydrochloric acid resulted in a significant decrease in the shoulder peak in the extinction spectra of the gold nanorods as shown in Figure 3.3. Thus, not only can the localized surface plasmon resonance of the gold nanorods be tuned by altering the concentration of hydrochloric acid in the growth solution, but also the shape of the gold nanorods can be adjusted through the addition of the hydrochloric acid.
3.3.3 Overall Trend

By adjusting the concentration of chloroauric acid and hydrochloric acid in the growth solution, we were able to tune dimensions and localized surface plasmon resonance of the gold nanorods to improve their use in applications requiring both cellular uptake and photothermal triggered processes through the use of localized surface plasmon resonance bands in the near infrared window. As mentioned above, the volume of 10 mM chloroauric acid introduced into the growth solution adjusted from 1.50 mL to 0.30 mL, the dimensions of the gold nanorods on average decreased from $37 \pm 7$ nm in length and $10 \pm 1$ nm in width, to $24 \pm 4$ nm in length and $7 \pm 1$ nm in width, respectively. The concentration of hydrochloric acid was adjusted to further tune the localized surface plasmon resonance of these gold nanorods. As described above, an increased concentration of hydrochloric acid added to the growth solution resulted in a red shift to the central peak position of the localized surface plasmon resonance band for the gold nanorods. These changes in the central peak position of the localized surface plasmon resonance band for the gold nanorods from the adjustment in the concentration of chloroauric acid and hydrochloric acid are summarized in Table 3.3.
Table 3.3 The change in the central peak position of the localized surface plasmon resonance band of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HAuCl₄</th>
<th>0</th>
<th>42</th>
<th>84</th>
<th>126</th>
<th>168</th>
<th>210</th>
<th>315</th>
<th>420</th>
<th>525</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 µmol</td>
<td>725</td>
<td>740</td>
<td>762</td>
<td>790</td>
<td>803</td>
<td>811</td>
<td>847</td>
<td>850</td>
<td>851</td>
<td>865</td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>748</td>
<td>760</td>
<td>795</td>
<td>808</td>
<td>830</td>
<td>836</td>
<td>850</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>778</td>
<td>801</td>
<td>820</td>
<td>840</td>
<td>842</td>
<td>845</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>780</td>
<td>805</td>
<td>832</td>
<td>835</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>800</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The changes in the central peak position of the localized surface plasmon resonance band for the gold nanorods suggest the change in the aspect ratio of the gold nanorods. The samples of these gold nanorods were characterized by transmission electron microscopy to observe the change in dimensions and the relative number of gold nanorods produced from each synthesis in contrast to the non-rod shaped gold nanoparticles as shown in Figure 3.10.
Figure 3.10 Transmission electron microscope (TEM) images of gold nanorods respect to the change in the moles of the hydrochloric acid and chloroauric acid.
The changes in the central peak position of the localized surface plasmon resonance band for the gold nanorods were due to the change in the aspect ratio of the gold nanorods as expected. The changes in the average dimensions of the gold nanorods are summarized in Table 3.4, 3.5, 3.6, and 3.7.

Table 3.4 The change in the aspect ratio (length to width) of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HCl (µmol)</th>
<th>Aspect Ratios of Gold Nanorods (Length to Width)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>42</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>84</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>126</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>168</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>210</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>315</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>420</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>525</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>630</td>
<td>3.4±0.8</td>
</tr>
</tbody>
</table>

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Table 3.5 The change in the central transverse width of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HAuCl₄</th>
<th>Width of Gold Nanorods (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3.0 µmol</td>
<td>7±1</td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>8±1</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>9±2</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>10±2</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>10±1</td>
</tr>
</tbody>
</table>
Table 3.6 The change in the longitudinal length of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HAuCl₄ (µmol)</th>
<th>Length of Gold Nanorods (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moles of HCl (µmol)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3.0 µmol</td>
<td>24±4</td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>30±4</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>32±6</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>39±7</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>37±7</td>
</tr>
</tbody>
</table>
Table 3.7 The change in the volume of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HAuCl₄</th>
<th>Volumes of Gold Nanorods (X10³ nm³)</th>
<th>Moles of HCl (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 µmol</td>
<td>5±1 8±2 8±2 6±2 8±2 10±3 11±3 9±3 10±3 10±3</td>
<td></td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>8±2 11±3 11±3 12±3 13±3 12±3 21±5 - - -</td>
<td></td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>11±4 17±6 13±5 17±6 28±8 18±7 - - - -</td>
<td></td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>16±6 22±7 17±9 23±8 - - - - - -</td>
<td></td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>15±3 - - - - - - - - - -</td>
<td></td>
</tr>
</tbody>
</table>

The changes in the dimensions of the gold nanorods were compared based on adjustments to the concentration of chloroauric acid and hydrochloric acid. As mentioned earlier, both length and width of the gold nanorods decreased with a decrease in the concentration of chloroauric acid in nanorods growth solution. On the other hand, as the concentration of hydrochloric acid in nanorods growth solution increased, the length of the nanorods increased while the width of the nanorods remained relatively consistent. We had expected the width of the gold nanorods to decrease as well because the same amount of gold ions were available for the growth of the gold nanorods, and there would be less gold deposition onto the sides of the nanorods if more gold deposition was found at the ends of the nanorods. The change in the volume of the individual gold nanorods with increase in the concentration of hydrochloric acid in the nanorod growth solution is likely due to the change in the yield or number of gold nanorods produced from each
synthesis in contrast to the non-rod shaped gold nanoparticles. As described above, the yield/number of the gold nanorods improved with the addition of the hydrochloric acid. In this way, more gold ions can be contributed to the gold nanorods than other forms of gold nanoparticles. The changes in the yield/number of gold nanorods produced from each synthesis as opposed to the non-rod shaped gold nanoparticles are summarized in Table 3.8. In addition, full width at half maximum (FWHM) was measured to determine population dispersity in the gold nanorod samples as shown in Figure 3.11. These FWHM and their ratio to the centroid of localized surface plasmon resonance, aspect ratio, and length of the gold nanorods are summarized in Table 3.9, 3.10, 3.11, and 3.12.

**Table 3.8** The change in the ratio of the gold nanorods to the non-rod shaped gold nanoparticles from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Ratio of Gold Nanorods to Non-Gold Nanorods Shape Nanoparticles (%)</th>
<th>Moles of HAuCl₄ (µmol)</th>
<th>0</th>
<th>42</th>
<th>84</th>
<th>126</th>
<th>168</th>
<th>210</th>
<th>315</th>
<th>420</th>
<th>525</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 µmol</td>
<td>3.0 µmol</td>
<td>57</td>
<td>%</td>
<td>77</td>
<td>%</td>
<td>93</td>
<td>%</td>
<td>94</td>
<td>%</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>6.0 µmol</td>
<td>6.0 µmol</td>
<td>86</td>
<td>%</td>
<td>92</td>
<td>%</td>
<td>90</td>
<td>%</td>
<td>94</td>
<td>%</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>9.0 µmol</td>
<td>9.0 µmol</td>
<td>88</td>
<td>%</td>
<td>96</td>
<td>%</td>
<td>95</td>
<td>%</td>
<td>96</td>
<td>%</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>12.0 µmol</td>
<td>12.0 µmol</td>
<td>93</td>
<td>%</td>
<td>95</td>
<td>%</td>
<td>94</td>
<td>%</td>
<td>94</td>
<td>%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>15.0 µmol</td>
<td>15.0 µmol</td>
<td>97</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

56
Figure 3.11  Representative full width at half maximum (FWHM) as measured for a suspension of gold nanorods. Dotted lines indicated the measurement of the centroid of the longitudinal surface plasmon resonance band, and the baseline measurement for this peak.
Table 3.9 The change in the full width at half maximum (FWHM) of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HCl (µmol)</th>
<th>0</th>
<th>42</th>
<th>84</th>
<th>126</th>
<th>168</th>
<th>210</th>
<th>315</th>
<th>420</th>
<th>525</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 µmol</td>
<td>160</td>
<td>140</td>
<td>137</td>
<td>139</td>
<td>140</td>
<td>144</td>
<td>159</td>
<td>161</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>122</td>
<td>119</td>
<td>117</td>
<td>125</td>
<td>126</td>
<td>128</td>
<td>131</td>
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<tr>
<td>9.0 µmol</td>
<td>118</td>
<td>114</td>
<td>118</td>
<td>122</td>
<td>123</td>
<td>122</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>122</td>
<td>123</td>
<td>123</td>
<td>124</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>126</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.10 The change in the ratio of the full width at half maximum (FWHM) over the centroid of localized surface plasmon resonance of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HCl (µmol)</th>
<th>0</th>
<th>42</th>
<th>84</th>
<th>126</th>
<th>168</th>
<th>210</th>
<th>315</th>
<th>420</th>
<th>525</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 µmol</td>
<td>0.22</td>
<td>0.19</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>6.0 µmol</td>
<td>0.16</td>
<td>0.16</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td>0.15</td>
<td>0.14</td>
<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td>0.16</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15.0 µmol</td>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.11 The change in the ratio of the full width at half maximum (FWHM) over the aspect ratio of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of HCl (µmol)</th>
<th>0</th>
<th>42</th>
<th>84</th>
<th>126</th>
<th>168</th>
<th>210</th>
<th>315</th>
<th>420</th>
<th>525</th>
<th>630</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 µmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50±10</td>
<td>41±8</td>
<td>39±9</td>
<td>35±9</td>
<td>35±9</td>
<td>50±20</td>
<td>36±9</td>
<td>40±10</td>
<td>32±6</td>
<td>33±7</td>
<td></td>
</tr>
<tr>
<td>6.0 µmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32±6</td>
<td>33±7</td>
<td>34±7</td>
<td>31±6</td>
<td>29±6</td>
<td>32±8</td>
<td>36±9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.0 µmol</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33±9</td>
<td>29±7</td>
<td>30±7</td>
<td>31±8</td>
<td>32±8</td>
<td>31±8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.0 µmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>32±8</td>
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<tr>
<td>15.0 µmol</td>
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<td></td>
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<tr>
<td>34±7</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.12 The change in the ratio of the full width at half maximum (FWHM) over the length of the gold nanorods from the adjustment in the moles of hydrochloric acid and the moles of chloroauric acid in the growth solutions of the gold nanorods.

<table>
<thead>
<tr>
<th>Moles of H\text{AuCl}_4</th>
<th>\text{Moles of HCl (\text{\textmu}mol)}</th>
<th>\text{Ratio of the FWHM over Length of Gold Nanorods}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>3.0 \text{\mu}mol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1\pm0.5</td>
<td>3.7\pm0.7</td>
<td>2.9\pm0.5</td>
</tr>
<tr>
<td>9.0 \text{\mu}mol</td>
<td>3.7\pm0.7</td>
<td>2.9\pm0.5</td>
</tr>
<tr>
<td>12.0 \text{\mu}mol</td>
<td>3.2\pm0.6</td>
<td>2.8\pm0.5</td>
</tr>
<tr>
<td>15.0 \text{\mu}mol</td>
<td>3.4\pm0.6</td>
<td>-</td>
</tr>
</tbody>
</table>

The non-rod shaped gold nanoparticles were found to be spherical, cubic, and dogbone shaped nanoparticles. Spherical gold nanoparticles are the most common shape of nanoparticles that are found as the by-product of the gold nanorod synthesis. Spherical gold nanoparticles are formed when the truncated octahedral shape of the gold seeds grows isotropically into larger particles. As the gold deposition rate increased with increase in the ratio between the L-ascorbic acid and gold ions, more gold seeds grew isotropically into larger particles. In addition, spherical gold nanoparticles are predominantly found when the reduction capacity of L-ascorbic acid was increased with an increase in the pH of the nanorods growth solution. These results suggest that a rapid growth of the gold seeds reduces the number of asymmetrical truncations that leads to
anisotropic growth of gold seeds into nanorods as indicated in Table 3.8. The dogbone shapes of the gold nanoparticles were observed as the by-product of the gold nanorod synthesis for the reason described in Section 3.3.2. Similar to spherical gold nanoparticles an increase in the rate of gold deposition with a change in the ratio between the L-ascorbic acid and gold ions, and the pH of the nanorods growth solution both increased the formation of dogbone shapes of the gold nanoparticles. Cubic gold nanoparticles are sometimes observed as the by-product of the gold nanorod synthesis. Cubic gold nanoparticles are formed as gold deposit much faster onto the (111) facets in the truncated octahedron structure than the (100) facets due to the difference in packing density of the surfactants as discussed in Section 1.2.1. Schematic of this growth process is illustrated in Figure 3.12.62

Figure 3.12 Illustration for the truncated octahedron structure of a gold nanoparticle and its geometrical change into cubical structure of a gold nanoparticle (Reprinted with permission from Pal J. et al. 2015. Copyright Royal Society of Chemistry).62

There were only few cubic gold nanoparticles formed in each of the gold nanorods synthesis, but cubic gold nanoparticles were clearly one of the shapes that formed as by-product of the gold nanorod synthesis. In general, however, prepared gold nanorods had
relative low amount of these byproducts and produced gold nanorods had relatively consistent size distribution as shown in Table 3.8 and 3.9 except few samples with low amount of chloroauric acid and hydrochloric acid. The reason for the samples with low amount of chloroauric acid and hydrochloric acid had relatively high amount of byproduct and polydispersity were, as mentioned above, due to the increased reactivity from the increase in the L-ascorbic acid to chloroauric acid ratio and reduction capacity of L-ascorbic acid.
Chapter 4.

Conclusion

4.1 Summary

Gold nanorods have unique optoelectronic properties, which can be utilized in many potential applications. We sought to prepare gold nanorods that would potentially be used in biomedical applications, such as bio-imaging, photothermal therapies, and drug delivery systems. Typically in biomedical applications, gold nanorods with a localized surface plasmon resonance band that lies in the near infrared window between 650 to 1350 nm is highly desirable to obtain better images and an efficient photothermal effect over a range of depths within biological tissues. In addition the dimensions and overall size of gold nanorods also plays an important role in terms of cellular uptake and retention, as well as controlling the ratio between their absorbance and scattering properties. Thus, a primary goal of our study was to regulate dimension and localized surface plasmon resonance of the gold nanorods to improve their potential utility in applications requiring both cellular uptake and photothermal triggered processes through the use of localized surface plasmon resonance bands in the near infrared “window”. We have modified the seed-mediated method originally published by Murphy et al, by sequentially varying concentration of hydrochloric acid and chloroauric acid to tune the dimensions and localized surface plasmon resonance of the gold nanorods. By adjusting the volume of 10 mM chloroauric acid introduced into the growth solution from 1.50 mL to 0.30 mL, we were able to tune the average dimensions of the gold nanorods from 37±7
nm in length and 10±1 nm in width, to 24±4 nm in length and 7±1 nm in width, respectively. Localized surface plasmon resonances of the gold nanorods were further tuned by altering the concentration of hydrochloric acid introduced into the growth solution. Although tunability of the localized surface plasmon resonance depended on the volume of chloroauric acid added to the growth solution as shown in Table 3.3, in general they exhibited a red-shift in the localized surface plasmon resonance until a high concentration of chloride ions destabilized the CTAB surfactants on the surfaces of gold nanorods. Overall on average, we were able to show a tunability of the nanorod dimensions from 24±4 nm in length and 7±1 nm in width, to 47±10 nm in length and 11±2 nm in width. We also demonstrated a tunability in the localized surface plasmon resonance of the gold nanorods from 725 nm to 865 nm by altering concentration of hydrochloric acid and chloroauric acid concentrations in the growth solution. More importantly, these properties were individually controlled, which would improve further in optimizing properties of gold nanorods for the used in applications requiring both cellular uptake and photothermal triggered processes through the use of localized surface plasmon resonance bands in the near infrared “window”.

4.2 Future Direction

For future studies, it would be very interesting to utilize the gold nanorods for biomedical application, and in particular as a platform for drug delivery. Drug delivery systems are widely relied upon to establish safe, effective means of delivering therapeutics to patients, as well as to regulate a time dependent dose of drug to patients. Systems have been developed for drug delivery that are tuned based on the desire to
improve their efficacy, which includes minimizing their impact on healthy tissues, specifying the targeted region within the patient for drug delivery, improving and regulating the circulation time of drug in vivo, and controlling the rate and concentrations of released drug. Drug delivery systems are sought that will maximize the therapeutic effects of the drug, while minimizing adverse side effects. For example, liposomes are commonly used as drug delivery vehicles because they are well known to be biocompatible and they can provide a biocompatible shell around drugs, which minimize drug exposure to healthy tissues that may otherwise result in adverse side effects. As the temperature of the solution surrounding a liposome is raised, the retention properties of the liposome drops since the liposomal membrane fluidity will increase with elevated temperatures. Conventional liposomal drug delivery systems relied on slight elevation of local temperature through ultrasound or hyperthermia to release drug contents, but these methods were not efficient that they were slow in releasing drug contents. The heat released from gold nanorods through the photothermal effect can elevate the local temperature of a liposome significantly and, thereby, gold nanorods can assist in the sufficient release of drug contents from the liposome. Thus, it would be fascinating to associate liposomes with gold nanorods for more controlled release of drug contents. In addition, this association would be a good demonstration for how gold nanorods can be utilized to improve drug delivery systems that requires photothermal triggered processes to release drug contents. Moreover, it would be very interesting to see how a change in dimensions of the gold nanorods can influence the association of these materials and how these changes affect the efficiency of this photothermal trigger release of the drug contents.
References


Appendix A.

TEM images and Histograms of Gold Nanorods

TEM images and histograms for each of gold nanorod samples synthesized with different amount of chloroauric acid and hydrochloric acid introduced to the growth solution of the nanorods are represented in this Appendix A.

![TEM image and histograms](image)

Figure A1. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 0 M hydrochloric acid.
Figure A2. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 0.2 M hydrochloric acid.
Figure A3. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 0.4 M hydrochloric acid.
Figure A4. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 0.6 M hydrochloric acid.
Figure A5. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 0.8 M hydrochloric acid.
Figure A6. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 1.0 M hydrochloric acid.
Figure A7. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 1.5 M hydrochloric acid.
Figure A8. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 2.0 M hydrochloric acid.
Figure A9. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 2.5 M hydrochloric acid.
Figure A10. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.3 mL of a 10 mM chloroauric acid and 210 µL of a 3.0 M hydrochloric acid.
Figure A11. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 0 M hydrochloric acid.
Figure A12. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 0.2 M hydrochloric acid.
Figure A13. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 0.4 M hydrochloric acid.
Figure A14. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 0.6 M hydrochloric acid.
Figure A15. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 0.8 M hydrochloric acid.
Figure A16. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 1.0 M hydrochloric acid.
Figure A17. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.6 mL of a 10 mM chloroauric acid and 210 µL of a 1.5 M hydrochloric acid.
Figure A18. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 0 M hydrochloric acid.
Figure A19. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 0.2 M hydrochloric acid.
Figure A20. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 0.4 M hydrochloric acid.
Figure A21. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 0.6 M hydrochloric acid.
Figure A22. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 0.8 M hydrochloric acid.
Figure A23. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 0.9 mL of a 10 mM chloroauric acid and 210 µL of a 1.0 M hydrochloric acid.
Figure A24. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 1.2 mL of a 10 mM chloroauric acid and 210 µL of a 0 M hydrochloric acid.
Figure A25. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 1.2 mL of a 10 mM chloroauric acid and 210 µL of a 0.2 M hydrochloric acid.
Figure A26. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 1.2 mL of a 10 mM chloroauric acid and 210 µL of a 0.4 M hydrochloric acid.
Figure A27. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 1.2 mL of a 10 mM chloroauric acid and 210 µL of a 0.6 M hydrochloric acid.
Figure A28. Representative TEM image and histograms of aspect ratio, width, and length for gold nanorods synthesized from the growth solution contained 1.5 mL of a 10 mM chloroauric acid and 210 µL of a 0 M hydrochloric acid.

The yield/number of gold nanorods produced from each synthesis as opposed to the non-rod shaped gold nanoparticles are measured by segregating particles using MountainMap program as shown in Figure A29.
Figure A28. Representative particle segregation work using Mountain Map Program for the yield measurement.