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# Effects of Silver Nanoparticles on Photochemical Processes Focusing on Luminol Chemiluminescence

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# **Effects of silver nanoparticles on photochemical processes focusing on luminol chemiluminescence**

by

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Thesis

Submitted to the Department of Chemistry

Eastern Michigan University

In partial fulfillment of the requirements

for the degree of

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in

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**Abstract:** Silver nanoparticles of varying sizes were synthesized. Their effects on enhancing or quenching the photochemical processes of tryptophan fluorescence, a ruthenium complex decay constant and chemiluminescence of luminol and ferricyanide, were studied. The tryptophan fluorescence was slightly enhanced by smaller nanoparticles and showed slight quenching in the presence of larger nanoparticles. The tris (2,2'-bipyridyl ruthenium (II) chloride) complex was excited using a 532 nm laser, and the decay constant was measured. Neither gold nor silver nanoparticles showed an impact on this process. The luminol chemiluminescence was studied with an emphasis on holding the concentration of nanoparticles, surface area, and silver constant in separate trials to assist in understanding of the interaction between the properties of nanoparticles and light enhancement. Nanoparticles that are of a medium size of 30 to 50 nm in diameter show the greatest enhancement regardless of the parameter held constant.

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## **Introduction**

The intention of this project was to study nanoparticles' influence on light emitting processes. The properties of metals change as the size approaches the atomic scale. Nanoparticles can interact with fluorophores by enhancing or quenching the amount of light emitted.<sup>1</sup> They can also be used as catalysts for luminescent reactions.<sup>2</sup> The exact mechanisms of how nanoparticles interact with chemicals are not entirely known. This project investigated fluorescence and chemiluminescence to attempt to unlock some of the mechanisms of how nanoparticles work.

Fluorescence is a process that occurs when light is absorbed by a chemical, excites an electron, and in a very short timeframe, usually in the nanosecond range, the electron relaxes down to its ground state emitting light. Nanoparticles interact with the excited state via electric field or collisions. This project studied the kinetics of the fluorescence of a ruthenium complex as well as the fluorescence intensity of tryptophan. The experiments were designed to determine whether the nanoparticles would enhance the light intensity or quench it, which results in a reduction of light intensity.

Chemiluminescence is similar to fluorescence, only instead of exciting the electrons with a light source, a chemical reaction occurs which produces a chemical with an electron in the excited state. Luminol, a common example, produces a product in the excited state that relaxes to the ground state along with emission of blue light.<sup>3</sup> The preliminary studies conducted led to other projects such as the effects of polyvinyl alcohol on nanoparticle interactions with tryptophan fluorescence, the impact of nanoparticles on the reaction between luminol and ferricyanide, and improving synthesis consistency. The beginning of this research was only supported by basic synthetic

procedures, studies performed earlier by the research group, and the concept that it was unknown how the nanoparticles influence many of the processes of luminescent materials.

Nanoparticles have many significant applications in scientific research. Nanoparticles are used for a multitude of practical applications including curing diseases such as HIV,<sup>4</sup> binding analytes for separation and qualitative analysis,<sup>5,6</sup> reaction catalysis and tracking of molecules.<sup>7</sup> Historically, nanoparticles were used as dyes in glass for red or yellow colors.<sup>8</sup> Understanding not only the uses of nanoparticles, but also their different behaviors from larger metal samples of the same element and their interactions with other particles and light, could prove crucial to furthering the application of these particles. There is a limited amount of research on the synthesis of nanoparticles, their impact on chemiluminescence of luminol, and their interactions with fluorophores. This thesis covers a few of these topics in hopes of confirming previously reported results or challenging them in addition to improving understanding of the interactions between nanoparticles and other compounds. Specifically, these investigations involved the synthesis of the nanoparticles, testing their interactions with tryptophan, and studying their catalytic effect on the chemiluminescence reaction of luminol and ferricyanide.

Last, it is partially an intention to utilize some of the concepts in both high school and college level chemistry experiments. The project involved experiments that are simple enough for advanced placement chemistry classes and are suitable for the designated curriculum to meet several Michigan High School Content Expectation Standards. The students synthesized a material and investigated the success of the

synthesis by studying the properties of the material. Using an experiment of this type in high school would be largely beneficial at bridging content-driven learning in secondary science classrooms with the research done in undergraduate and graduate level science programs. This experiment incorporated many of the concepts taught in an advanced placement chemistry classroom such as stoichiometry, redox reactions, and solutions. It also covered more advanced topics such as the Tyndall effect, reducing agents, limiting reagent, concentration, light, and qualitative analysis. This experiment also incorporated scientific papers into the high school curriculum. These topics, combined with data analysis, can be incorporated to teach an excellent experiment in an upper level high school chemistry course.

### **Synthesis of Silver Nanoparticles**

Silver and gold nanoparticles were synthesized, and their sizes were then characterized. Solomon provided a procedure for synthesizing small (5-20 nm) silver nanoparticles using silver nitrate and sodium borohydride.<sup>8</sup> In order to make larger (10-100 nm) silver nanoparticles, a silver solution with a reducing agent was added to a silver nanoparticle seed solution. This was accomplished successfully (Schneider) by using additional silver nitrate, ascorbic acid in sodium hydroxide, and a silver seed solution in sodium hydroxide.<sup>9</sup> The fundamental reaction is a reduction of the silver ion to the metallic form. In this process, silver atoms naturally form spherical collections of thousands of atoms called nanoparticles. Silver nitrate is used to supply the silver ion,  $\text{Ag}^+ + \text{e}^- \rightarrow \text{Ag}$  and the reducing agent used varied typically between sodium borohydride, sodium citrate, or ascorbic acid. The gold reaction,  $\text{Au}^{3+} + 3\text{e}^- \rightarrow \text{Au(s)}$ , is

similar and usually uses sodium citrate as the reducing agent, and the reaction must be heated to produce nanoparticles.

Synthesizing the nanoparticles was somewhat new for the research group. Earlier research in this group had been performed using expensive, purchased nanoparticle samples that had already been synthesized and characterized based on size. A small vial of nanoparticles can range in cost from twenty to sixty dollars but may only contain a few cents worth of chemicals. While silver nitrate and gold (III) chloride are both very expensive, the concentrations used are so minimal that it significantly reduces costs to be able to synthesize these nanoparticles. There are various methods to synthesize silver and gold nanoparticles as explained below in the Methods section.

## **Methods**

Chemicals were purchased from Sigma-Aldrich. Glassware was cleaned using an ethanol/hydroxide solution prior to cleaning using distilled water. All water used had a purity of 18.2 MΩ·cm.

### **Silver Nanoparticle Synthesis**

#### **Method 1. (6:1 borohydride/silver ratio)<sup>8</sup>**

2 mM NaBH<sub>4</sub> was prepared and kept on ice to prevent the solution from decomposing. To a 50 mL beaker, 30 mL of 2 mM NaBH<sub>4</sub> was added and stirred, followed by the dropwise addition of 10 mL of 1 mM AgNO<sub>3</sub> to the NaBH<sub>4</sub> while stirring. It was optional to add a stabilizing agent of 1 mL of 0.3% polyvinyl alcohol.

#### **Method 2. (Citrate method)**

Various amounts, ranging from 1 to 10 mL of 0.6 mM silver nitrate and 0.3 mM

sodium citrate, were mixed in a clean test tube. The amounts of the two solutions do not necessarily need to be the same. The mixture was boiled for approximately 5-10 minutes at 100°C until a faint yellow color change occurred and then allowed to cool to room temperature.

### **Method 3. (1:6 borohydride/silver ratio)<sup>9</sup>**

10 mL of 0.1 mM NaBH<sub>4</sub> stored on ice was combined with 10 mL of 0.6 mM AgNO<sub>3</sub> in a clean beaker. The silver nitrate was added dropwise over a period of 2-4 minutes with continuous stirring.

### **Method 4. (Citrate method 2)<sup>10</sup>**

A solution was made by combining 25 mL of 5 mM AgNO<sub>3</sub> with 50 mL of 35 mM sodium citrate. The mixture was heated until a color change from clear to faint yellow was evident and then removed from the heat. Typically the heating lasted between 5 and 10 minutes, and the solution boiled before the color change occurred.

The synthesis of silver nanoparticles was repeated many times with several batches of nanoparticles made with varying success and stability. Three solutions of reducing agents—sodium borohydride, sodium citrate and ascorbic acid—were used in the synthesis. The ascorbic acid was used solely for increasing the size of the nanoparticles from a seed solution. The sodium borohydride solution quickly decomposed at room temperature conditions, and care was taken to keep the solution on ice. The sodium citrate was much more stable than the borohydride but required the addition of heat to complete the reduction of silver nitrate. This sometimes led to aggregation of the

nanoparticles after prolonged heating.

The successes and failures of the reactions were measured using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer but were also tracked visually by the color of solution. A yellow color signified a success, a clear solution indicated no reaction had occurred, and a grey murky color indicated that the reaction had reacted for a long time and the nanoparticles had aggregated. The spectrum of a successful yellow colloid would have an absorbance peak that fell between 380 and 460 nanometers, with smaller particles exhibiting lower wavelength absorption peaks.

Many factors were influential upon the success of the synthesis. The most important was the choice of reducing agent. The timing of the addition of the reducing agent to the silver nitrate affected the stability of the particles. Previous papers indicate that adding ten mL of silver nitrate should take approximately three minutes.<sup>1</sup> For the initial synthesis, a glass pipette was used to administer the silver nitrate solution drop wise at a rate of one to three drops per second.

A stabilizing agent such as polyvinyl alcohol was helpful at preventing aggregation of nanoparticles. This was modeled after the addition of PVP (polyvinylpyrrolidone) as a stabilizing agent.<sup>8</sup>

The most successful synthesis procedure was the method that used a 6:1 ratio of sodium borohydride to silver. This ratio was determined previously in the Solomon article, and they theorized that the excess borohydride anions will crowd around the surface of the nanoparticles, and this excess negative charge repels nanoparticles from one another.<sup>8</sup> This makes the resulting colloid very stable because the nanoparticles do not interact and are less likely to bind together forming a larger metal particle. The 6:1

ratio of borohydride to silver nitrate is important for this stability to exist.

It should be noted that Method 1 produced a very high concentration of silver nanoparticles. The absorbance readings were often so large that a dilution was needed to produce more reliable results. This large concentration of nanoparticles impacts photochemical processes. For example, a dilution of the silver nanoparticles by a factor of five led to a larger increase in the fluorescence of the tryptophan molecules.

There were other methods used to synthesize silver nanoparticles. The procedure used by Schneider resulted in successful batches of nanoparticles where the maximum absorbance wavelengths ranged from 413-420 nm.<sup>9</sup> The difference between this synthesis and Solomon's was that this reverses the 6:1 ratio and has a large excess of silver nitrate. There was no justification for this ratio, and this project found the resulting nanoparticles made using this method were less reproducible and tended to aggregate particles.

The citrate reduction method was also used with various amounts of citrate, silver nitrate, and distilled water. The citrate method required heat to reduce the silver, and the yellow color became evident after five to ten minutes of heating. This method was also less consistent, and the nanoparticles were less stable, than the Solomon procedure. The range of maximum absorbance wavelengths for the silver nanoparticles was 407-420 nm, similar to the Schneider synthesis.

The main reason for utilizing the Solomon synthesis was that the silver nanoparticles were highly concentrated. It was not easy to concentrate nanoparticles, but it was very easy to dilute them. Because nanoparticles are often tested by their effects on a reaction or fluorescence, it was helpful to begin with a concentrated solution that could be diluted if needed. Additionally, the process was very consistent, typically producing

maximum absorbance peaks between 390 nm and 400 nm. A third advantage was that this synthesis produced a larger quantity of nanoparticles than the other methods. The drawbacks to this synthesis were that the sodium borohydride was unstable, must be kept cold, and must be made fresh each time the synthesis was done. If the proper ratio of silver nitrate to borohydride was not used, the nanoparticles could aggregate, although this can be avoided by capping the nanoparticles with some stabilizing agent such as polyvinyl alcohol.

### **Gold nanoparticles synthesis**

The first trial of synthesizing gold nanoparticles worked but did not stop at the appropriate size, and the solution aggregated into very large particles within a few minutes. In this method, 20.0 mL of 1.0 mM gold (III) chloride was added to a 50 mL Erlenmeyer flask. While stirring, this solution was heated until boiling, and then 2 mL of 1% sodium citrate was added.<sup>11</sup> The solution turned gray within fifty seconds, dark gray within ninety seconds and purple within 2 minutes, and the solution stayed dark purple until it was removed from heat after seven minutes. Ten drops of 1.0 M sodium chloride was added to a portion of this solution resulting in aggregation of nanoparticles. This method was not successful, and the ruby red color associated with successful gold nanoparticles was not seen at any point.

The more successful procedure was to add 2.0 mL of the gold chloride to 5.0 mL of sodium citrate in a test tube suspended in boiling water.<sup>12</sup> The mixture was heated without stirring. After five minutes, a red color was achieved that lasted approximately ten minutes until a brown hue became evident. After fifteen minutes the red faded into a dirty gray color, and after twenty minutes aggregated particles were visible. This



procedure was run several times, and samples were pulled throughout the heating process to determine the best time to stop heating. Within three days, most had aggregated, and within three weeks, none were without a significant aggregation of nanoparticles.

Sodium citrate is not capable of fully reducing gold (III) at room temperature,<sup>12</sup> which accounts for heating the previous solutions. Sodium borohydride is supposed to be capable of reducing gold (III) to gold metal.<sup>12,13</sup> Unfortunately, adding the borohydride to the gold (III) solution did not produce nanoparticles. It required heating and/or the addition of sodium citrate, but resulted in solutions that were not stable.

Despite some success with synthesizing gold nanoparticles, there were issues, doubt, and expense that led the group to focusing on silver nanoparticles. Later it was found that the resulting gold nanoparticles were not stable, and different procedures would need to be used if stable gold nanoparticles were to be tested.

### **Synthesis of larger silver nanoparticles**

The concept to make larger silver nanoparticles involved a small amount of seed nanoparticles and a method to coat them further with silver atoms to obtain larger nanoparticles. The procedure involved a small amount of seed nanoparticles (0.1-8 mL) and the addition of 10.0 mL of 0.1 mM sodium hydroxide solution. While stirring this solution, 10.0 mL of 0.3 mM ascorbic acid in 1 mM NaOH and 10.0 mL of 0.3 mM AgNO<sub>3</sub> were added simultaneously over the course of about a minute. By starting with different amounts of seed, different sized nanoparticles resulted. The theory stated that all of the added silver would be added onto those nanoparticles, making larger nanoparticles. The theoretical formula confirmed by Schneider was  $d_{\text{calc}} = d_{\text{starter sol}} \times [(m(\text{Ag}^+) + m(\text{Ag}_s) / m(\text{Ag}_s))]^{1/3}$  where Ag<sub>s</sub> is the amount of silver in the seed solution, and

$\text{Ag}^+$  is the amount of silver added.<sup>9</sup>

By adding 0.1, 0.3, 0.5 and 0.7 mL of seed solution and following the procedure stated above, the nanoparticles' maximal absorbance wavelength shifted from 390 nm to between 410 nm and 415 nm, depending upon the amount of seed. The 0.3 mL sample shifted furthest, but all four seed solutions were within a very short range. This indicates that a larger amount of seed is necessary to create a larger range of particle sizes indicated by the peak wavelength of the absorbance. Frequently, a very small amount of seed was used, compared to the total amount of solution, and very large and dilute nanoparticles were frequently produced. Later in this research project, more seed solution syntheses was used with some success. Samples were made using 4 mL of seed solution with 4 mL each of the silver nitrate and ascorbic acid solutions. Variations were also successful by using 6 mL of seed solution instead of 4 mL.

From this it would appear that using the Schneider synthesis resulted in silver nanoparticles that absorb mainly between 410 nm and 415 nm. By using these nanoparticles as a seed, larger particles were obtained. There are limits on these synthetic methods and how the variation in the amount of seed solution used affected the resulting size of the nanoparticles.

Greater success was found by using a very large amount of seed solution relative to previous methods. Mixing similar amounts of seed solution with silver nitrate and ascorbic acid produced stable nanoparticles. Most mixtures consisted of 6-10 mL of each solution. This method created more concentrated and stable nanoparticles. Typically the size of the nanoparticles increased by 10-20 nm, but the seed solution could be changed to create larger nanoparticles with sizes of about 50-60 nm.

### **Determination of nanoparticle size**

The sizes of nanoparticles were typically classified using transmission electron microscopy (TEM) and UV-Vis spectroscopy. The general trend is that as the diameters of the nanoparticles increased, the maximum absorbance wavelength shifted towards the red. Visually the bright yellow color darkened towards a yellow-gray as the particles became larger. The TEM profiles were performed by sampling a given area and then creating a range of diameters of the nanoparticles from the data from the selected area. For example, in Solomon's work, a set of nanoparticles had an average diameter of 12 nm, but the range on the individual nanoparticles went from 5 nm to 20 nm, with a somewhat bell-shaped curve peaking near 12 nm.<sup>8</sup>

One of the most important things to realize when discussing the size of nanoparticles is that the sizes will vary within a sample. A transmission electron microscopy unit is typically used to determine the size of the nanoparticles. By measuring the size of several nanoparticles, an appropriate range can be determined. However, this method requires very expensive equipment, so researchers have correlated the results using microscopy with absorbance spectra of the nanoparticles to determine relationships between the diameter of nanoparticles and the peak absorbance wavelength. This correlation allowed researchers to determine the average size using the less expensive UV-Vis spectrophotometer instrumentation.

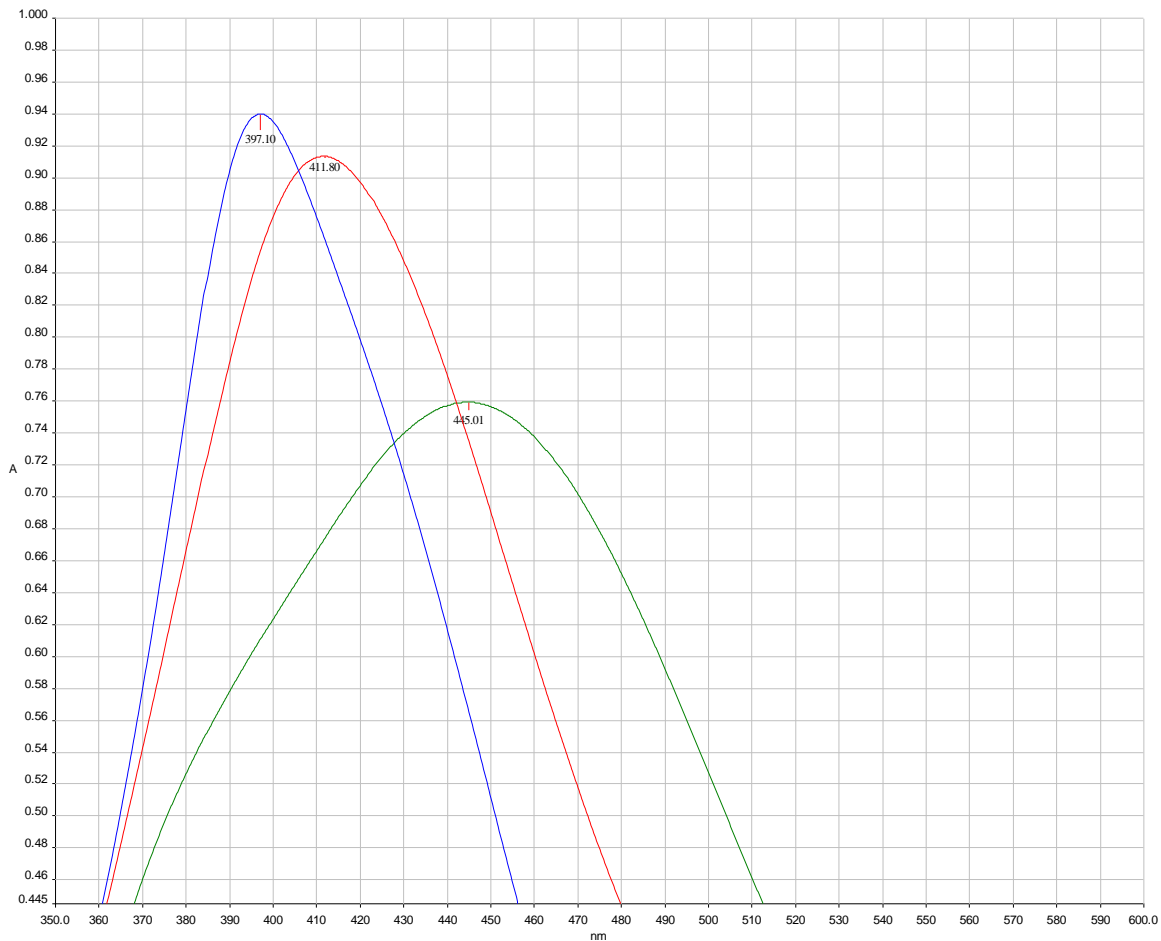


Figure 1: UV-Vis spectrum for three samples of silver nanoparticles. The blue peak is at 397.1 nm, the red is at 411.8 nm and the green is at 445.0 nm. The larger the nanoparticles, the further red-shifted the spectrum peak.

Figure 1 shows the spectra for three samples of silver nanoparticles; the spectrum shown in blue with an absorbance maximum at 397.1 nm contains the smallest nanoparticles. Depending upon which table is referenced, as shown in Tables 1-4 below, the nanoparticles can be characterized with an average diameter between 5 and 30 nm. The second peak shown in red with an absorbance maximum of 411.8 nm comes from

slightly larger nanoparticles with a diameter between 30 and 50 nm based upon the reference tables shown below. The largest nanoparticles, spectrum shown in green with an absorbance maximum at 445.0 nm, have a diameter between 60-80 nm. These larger nanoparticles have the broadest and the furthest red-shifted peak. For silver nanoparticles this is relatively simple; as the peak absorbance shifted towards the red, the nanoparticle size was increased. Results from four sources were shown below in Tables 1-4 to characterize the size of nanoparticles.

**Table 1: Gold nanoparticle diameters and peak absorbance wavelengths**

Maximum absorbance wavelength (nm)	Diameter of gold nanoparticles (nm)	Range of diameters (+/- nm)
519	6.5	1.5
524	18.6	3.5
531	48.1	5.8
545	96.7	15.4

The data in Table 1 are taken from Xu and compare diameters of gold nanoparticles with their peak absorbance wavelengths.<sup>14</sup>

**Table 2: Silver nanoparticle diameters and peak absorbance wavelengths 1**

Maximum absorbance wavelength (nm)	Diameter of silver nanoparticles (nm)
400	38
410	56
440	75
470	110

The data in Table 2 are taken from Schneider for silver nanoparticles of varying size.<sup>9</sup>

**Table 3: Silver nanoparticle diameters and peak absorbance wavelengths 2**

Particle size (nm)	Maximum absorbance wavelength (nm)	PWHM (nm)
10-14	395-405*	50-70
35-50	420	100-110
60-80	438	140-150

The data in Table 3 are taken from Solomon and show  $\lambda_{\max}$  and peak width at half maximum for various particle sizes. \* ( $\lambda_{\max}$  may vary from sample to sample)<sup>8</sup>

**Table 4: Silver nanoparticle diameters and peak absorbance wavelengths 3**

Diameter of nanoparticle (nm)	Maximum absorbance wavelength (nm)
20	404.8
30	410.2
40	416.9
50	423.7
60	441.7
70	450.5
80	466.7

The data from Table 4 illustrate UV-Vis data and are from the Nanocomposix website.<sup>15</sup>

Table 4 was used most frequently in this project, but because of its limitations of not being very useful for 380-400 nm peaks, the smallest maximum absorbance wavelength of 380 nm was estimated to consist of very tiny nanoparticles about 4 nm in diameter, and a linear relationship was made between size and peak absorbances occurring between 380 nm and 400 nm. This relationship yields results consistent with Table 2 giving 12 nm diameter size particles for a peak absorbing at 400 nm. The typical samples of silver nanoparticles had maximum absorbance wavelengths between 380 nm and 400 nm, using the first synthetic method. Using the seed method,<sup>9</sup> the maximum

absorbance wavelengths shifted enough to increase the size by about 10 or 20 nm each time an addition was done.

Some important points to note are that the silver nanoparticles had a much larger range of wavelengths, whereas the gold nanoparticles tended to have a much narrower range of wavelengths. The larger range for silver made it easier to characterize than gold. In addition, the Solomon data included the peak-width at half maximum values, which indicated that as the size of the nanoparticles increased, not only did the peak shift towards the red but the absorbance spectrum also broadened.

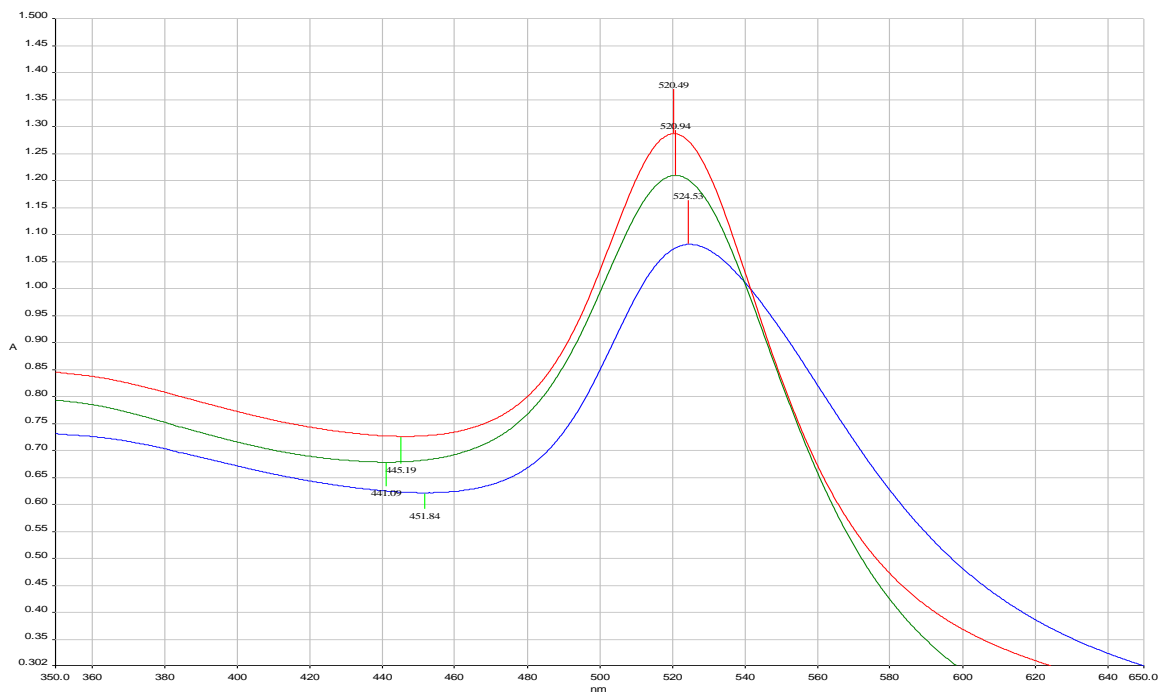


Figure 2: UV-Vis Spectrum for three samples of gold nanoparticles. Peaks are located at 520.5 nm (red), 520.9 nm (green) and 524.3 nm (blue). Red shifting of the maximum absorbance indicated an increase in size of the nanoparticle

Figure 2 shows results for three different samples of gold nanoparticles. According to Table 1, the nanoparticles having a spectrum with an absorbance maximum at 520.5 nm had a diameter of approximately 8-10 nm. The nanoparticles yielding the green spectrum with a peak at 520.9 nm had a similar diameter. The spectrum indicated in blue with an absorbance maximum at 524.3 nm comes from nanoparticles with a diameter of around 20 nm, double the size of the other two samples. An absorbance maximum shift of only 4 nm indicated a much larger increase in size for gold nanoparticles than for silver nanoparticles.

### **Tryptophan Fluorescence**

Tryptophan will fluoresce in the 300 nm range when excited by a 280 nm light source.<sup>16</sup> Silver nanoparticles interact with electric fields and light, producing the fluorescence enhancement or quenching effects seen in many silver nanoparticles systems. This project focused on the nanoparticles' effect on the fluorescence properties of tryptophan. Previous studies showed that nanoparticles influenced the relaxation processes of tryptophan fluorescence, causing an enhancement of emission.<sup>1</sup> Tryptophan had also been successfully used as a reducing agent in the production of silver nanoparticles.<sup>17</sup>

### **Methods**

Fluorescence studies of tryptophan with a concentration of 250  $\mu\text{M}$  were performed using a Jasco fluorimeter. The fluorescence intensities were compared in the presence of various silver nanoparticles. For all tryptophan samples, the excitation wavelength was 290 nm, and the emission wavelength was 310 nm. Tryptophan was



purchased from Sigma Aldrich. The fluorescence data were compared to the blanks that contained a solution of tryptophan in distilled water.

### Data

In the first experiment, four batches of silver nanoparticles ranging from small to large were tested with tryptophan to determine their effects on the fluorescence intensity. Two milliliters of 100  $\mu\text{M}$  tryptophan solution was mixed with two milliliters of 18.2  $\text{M}\Omega\cdot\text{cm}$  water and two milliliters of a solution of synthesized nanoparticles. In the first experiment, the only parameter that was varied was the diameters of the nanoparticles.

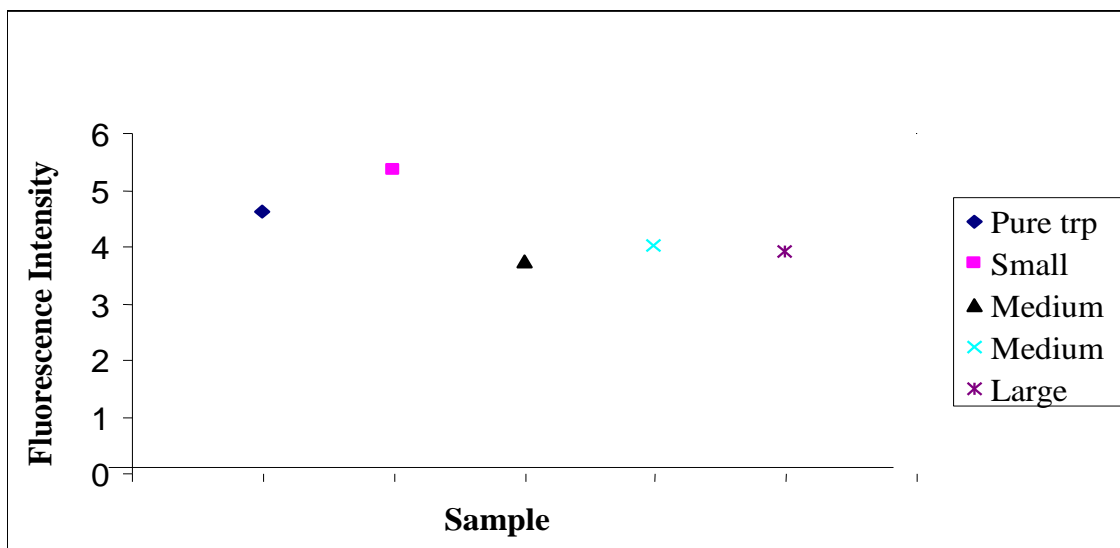


Figure 3: Fluorescence of tryptophan with varying sizes of silver nanoparticles. 2 mL of 100  $\mu\text{M}$  Tryptophan were mixed with 2 mL of various sized silver nanoparticles and 2 mL of distilled water. The fluorescence values show little variation despite the different sized nanoparticles.

No definitive trends were identified in Figure 3, but there is a possibility that larger nanoparticles quenched the fluorescence slightly and smaller nanoparticles enhanced. In the next experiment, the amount of tryptophan was varied while keeping the volume of silver nanoparticles solution and total solution constant. The amount of tryptophan increased from 0 mL to 2.0 mL in 0.5 mL increments. The amount of distilled water started at 2.5 mL and decreased to 1.0 mL in 0.5 mL increments for each sample. This procedure kept the total volume of solution constant while increasing the amount of fluorophore. In Figure 4 a linear increase was seen for the pure sample as expected due to Beer's Law for fluorescence. The results were inconclusive but one possibility emerged that led to further testing. The smallest silver nanoparticles gave different results in both Figures 3 and 4 from the larger nanoparticles. At this point it was possible that they were different due to having PVA added as a stabilizing agent. Testing the effect of the stabilizing agent could help determine the actual mechanism of the silver nanoparticles interactions with fluorophores to either increase or quench fluorescence. If the PVA interfered with the process, it could support a mechanism in which the nanoparticle must contact the fluorophore instead of interacting via electric field.

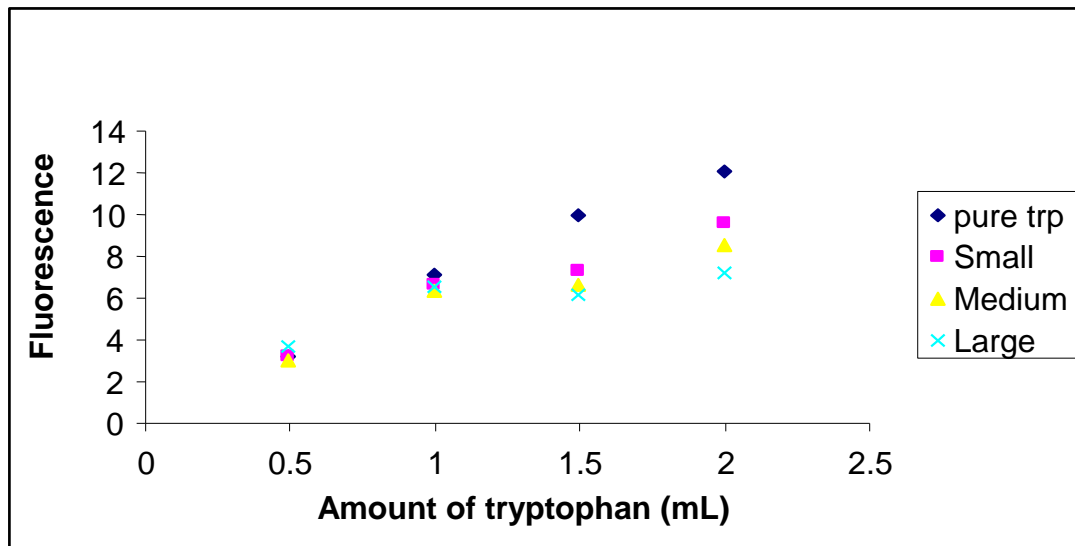


Figure 4: Tryptophan fluorescence with silver nanoparticles. As three different samples of silver nanoparticles were added to tryptophan solution, the effects on fluorescence intensity were monitored. Each sample consisted of 0.5 mL of nanoparticles, and the proper amount of distilled water was added to make the total volume of the sample 3.5 mL.

Additional experiments tested the effects of silver nanoparticles with and without PVA added and the effect of varying the amount of PVA added in samples. The first experiment increased the amount of PVA for small silver nanoparticles, and the hypothesis that PVA interferes with nanoparticles interaction appeared to have merit, as seen in Figure 5. As more PVA is added, the enhancement of fluorescence due to the nanoparticles is diminished, and the fluorescence approached that of pure tryptophan.

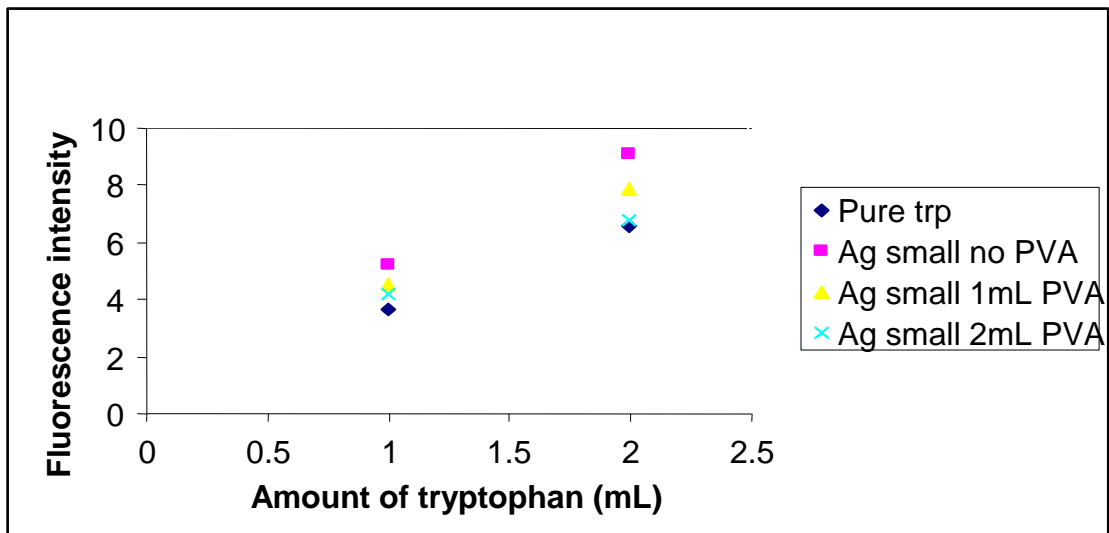


Figure 5: Effect of varying the amount of polyvinyl alcohol attached to small silver nanoparticles on the fluorescence of tryptophan. Increasing the amount of PVA added to silver nanoparticles reduced the enhancement of fluorescence for small silver nanoparticles, which had a maximum absorbance wavelength of 380nm-390nm.

The next two experiments shown in Figures 6 and 7 agree with the hypothesis that PVA interferes with the fluorescence, but showed a less pronounced effect. Figures 6 and 7 both show quenching, but once again the trend was that the data approached the blank as the amount of PVA increased.

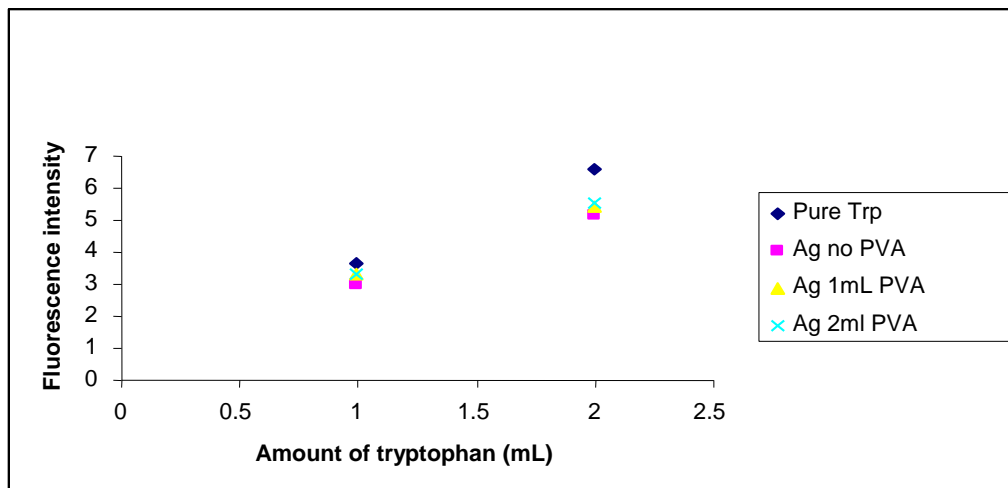


Figure 6: Effect of varying the amount of polyvinyl alcohol attached to medium silver nanoparticles on the fluorescence of tryptophan. The silver nanoparticles quenched the fluorescence slightly and the PVA reduced this quenching slightly. The nanoparticle diameter was approximately 30 nm for this experiment.

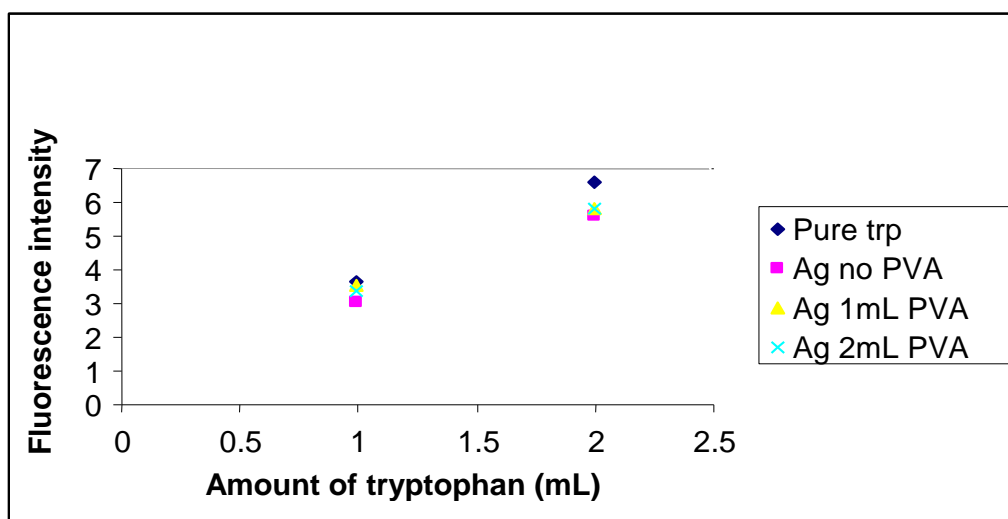


Figure 7: Effect of varying the amount of polyvinyl alcohol attached to large silver nanoparticles on the fluorescence of tryptophan. Pure tryptophan fluoresced with the largest intensity. A slight increase in fluorescence was evident upon addition of polyvinyl alcohol when compared to the nanoparticles without.

Despite the inconsistency, it certainly appeared possible that PVA molecules attached themselves to silver nanoparticles as a stabilizing agent. These molecules limited the ability of the nanoparticles to either collide with fluorophores or become close enough to the fluorophore to interact via an electric field.

Next, the effects of silver nanoparticles were studied by comparing more dilute specimens of nanoparticles. The samples were diluted by factors of 5 and 25 and compared against a blank solution and a solution containing the original concentration of nanoparticles. Trends from this data were not evident; outliers were prevalent and the inability to distinguish trends cast doubts onto the original theory that the nanoparticles must collide with the fluorophore to influence the fluorescence intensity. As the concentration of nanoparticles decreased, fewer collisions should have occurred, limiting the effects of the nanoparticles. Figures 8-10 show that typically the concentrated silver nanoparticles and samples diluted by a factor of 5 tended to slightly enhance the fluorescence, but the variations in the data made proposing any conclusions very tenuous.

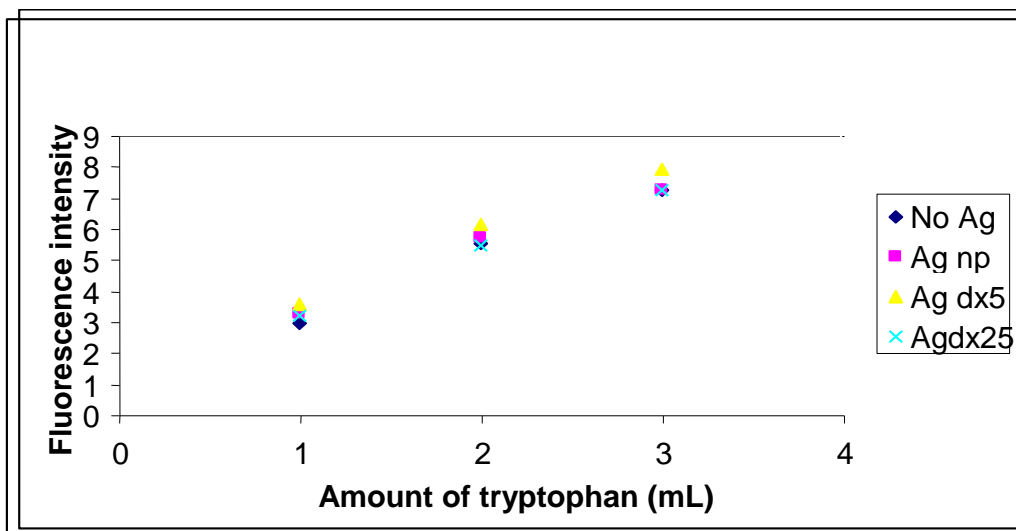


Figure 8: Dilution of small silver nanoparticles' effect on the fluorescence of tryptophan. Maximum fluorescence occurred when the silver nanoparticle sample was diluted by a factor of five. Dilution by a factor of 25 approached the control with no silver, and a small dilution increased the fluorescence intensity.

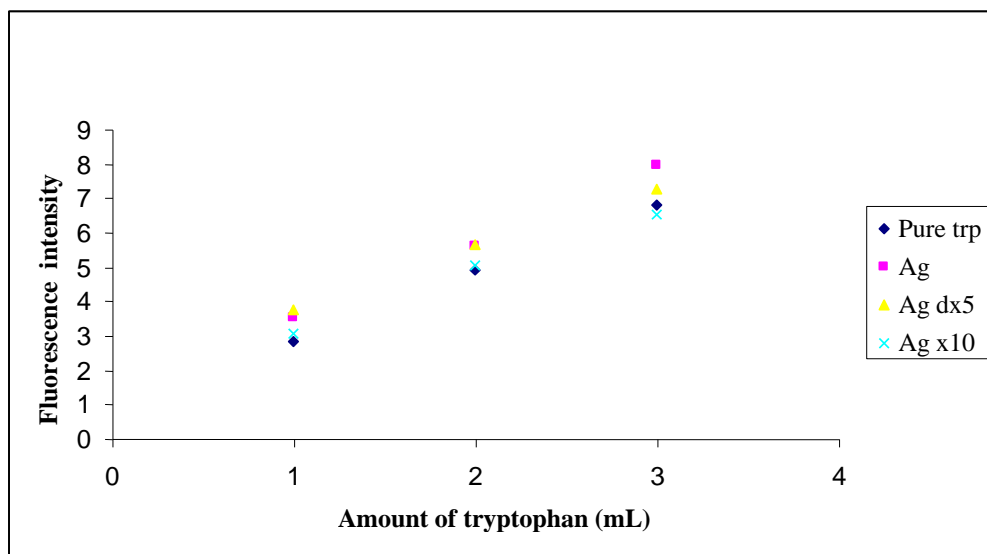


Figure 9: Dilution of medium silver nanoparticles' effect on the fluorescence of tryptophan. Maximum fluorescence occurred when the silver nanoparticle sample is not diluted or diluted by a factor of five. Dilution by factor of 10 approached the control with no silver.

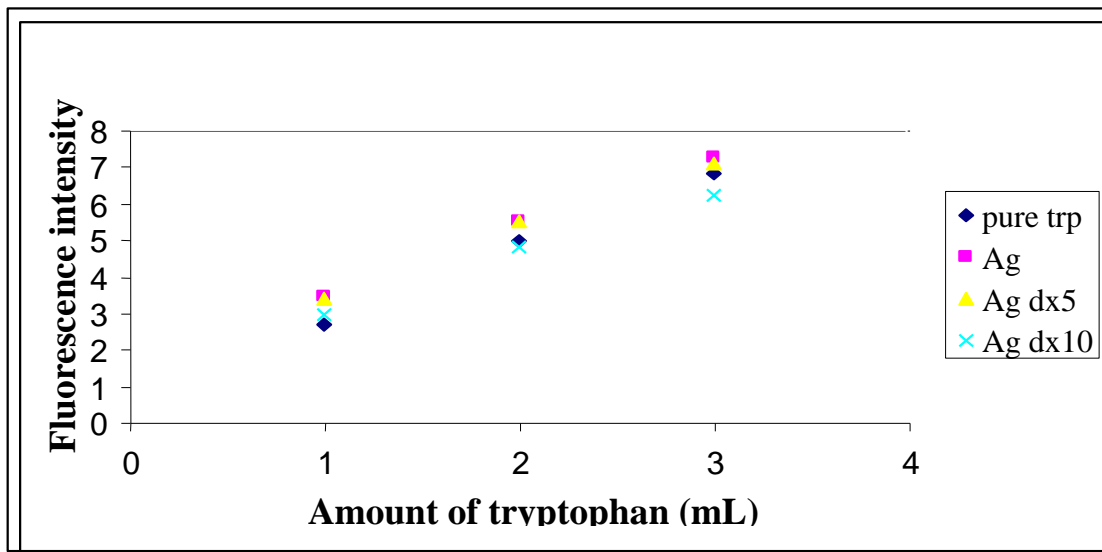


Figure 10: Dilution of large silver nanoparticles' effect on the fluorescence of tryptophan. Maximum fluorescence occurred when the silver nanoparticle sample was not diluted or diluted by a factor of five. Dilution by a factor of 10 approached the control or even quenched the intensity slightly.

At this point the lack of precise data does not allow for a solid conclusion.

Figures 8-10 showed some agreement that the nanoparticles enhanced the fluorescence of tryptophan, but there was a lack of consistency at various concentrations of nanoparticles. It was possible that adding the organic molecules to nanoparticles did impede the interaction of the nanoparticles in various processes, but no solid conclusion could be reached with this sets of experiments. Additionally, it would be very difficult to design an experiment that would isolate the collision factors. None of the trials show a very large effect on the fluorescence that might make it easier to observe trends for the attachment of polyvinyl alcohol. If more research were to be continued on this topic, it



would be recommended to find a process that has a large and consistent enhancement from silver particles of multiple sizes.

### **Laser fluorescence decay rates**

In this study, a pulsing green (532 nm) laser was used to excite tris (2,2'-bipyridyl)ruthenium(II)chloride with and without silver or gold nanoparticles. The structure of the Ruthenium complex is shown below in Figure 11.<sup>19</sup> It had previously been determined that the Ruthenium complex is capable of binding gold nanoparticles<sup>18</sup> and inducing a change in the nanoparticles evident via a color change.<sup>14</sup> The excitation source for all lifetime measurements was a New Wave Research Minilase II/10 Hz doubled Nd:Yag laser operated at 532 nm with a 16 nanosecond pulsewidth. The fluorescent signal was detected by a Hamamatsu photomultiplier tube, which was connected to a Hewlett Packard 54615B oscilloscope to record. The resulting trace was then recorded to a computer using the BenchLink Scope software.

The fluorescence decay data were analyzed by fitting data to an exponential function,  $Ae^{-Bt} + C$ . The decay constants (B) were determined three times on three different trials for a total of nine data points per solution. A was the amplitude of the curve, and C was the baseline value. The solutions were prepared with 1.0 mL of 250  $\mu$ M Ru complex and varying amounts of small (diameter near 10 nm) nanoparticles in 18.2 M $\Omega$ ·cm water. The total volume of each solution was 3.5 mL as seen in Table 5, but with varying amounts of nanoparticles, and the Ru complex was held constant.

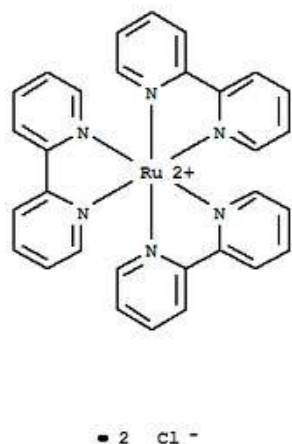


Figure 11: Structure of the tris (2,2'-bipyridyl)ruthenium(II)chloride compound

**Table 5: Decay constants of Ru in presence of Ag nanoparticles**

Volume of nanoparticles added (mL)	Average decay constant ( $\times 10^7 \text{ s}^{-1}$ )	Confidence range
1.0	4.33	0.0739
0.7	4.28	0.115
0.5	3.72	0.108
0.3	4.44	0.240
0.0	4.36	0.251

Table 5 displays the results of silver nanoparticles with 250  $\mu\text{M}$  tris (2,2'-bipyridyl)ruthenium(II)chloride excited with a 532 nm laser and the decay constants for the resulting fluorescence in the presence of  $\text{N}_2$  gas. There was no consistent quenching or enhancement trend. The confidence range for the decay constants was calculated using an alpha value of 0.05 on the nine data points taken for each sample.

**Table 6: Decay constants of Ru in presence of Au nanoparticles**

Volume of nanoparticles added (mL)	Average decay constant ( $\times 10^7 \text{ s}^{-1}$ )	Confidence range
1.0	3.24	0.214
0.7	4.30	0.135
0.5	4.74	0.271
0.3	4.29	0.0848
0.0	4.09	0.0506

Table 6 displays the results of 20 nm gold nanoparticles with 250  $\mu\text{M}$  tris (2,2'-bipyridyl)ruthenium(II)chloride excited with a 532 nm laser and the decay constants for the resulting fluorescence in the presence of  $\text{N}_2$  gas. The data shows less consistency and no trend, similar to the silver nanoparticles.

These results indicated that it is unlikely that either gold or silver impacted the fluorescence lifetime of the ruthenium complex. It was possible that high concentrations of gold nanoparticles interfered with the fluorescence lifetime, but further investigations were needed to verify this conclusion.

#### **Luminol and Ferricyanide reaction enhanced by silver nanoparticles**

The characteristic blue glow from the chemiluminescence of luminol is seen on television's forensics shows. Because the light emitted has a 425 nm wavelength,<sup>3</sup> it was a prime candidate to be impacted in the presence of silver nanoparticles, which absorbed light in that region. The key steps in this chemiluminescence process are the luminol first formed a radical by reacting with a reducing agent, then an oxygen molecule was attached, and finally a nitrogen molecule was removed forming the excited state of the 3-Aminophthalate before light is emitted. Zhang reports on this reaction and its interactions with gold nanoparticles.<sup>20</sup> The basics of the mechanism are illustrated below

in Figure 12.<sup>20</sup>

**Scheme 1. Schematic CL-Generating Mechanism for the Oxidation of Luminol with Three Major Steps**

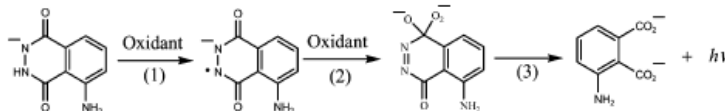


Figure 12: Key steps in luminol chemiluminescence

Three studies similar to this research included chemiluminescence studies of luminol-ferricyanide enhancement by gold nanoparticles,<sup>13</sup> luminol-peroxide enhancement by silver nanoparticles,<sup>2</sup> and luminol-peroxide enhancement by gold nanoparticles.<sup>20</sup>

Zhang studied the effects of different sizes of gold nanoparticles on the reaction between luminol and hydrogen peroxide. It was found that all sizes of nanoparticles enhanced the chemiluminescence, with 38 nm diameter nanoparticles being the most effective. A mechanism was proposed by Zhang of how the gold nanoparticles influenced the reaction. It was concluded that the hydrogen peroxide bond is broken at the surface of a nanoparticle, forming two radicals that are stabilized by the nanoparticle until they can react with hydroxide, oxygen molecules, or the luminol itself. This was tested by introducing a variety of organic molecules that were capable of interacting with the nanoparticle, limiting the nanoparticles' available space to bind the peroxide and radicals. All tested organic molecules inhibited the chemiluminescence to some degree.<sup>20</sup>

Duan investigated the effects of different sized gold nanoparticles on the reaction between luminol and ferricyanide. The results were that small gold nanoparticles, 2.5 nm – 11 nm, inhibited the reaction, but nanoparticles 11 nm and larger enhanced the reaction.

In addition, 25 nm nanoparticles led to the greatest enhancement of chemiluminescence. The theory proposed was that smaller gold nanoparticles were more reactive and were able to compete with the luminol to react with the ferricyanide. This theory was supported by UV-Vis spectra of a mixture of the smaller gold nanoparticles and ferricyanide over time. The UV-Vis absorbance peak for the gold nanoparticles dropped drastically, and the ferricyanide peak would disappear. The mechanism of enhancement proposed in this paper involved the gold nanoparticle assisting in the transfer of the radical electron on the nitrogen in luminol to the oxygen molecule. This occurred after the ferricyanide had produced the nitrogen radical by being reduced to ferrocyanide. The benefits of this mechanism were that the mechanism upholds the notion of the nanoparticle acting as a catalyst by gaining electrons then transferring electrons to the molecular oxygen before the nitrogen is removed to form the 3-aminophthalate. Thus the nanoparticle remains unchanged. It also had the nanoparticle interacting with radicals, which has been previously shown to be possible.<sup>20</sup> The paper also proposed that the smaller nanoparticles can be oxidized by the ferricyanide, and the competition is what quenched the reaction. The paper stated that the nanoparticles smaller than 25 nm in diameter have a lower surface plasmon resonance peak, and that may correlate to less surface energy, reducing the catalytic abilities of the nanoparticles.<sup>13</sup>

Chen studied the impact of silver nanoparticles of varying size on the chemiluminescence of luminol using hydrogen peroxide in place of ferricyanide. The results showed that silver nanoparticles enhanced the chemiluminescence and that the smaller nanoparticles did so more efficiently. The article stipulated that the efficiency of the small nanoparticles was due to an increase in surface area of smaller nanoparticles

and surface electron density increased in smaller nanoparticles. The article proposed silver nanoparticles catalyzed the reaction by splitting the peroxide molecules into two radicals, which rapidly oxidized the luminol. The article supported this theory by performing the same experiment with Bovine Serum Albumin (BSA) attached to the nanoparticles. By attaching molecules to the surface of the nanoparticles, the peroxide was limited in binding to the silver nanoparticles. The possibility existed that the BSA interfered with radical formation, as may the acid used to attach the BSA to the nanoparticles. This paper concluded by suggesting that other researchers study the effects of silver nanoparticles on luminol using other oxidizing agents.<sup>2</sup>

All of these papers were very well done in their experiments using highly controlled systems. Multiple sources were used to characterize the sizes of nanoparticles and some specified the concentrations of their nanoparticles. They used apparatus to mix their solutions in the appropriate volume and timeframe automatically and tested pH effects and concentration variations. There are two aspects in which these papers were inadequate. The mechanism proposed by Zhang, which is the same and supported by Chen, is possible, but concerns arise that experiments done were not unique to the conclusions. Tests were not created that eliminated other possible mechanisms but rather justified situations such as the nanoparticle being involved in the reaction. Confirming a mechanism is very difficult; however, the tests performed thus far have created a false sense of security of the mechanism being correct. The second issue was the size of nanoparticles was explored, yet no constants were defined. The articles either did not specify concentrations of nanoparticles, or varied concentrations were used. In this thesis there were two important factors considered for nanoparticles: the number of them and

their total surface area. One study divided chemiluminescence intensities by the total surface area, but this again assumes a linear impact of concentration on intensity, which is later disproved.<sup>2</sup> Something to consider is that if the same amount of silver is used to make large nanoparticles and small nanoparticles, it results not only in different sizes of nanoparticles, but also in the number of nanoparticles made and the total surface area. It also was possible that the total amount of silver or gold was not the same in these previous studies.

Therefore this project tested the effect of silver nanoparticles on the luminol reaction, varying the size of the nanoparticles and changing only one of the parameters of the number of nanoparticles, the surface area, or the total amount of silver atoms during an experiment.

There are some assumptions to be made for this to be possible; first, because an electron microscopy unit was not available, it was assumed that the wavelengths given by Nanocomposix (see Table 4) matched the nanoparticle samples in determining the sizes of the nanoparticles synthesized. If a sample of nanoparticles had a peak absorbance at a wavelength of 410 nm, it was assumed the nanoparticles were 30 nm in diameter.<sup>15</sup> When the absorbance peaks were below 405 nm, a 12 nm diameter was used for peak absorbance at 400 nm and then decreased by 4 nm in diameter for every 10 nm in peak absorbance blue shift, which was the best fit with the Solomon data in Table 2. There are some discrepancies amongst the different sources used, so this is not certain but is a reasonable approximation. All of the nanoparticles were assumed to be spherical, which was typical, but it was possible that rod shapes or other shapes can form which changes the calculations of the surface area and number of nanoparticles. Nanoparticles were

assumed to have no distribution in size when determining the number of nanoparticles and surface area. Though not true,<sup>8,12,14</sup> this assumption should have minimal impact on data. A report of nanoparticles of diameter 10 nm could actually have nanoparticles ranging from 5 to 20 nm, but all were assumed to be 10 nm to simplify calculations. Another assumption was that all the silver of the silver nitrate was converted into nanoparticles. To see if there was leftover silver nitrate, it would be necessary to determine the molar absorptivity constants of the nanoparticles based on size and compare concentrations, but even in the event all of the silver was not made into nanoparticles, it was unlikely that an uneven amount of excess based on size would have occurred.

### **Methods**

Chemiluminescence data were obtained using a Jasco FP-6300 Spectrofluorometer, as seen in Figure 13, with the excitation lamp turned off and the emission detector set to 425 nm. A solution containing 1.0 mL of nanoparticles or blank and 1.0 mL of luminol was inserted into the cuvette. After five seconds, 1.0 mL of ferricyanide solution was injected. The chemiluminescence intensity for the reaction was collected for between 20 and 150 seconds. The concentrations of luminol and ferricyanide were 0.010 M, and the luminol was in 0.10 M NaOH. Water for making solutions was 18.2 M $\Omega$ -cm. The chemicals were purchased from Sigma-Aldrich. The average peak intensity of the trials was used for data analysis as seen in Figure 12. The experiments were run several times to ensure reproducibility. There was a large amount of variation in a set of data, but general trends were determined from averages.





Figure 13: Jasco FP-6300 Spectrofluorometer used to detect chemiluminescence intensity of luminol reactions.

Calculations were performed to determine the total number of silver atoms, silver nanoparticle concentration, and surface area of the nanoparticles. The total silver calculation involved determining the total mass of Ag from the amount of silver nitrate and the percent composition of silver in silver nitrate. The volume of the nanoparticle was calculated by assuming the nanoparticle was a perfect sphere and matched diameters from UV-Vis data on the Nanocomposix website. Once the volume of a single nanoparticle was determined, the mass of silver in one nanoparticle was found using the density of silver ( $10.49 \text{ g/cm}^3$ ). This assumed the packing method of the nanoparticles is similar to the bulk metal, which is a good assumption. The third piece of information required was the total volume of solution, which was used to analyze surface area and

nanoparticle concentration since some synthesis methods produced different solution volumes. The surface area concentration was found from the surface area on a single nanoparticle and multiplying it by the concentration of nanoparticles. This was done using Excel spreadsheets; a sample is attached in Appendix 1 with explanation in Appendix 5.

When analyzing samples, the peak intensity of light emission was the basis for determining enhancement or quenching effects. The intensities showed some variation within a single sample, but average data showed consistency. Figure 14 shows the black curve with nanoparticles added, which represented a single data point of around 270 from the peak, and the blue curve, a blank, would be a point near 60 for their relative chemiluminescence intensities.

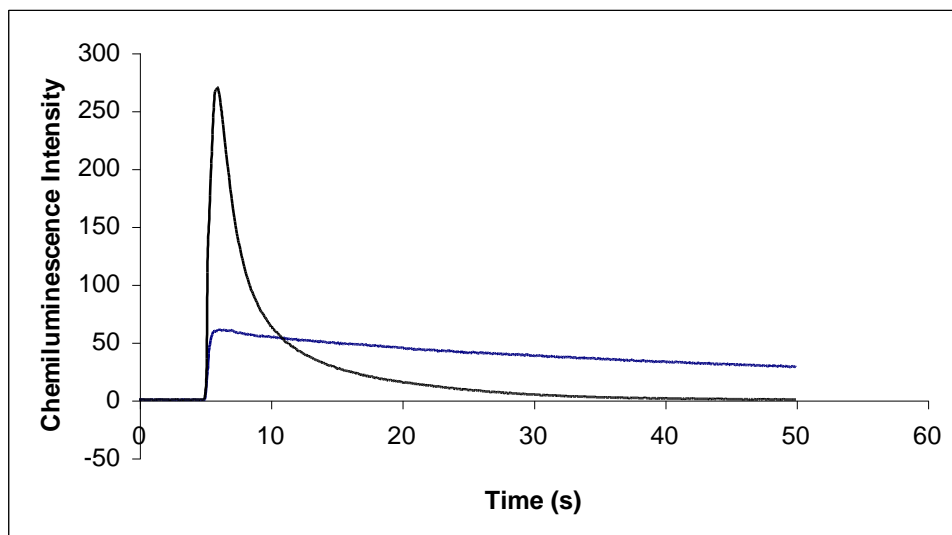


Figure 14: Chemiluminescence intensity for the reaction between luminol and ferricyanide. The quickly decaying curve in black has nanoparticles added, and the slower decay curve in blue had distilled water added to the luminol and ferricyanide.

Different size nanoparticles were combined with luminol and added to ferricyanide. At this point none of the three parameters of surface area, number of nanoparticles, or silver concentration were held constant. Despite not holding anything constant, the results seen in Figure 15 were very similar to those of the Chen paper. Figure 15 shows that adding the nanoparticles did impact the peak light intensity, and the smaller nanoparticles had the greatest intensity. This did agree with the Chen article, but this preliminary study only studied nanoparticle size. Concentration of surface area, number of nanoparticles, and amount of silver all vary in the three samples. These factors will be held constant in later experiments.

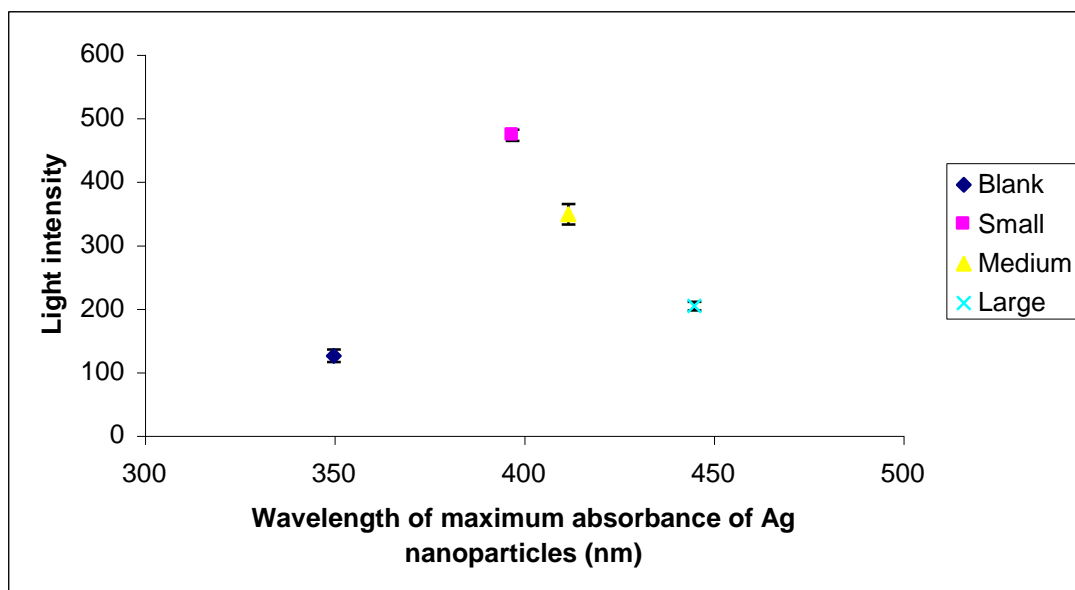


Figure 15: Chemiluminescence intensity of luminol with varying sizes of silver nanoparticles (small nanoparticles, medium nanoparticles, and large nanoparticles). This graph shows the relationship between wavelength of maximum absorbance of the silver nanoparticles and the effect on the chemiluminescence reaction of luminol and potassium

ferricyanide.

Some inconsistency of results might have been due to the method of mixing the three solutions. The luminol and nanoparticle solution could emit light before the ferricyanide was added. Samples were tested with varying times between the addition of the nanoparticles to the luminol, and the addition of the ferricyanide to the mixture.

Figure 16 illustrates the impact of mixing the nanoparticles with the luminol. When the addition of the ferricyanide is delayed, the luminol and silver nanoparticles reacted, reducing the overall intensity of emitted light.

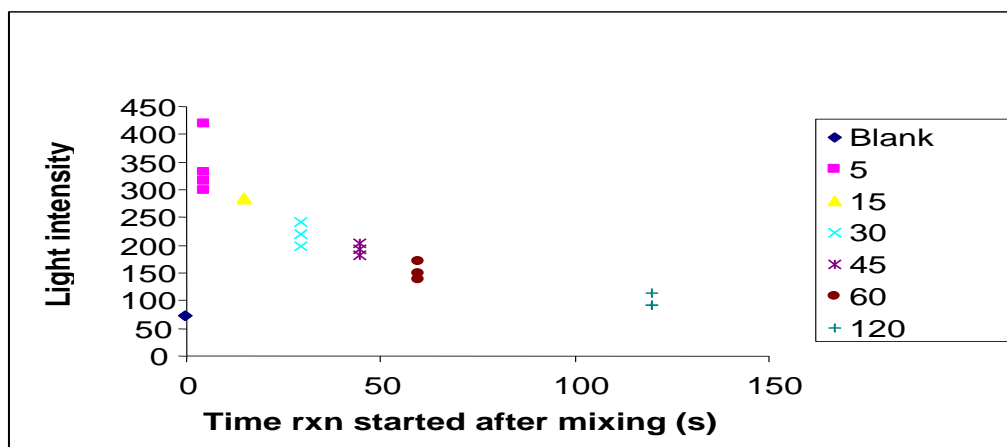


Figure 16: Chemiluminescence intensity with delayed addition of ferricyanide. The luminol reaction with ferricyanide in presence of silver nanoparticles was analyzed while the time between adding ferricyanide to the luminol nanoparticle mixture was varied.

Results of mixing the nanoparticles with the ferricyanide showed that the ferricyanide had a larger impact on the results than the luminol. Figure 17 shows that adding luminol to the nanoparticles first will lead to less side reaction than adding the

ferricyanide first. The ferricyanide reacted faster with the nanoparticles than the luminol, as evidenced by the greater decrease in chemiluminescence. The process outlined in the methods section of mixing the nanoparticles and luminol before adding the ferricyanide after a consistent five-second delay was then utilized to mitigate the impact of the side reaction. The studies aside from Figures 15-17 all show data collected where the mixing of the three solutions always had the same method, outlined above.

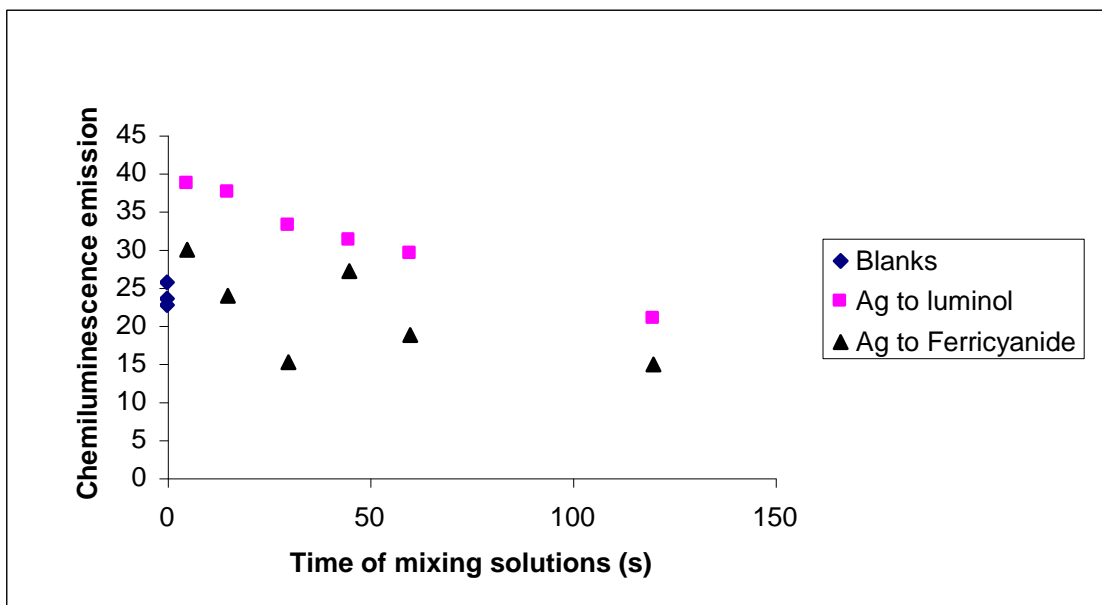


Figure 17: Chemiluminescence intensity of luminol ferricyanide reaction with varying delays between additions of third chemical. The pink series with larger intensity is when silver nanoparticles and luminol are mixed and ferricyanide is added after the stated time. The yellow series shows ferricyanide and silver nanoparticles mixed before addition of the luminol.

### **Assumptions made in calculations of surface area and nanoparticle amount**

1. All nanoparticles in the sample were the same size.
  - Nanoparticles will actually vary in size, but the averages that result should be sufficient for comparison of trends in properties.
2. Nanoparticles are large enough to ignore edging effects on the surface of the nanoparticle.
  - The smallest nanoparticles were around 4 nm in diameter, which would contain approximately 1000-10,000 atoms in the smallest nanoparticles.  
Therefore we can assume the surface to be smooth and ignore the variations in depth due to individual atoms.
3. All silver nitrate in synthesis has reacted to form nanoparticles.
  - If this is not true, it is assumed that the same amount of silver nitrate remains unreacted each time.
4. The size of the nanoparticles was correctly determined via UV-Vis spectroscopy and transmission electron microscopy was not necessary.
  - There are wide ranges of diameters of nanoparticles reported throughout the literature. One data source followed listed multiple diameters and multiple wavelengths that allowed a reasonable curve to be fit. Without the microscopy, sizes and shapes from synthesis procedures cannot be absolutely determined. This project assumed a comparison of small, medium, and large nanoparticles.

5. All nanoparticles are spherical.
  - If some of the nanoparticles were rod-shaped or other shapes, this would affect calculations on surface area and number of nanoparticles. Synthesis methods were modeled after established synthesis methods that produced spherical nanoparticles ascertained by microscopy data.
6. The peaks of the chemiluminescence intensity curves were sufficient to determine the catalytic effect on chemiluminescence.
  - The method used in determining an increase in chemiluminescence was taking the peak point of the graph. The decay function and total amount of light was not determined in this study. Future projects will investigate these aspects.

### **Comparisons of small/medium/large nanoparticles using constant number of nanoparticles**

The effectiveness on chemiluminescence with the same number of nanoparticles present in a given sample was compared. If the same number of nanoparticles gave the same increase in chemiluminescence, it can be assumed that surface area, which has different properties for different sizes of nanoparticles,<sup>2,13,20</sup> is not as important a factor in determining the effectiveness of nanoparticles. Instead it suggests that the analyte must bind to the nanoparticle whether the nanoparticles are small or large. If the largest nanoparticles gave the greatest increase in chemiluminescence when comparing equal numbers of nanoparticles, the increase in signal can be attributed to the increase in surface area. If the mid-sized nanoparticles were most efficient, that would suggest that two factors drove the efficiency of the nanoparticles' ability to enhance the chemiluminescence. First, the amount of surface area increased the effectiveness, and

second, the smaller nanoparticles, which had a greater surface energy, influenced the light-enhancing ability of the nanoparticle through more electron density.

There are other explanations for one size of nanoparticle enhancing light better than another. Surface plasmon resonance (SPR) is a phenomenon where propagating waves along the surface of a metal are enhanced due to resonance.<sup>21</sup> The peak wavelength of absorbance could account for a nanoparticle that would resonate with the surface plasmons in a nanoparticle causing enhanced luminescence.

**Table 7: Average light emission intensity for constant number of nanoparticles**

Diameter of nanoparticle (nm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0 (blank)	121.099	222.635	51.195	56.648	34.906
3					50.726
5		189.904	47.744		
10	116.487	307.164		42.183	
10				42.420	
20		185.490			32.985
20		<b>265.437</b>			
30			70.559		
35			<b>94.492</b>		44.493
40	<b>192.300</b>			<b>156.679</b>	
50			93.219	139.617	332.485
60	176.017	225.196	94.410		<b>381.947</b>

The various trials in Table 7 were performed on different days. The variation in nanoparticle size was dependent upon the samples made that day. A blank was in each trial to identify trends in quenching or enhancement within a trial set. The data in Table 7 show that there was some variation from sample to sample, possibly due to measurement inaccuracies or variations in size determination of the nanoparticles. Generally larger



nanoparticles were more effective than smaller nanoparticles. It seems that two key factors arose. First, nanoparticles 20-40 nm in diameter enhanced chemiluminescence best, despite having less surface area than the 50-60 nm nanoparticles. The very dilute concentrations of small nanoparticles typically quenched the chemiluminescence in Table 7. The highest light intensity in each trial was in bold to highlight the sizes of nanoparticles most effective at enhancing chemiluminescence. The trend that medium nanoparticles showed the greatest enhancement and larger nanoparticles enhanced light almost as well as medium nanoparticles was evident. There was some evidence of smaller nanoparticles quenching in all five trials and a few outliers where smaller nanoparticles did not quench or enhance chemiluminescence.

The implications of the results were very important for understanding the interactions of nanoparticles with light and the interactions made during catalysis in the luminol reaction. The smaller nanoparticles quenching the reaction showed that the small nanoparticles interfered by reacting with the luminol and/or ferricyanide. This explained the decrease in intensity when the nanoparticles were mixed with the luminol or ferricyanide, as in Figures 16 and 17. A similar conclusion where luminol reacts with small nanoparticles leading to quenching was proposed by Duan.<sup>13</sup> The larger nanoparticles did not enhance the chemiluminescence more than the medium-sized nanoparticles, indicating that smaller nanoparticles enhanced chemiluminescence better for a given amount of surface area. This eliminated surface area as the lone important feature of nanoparticles for intensity. There are three plausible options of explaining the results that mid-sized nanoparticles had a more effective surface for enhancing chemiluminescence. The first explanation was that the peak absorbance of the

nanoparticle influences chemical processes involving emissions of light of similar wavelengths. Because medium nanoparticles absorbed light of 425 nm the best, they may also have interacted with the excited state transition of the 3-aminophthalate the best. This could be rationalized by surface plasmons contributing electrical fields that impacted the relaxation time for a molecule in the excited state, such as the 3-aminophthalate in the luminol reaction. The second explanation was that larger nanoparticles lost the properties unique to nanoparticles and possessed properties closer to bulk metal. The last explanation was that the size of the nanoparticle could be key to most effectively catalyzing the reaction based on steric properties. Perhaps large nanoparticles and small nanoparticles are ineffective when binding to the luminol or its intermediates. To determine which of these is correct, further testing should be done with chemiluminescence reactions of different wavelengths to test the validity of the first explanation. If silver nanoparticles influence fluorescein, which chemiluminesces at a wavelength near 520 nm, with similar results based on size of the nanoparticles, the second or third explanations were most plausible.

### **Comparisons of small/medium/large nanoparticles using constant surface area**

Increasing the total surface area of the nanoparticles would increase the effect of chemiluminescence enhancement whether it was caused via electric fields or by binding luminol or ferricyanide to the nanoparticles. The importance of surface area highlighted a common error in previous studies where nanoparticles of varying concentrations were used. Varying concentrations led to the small nanoparticles having a much larger surface area than larger nanoparticles. Surface area variation complicated distinguishing between smaller nanoparticles causing greater enhancement or their larger relative surface area

being responsible for results. In Table 8, the data were taken from samples with constant surface area concentration. Calculations of surface area involved multiplying the number of nanoparticles in one milliliter by the surface area of a single nanoparticle. Sample calculations are shown in Appendix 1.

**Table 8: Average light emission intensity for constant surface area of nanoparticles**

Diameter of nanoparticle (nm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0	143.835		46.380	60.401	34.906
3					79.592
5			50.995		
10	119.424			96.458	
10				91.496	
20					33.453
20					
30			164.937		
35			<b>164.952</b>		82.807
40	<b>241.717</b>			<b>192.207</b>	
50			141.932	141.521	<b>483.949</b>
60	134.738		127.912		374.264

Table 8 is aligned with Table 7 in regard to trial numbers, which is why Trial 2 is left blank in Table 8. The nanoparticles used in Trial 1 in Table 8 were the same as used in Trial 1 in Table 7. Trial 2 was not completed due to insufficient amounts of nanoparticles synthesized. Table 8 displayed a very centralized set of peak enhancements in the range of 35-50 nm diameters. A slight drop in intensity occurred as nanoparticles became larger than 50 nm, but less of the quenching effects from smaller nanoparticles were seen compared to Table 7. The results from Table 8 showed that medium nanoparticles had the most effective surfaces for a given surface area. These results were

found in spite of significant dilutions to the smaller nanoparticles. Because it was not presently possible to synthesize medium nanoparticles at as high a concentration as the smaller nanoparticles, it is unknown how these results would compare if all of the nanoparticles were increased in concentration by a factor of 10 or 100 times.

**Comparisons of small/medium/large nanoparticles using constant concentration of silver mass**

Surface area and nanoparticle concentrations both relate to nanoparticles' influence on light-emitting processes. A constant silver concentration experiment was important because of the economics for larger industrial applications. Also this was a simple calculation that held less risk in errors from choosing a diameter of nanoparticle.

**Table 9: Average light emission intensity for constant concentration of silver metal**

Diameter of nanoparticle (nm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0		192.165	52.672	46.359	34.906
3					33.267
5		240.605	70.800		
10		275.424		<b>211.562</b>	
10				173.706	
20		252.988			75.747
20		<b>370.057</b>			
30			217.699		
35			<b>253.380</b>		102.314
40				180.465	
50			172.468	137.008	<b>396.040</b>
60		235.983	151.040		349.150

The trials in Table 9 do align with Tables 7 and 8. Trial 1 in Table 9 was not done due to limits on the amounts of nanoparticles synthesized, but was left in Table 9

for comparison purposes. Table 9 illustrated that medium nanoparticles were most efficient at enhancing chemiluminescence given constant amounts of silver. Slightly smaller nanoparticles showed an increase in their chemiluminescence intensities than the chemiluminescence data in Table 7 and Table 8. Only one instance of quenching effects was seen, and that was in Trial 5. The differences in dilutions were less apparent in these tests as the smaller nanoparticles were diluted least here. This change in the results validated concerns over previous papers' conclusions that did not consider factors of concentration of nanoparticles or surface area.

### **Discussion**

The results showed a difference in chemiluminescence intensity between the surfaces of small (3-15 nm), medium (15-40 nm), and large (40-100 nm) nanoparticles. The most effective at enhancing light processes were the nanoparticles around 35-50 nm in size. This was expected, as the smaller nanoparticles have less surface area for binding, limiting their contact. However, small nanoparticles did become better enhancers when they had a significantly larger number of nanoparticles. In fact, studies showed them to be the most effective at enhancing the chemiluminescence when no dilutions were performed, as shown in Figure 15. Experimentally, it is important to develop methods at reducing concentration as a variable by keeping the surface area or nanoparticle concentration constant.

Some quenching effects were observed for the very small nanoparticles, confirming Zhang's conclusions about gold nanoparticles. This research agrees that small nanoparticles are capable of reacting with the luminol without emitting light. This was upheld by data in Figure 16 where the chemiluminescence decreased as time passed

between mixing the luminol and nanoparticles before adding the ferricyanide. It also was visually evident that the color of the nanoparticles changed when small nanoparticles are mixed with luminol.

The amount of dilution necessary for some of these trials was large, with some samples being diluted 1000-10,000 times more than others. This dilution factor raised concerns over the variation within trials. The dilutions were necessary because of the larger concentration of smaller nanoparticles and also because of the vast number of nanoparticles in those samples. It would be beneficial in future studies to use nanoparticles within a smaller range such as 5 to 15 nm or 25 to 40 nm to limit the need for these large-scale dilutions. Methods of producing highly concentrated larger nanoparticles would be beneficial. Quenching frequently occurred in tests, although the concentration of nanoparticles had significant effects on quenching. The explanation for seeing quenching from dilute nanoparticles and enhancement from concentrated nanoparticles involved two competing processes. The nanoparticles reacted with the luminol, and the nanoparticles enhanced the luminol reaction with ferricyanide. In the dilute solutions, the non-chemiluminescence reaction dominated and reacted with the luminol, but when the nanoparticles were highly concentrated, the chemiluminescence is enhanced. The conclusion was that the best nanoparticle for enhancing the luminol-ferricyanide reaction was with a diameter near 40 nm. After 50 nm, the surface loses enough of its electron density to no longer enhance the chemiluminescence as well, and nanoparticles with diameters under 30 nm had too little surface area to enhance the chemiluminescence as effectively.

For constant surface area, the nanoparticles that were approximately 35-40 nm in

diameter produced the largest intensities with some variation. One study showed that a 50 nm nanoparticle sample functioned better than a sample of 40 nm diameter nanoparticles, but the trend was most peaks of chemiluminescence enhancement were from the nanoparticles with 35-40 nm diameters. If the quenching reaction could be eliminated, comparisons of small and medium nanoparticles would provide great insight into the interactions of the nanoparticles and luminol. Perhaps elimination of quenching could be accomplished by finding a selective protective attachment for the nanoparticle.

For constant concentration of silver used, the 30-35 nm nanoparticles were most effective at increasing the chemiluminescent intensity with one exception. Two sets of 20 and 30 nm nanoparticles showed very little enhancement and 50 nm nanoparticles enhanced greatly in trial 5 of Table 9, but the rest of the experiments showed that the medium nanoparticles were the best enhancers of chemiluminescence.

Throughout all three experiments, a range of 30-50 nm nanoparticles showed to be most effective at enhancing chemiluminescence. This may have been due to the large amount of dilution necessary for the smaller nanoparticles in this experiment. The data agree with the Zhang statement where the smaller nanoparticles reacted with the luminol in a competitive quenching, while the larger nanoparticles were less efficient.<sup>20</sup> The results disagreed with the results in Chen due to the fact that smaller nanoparticles were much more concentrated in that experiment.

It is important to specify what property of a nanoparticle was being tested. Many articles demonstrated differences in sizes of nanoparticles impacting various processes. Experiments should be developed that have methods which avoid many variables, such as using more concentrated nanoparticles for small nanoparticles and comparing their

effects with dilute large nanoparticles. Conclusions such as the competitive quenching effects were seen in this thesis but would have been missed otherwise. The calculations for determining ratios of surface area or number of nanoparticles should be done for all experiments comparing nanoparticle size in the future. The experiments should not be completed and then later have corrective factors applied to account for these differences, as the increase in chemiluminescence is not linear with concentration of the nanoparticles. The smaller nanoparticle samples are difficult to have made the number of nanoparticles constant or the amount of surface area constant because of their large concentrations and still obtain meaningful results. New synthesis methods might arise to mitigate this problem.

### **Luminol Reaction Mechanism**

A discussion of the luminol mechanism and the interactions of silver nanoparticles with this mechanism were presented in this section. The final step in the luminol chemiluminescence reaction converts the 3-aminophthalate from the excited state to the ground state by emitting a photon of wavelength 425 nm. Several steps were necessary to produce the excited 3-aminophthalate from the luminol as shown in Figure 18.<sup>22</sup>



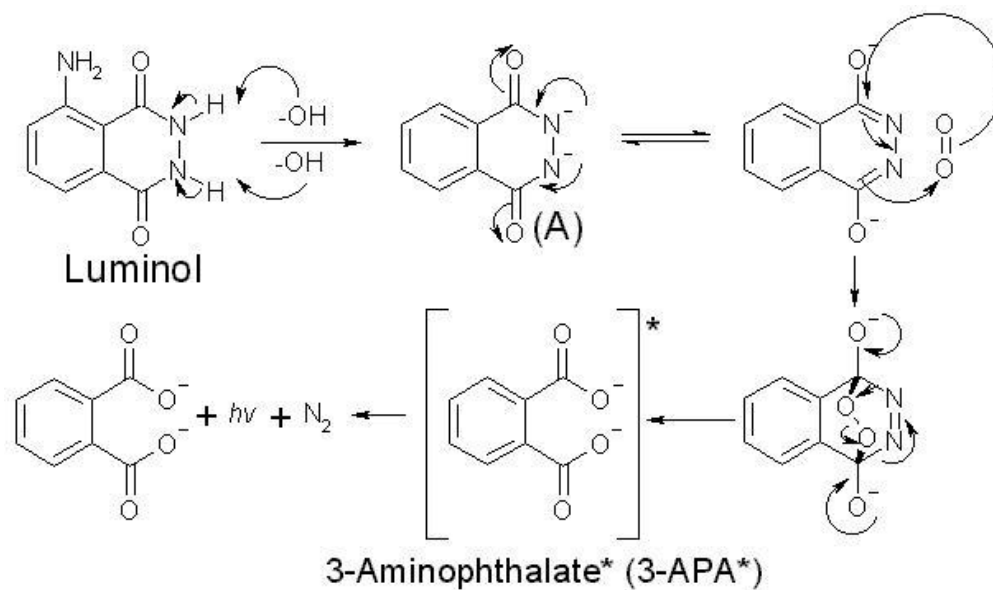


Figure 18: Mechanism of luminol

Zhang speculated gold nanoparticles catalyzed the reaction between the oxidant, hydrogen peroxide, and luminol intermediates. Gold nanoparticles cleaved the O-O bonds in the peroxides to form radicals stabilized by the electron density at the surface of the nanoparticle as shown in Figure 19.<sup>20</sup>

**Scheme 2. Possible Mechanism for the Luminol-H<sub>2</sub>O<sub>2</sub>-Gold Colloids CL System**

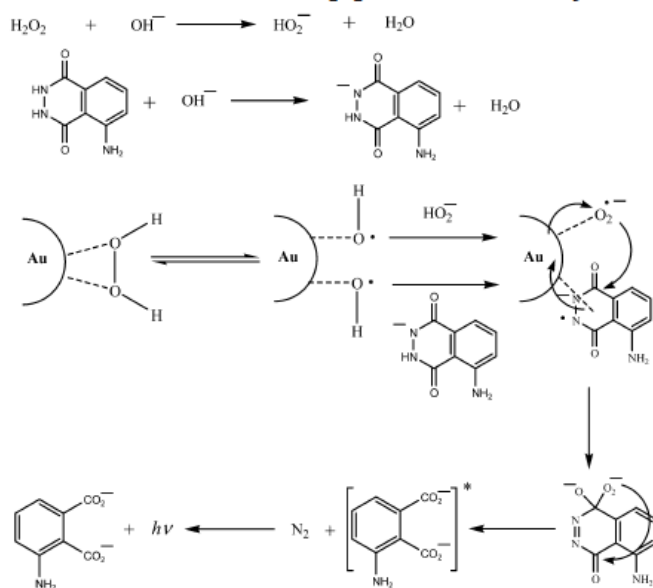
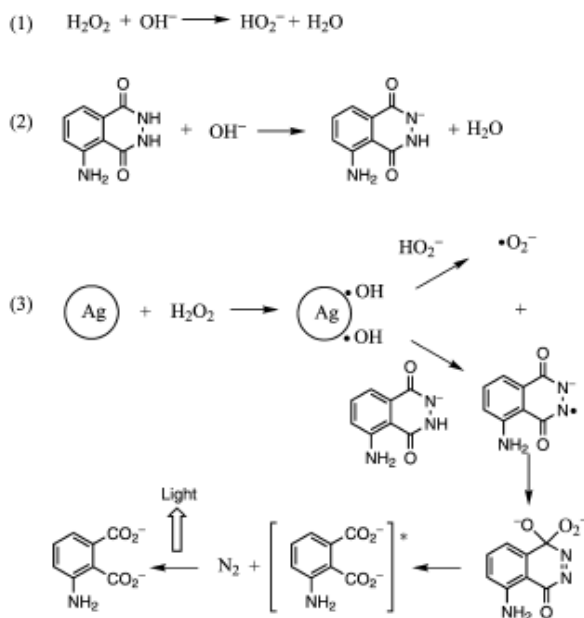


Figure 19: Scheme showing possible interactions of gold nanoparticles that catalyze the luminol reaction proposed by Zhang



Scheme 1. Possible mechanism of CL reaction catalyzed by AgNPs.

Figure 20: Suggested mechanism of luminol-peroxide in presence of silver nanoparticles, similar to Zhang mechanism, taken from Chen (2007)<sup>2</sup>

Zhang confirmed the nanoparticle as a catalyst by finding the nanoparticles intact before and after the reaction using X-ray photoelectron spectroscopy (XPS). However, no justification for cleaving the peroxide bond with the nanoparticle was presented, so an alternate mechanism was entirely plausible with a different role for the nanoparticle. Chen performed a test of adding molecules to the silver nanoparticles, which interfered with the catalysis as seen in Figure 20.<sup>2</sup> This mechanism was not confirmed, but only proposed that nanoparticles be close to an unknown reactant or intermediate. A future investigation of the reaction mechanism of this chemiluminescence reaction must occur to determine if the differences between luminol and ferricyanide allows for similar interactions to take place.

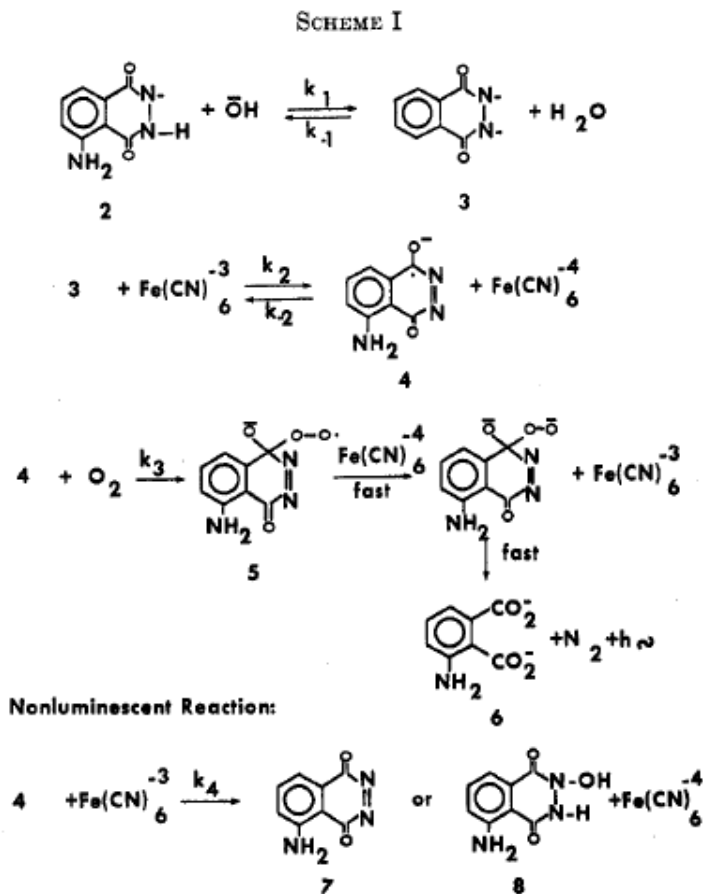


Figure 21: Scheme of luminol reacting with ferricyanide

The scheme in Figure 21 from Shevlin shows a mechanism similar to Chen's with a key difference in that the peroxide is cleaved forming two radicals, and the ferricyanide transfer an electron from the luminol to form 3-aminophthalate.<sup>3</sup>

The emission intensity graphs presented in Figure 14 show the curves with nanoparticles added approximately fit an exponential function. In Figure 20, the first step was already in equilibrium before the nanoparticles and ferricyanide were added to the luminol solution. In the second step, it would seem that the nanoparticle affects the

electron transfer from the luminol to the ferricyanide to make ferrocyanide, and this step likely accounted for the exponential decay curve. The last steps of the reaction are presumed to be too fast due to the instability of the radicals.

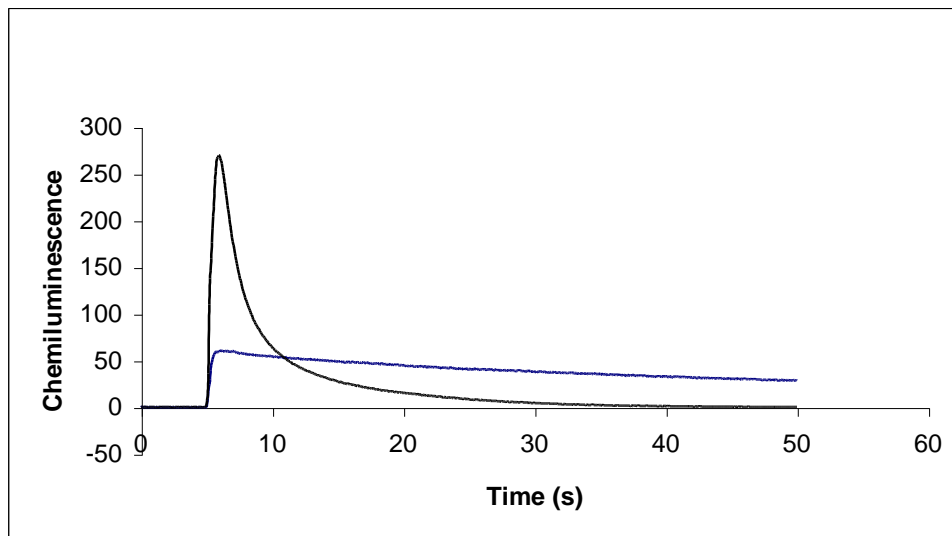


Figure 14: Chemiluminescence intensity for the reaction between luminol and ferricyanide. The quickly decaying curve in black has nanoparticles added and the slower decay curve in blue had distilled water added to the luminol and ferricyanide.

The silver nanoparticle interacts with luminol as evidenced by the decrease in chemiluminescence in Figures 16 and 17. It was possible the nanoparticle was attracting dissolved oxygen and supplied the oxygen to the luminol or the nanoparticle assisted in the electron transfer from the luminol to the ferricyanide to form the radical. There was no radical formation in this reaction similar to the radicals formed in the peroxide-luminol reaction. The luminol itself formed a radical, but the formation occurred in the peroxide-luminol reaction as well, without any support from the nanoparticle in the proposed mechanism from Zhang and Chen.

The similarity in size regardless of gold or silver nanoparticles supported the notion that steric effects played a key role in the nanoparticle interaction. If the nanoparticle's main role were to assist in the transfer of the oxygen molecule to luminol while displacing the nitrogen, the size of the nanoparticle would alter its effectiveness. Assuming the nanoparticle binds an oxygen molecule and the luminol radical, the surface curvature and available space could be crucial in providing the necessary distance for the oxidation of luminol to occur. This step was present in both the reaction with peroxide and ferricyanide. The small nanoparticles could be less effective at causing this transfer. At high concentrations their high surface energies might have overcome their poor efficiencies of catalyzation, and they would have sufficient nanoparticles to have two or more nanoparticles catalyzing the same luminol molecule in addition to more collisions. As the nanoparticle sizes increased beyond 50 nm, catalytic activity is lost because the larger nanoparticle becomes too large to assist in the transfers needed for reaction. Steric factors would explain the data in all three of the previous papers and this research. Three of the four papers came up with similar sizes of nanoparticles of 25 nm, 38 nm, and 30-50 nm for being most catalytic and the fourth used larger concentrations for smaller nanoparticles.

This research does raise questions as to whether the Zhang mechanism was correct. It does not disqualify the mechanism. However, similar results with gold and silver nanoparticles suggest that size plays a very key role. The nanoparticles appear to enhance the reaction in the same manner in both instances. The size dependency is also similar in that Zhang had the best results with medium nanoparticles and had some quenching effects with the small nanoparticles. The Chen results were not the same, but

the tests performed were different based on the parameters held constant. Chen states that the concentrations of nanoparticles were all different despite later showing that diluting a set of nanoparticles does affect the enhancement of chemiluminescence in a nonlinear fashion.

### **Educational aspects**

A high school experiment was developed that mimics the procedures and problems from this experiment. The experiment had the students synthesize the nanoparticles using the borohydride and silver nitrate method followed by two options of either determining the sizes of their nanoparticles using UV-Vis spectroscopy or performing the luminol-ferricyanide reaction with and without their nanoparticles being added to highlight the enhancement. The entire experiment handout can be found in Appendix 2.

The experiment includes a reading and writing portion that encourages high school students' exposure to scientific articles. The students were given a journal article by Chen, and guided questions are asked involving gathering information from the article. The guide aids the students in understanding the article that otherwise would be too difficult for students due to the complexities of the science. It is rare that a student will read anything in a chemistry classroom aside from a textbook, so this hopefully will be a great start to connecting scientific research to the high school educational experience.

### **High School Science Lab Benefits**

The benefits of doing this experiment in a high school setting were numerous, such as allowing students to work towards an actual lab problem that is not fully

developed and connecting students to a very current research topic in nanoparticles. Scientific writing and reading are introduced into the classroom, along with data analysis to interpret graphs and tables. Connections to spectroscopy and microscopy are probably introduced for the first time.

Presently, the experiment was done with one class. The physical lab experiment was completed, but shortage of time delayed the data analysis and writing component. The writing component, which had students answer questions about a scientific journal article, can be found in Appendix 3. A grant from the Hach Scientific Foundation was awarded for the 2010-2011 school year that will ensure multiple classes will perform the lab, analyze the data, and write summaries. Additionally, efforts will be made to distribute lab materials to other teachers in Michigan who are interested at the MSTA (Michigan Science Teachers Association) conference in February of 2011 and was presented to a group of local teachers at the SEMCTO (SouthEast Michigan Chemistry Teachers Organization) meeting in March 2010.

This particular experiment fits into the high school curriculum as multiple content areas are utilized such as redox chemistry, solutions, quantum chemistry, and stoichiometry. A list of Michigan state standards taught or assessed in this experiment can be found in Appendix 4. This project may set a trend of research being communicated to the secondary education level exposing high school students to research.

The goals of this experiment were to connect high-level students with real scientific research earlier so that it might influence them to pursue scientific careers. The experiment introduces literary skills of students by connecting between writing and



reading with science. The attention of students may be captivated by analyzing advanced research visually with graphs and charts that make connections with scientific research in chemiluminescence. Any success in this project will be disseminated to other teachers. Hopefully teachers will be encouraged to read journal articles and research that will positively impact their students. The success of this lab will be measured by keeping track of the number of students who go into scientific fields in college. The students who participate in this experiment will be encouraged to pursue science coursework, and their presence in AP and advanced courses will be noted and compared to other teachers. Results from informal or formal surveys given during meetings at SEMCTO or MSTA meetings and conferences may be used.

### **Future Studies**

This research reinforces the need to control variables of concentration of nanoparticles, surface area, and silver mass in an experiment. More research on these topics is warranted because of the inconsistent results from variations in size and concentration. Assumptions and background factors such as concentration may prove to be highly influential in the future and should be controlled in these chemiluminescence experiments.

Further studies in this lab include limiting the amount of oxygen supplied to the luminol reaction by purging solutions with nitrogen gas. More studies with constant surface area and the concentration of nanoparticles but different concentrations must be done to confirm results. More data should be released on synthesis methods, the ranges of diameters produced from these methods, and concentrations determined by molar absorptivity values. Studies similar to Zhang's study with molecules attached to the

nanoparticles and their effects on catalysis with different functional groups, different sizes of molecules, and different amounts of these molecules should be examined. A range of chemiluminescence reactions not exclusive to luminol should be examined experimentally. These reactions should all use various sizes of nanoparticles and compare the peak absorbance wavelengths of these nanoparticles to emission wavelengths to understand any relation to surface plasmon resonance effects. Nanoparticles could prove beneficial and data should be gathered about how they function, so that scientists might better understand their interactions with molecules and light.

## Thesis References

1. Rativa, Diego; Gomes, Anderson S. L.; Wachsmann-Hogiu, Sebastian; Farkas, Daniel L.; Araujo, Renato E.; Nonlinear Excitation of Tryptophan Emission Enhanced by Silver Nanoparticles *J Fluoresc* **2008** 18 pg. 1151-1155
2. Chen, Hao; Gao, Feng; He, Rong; Cui, Daxiang; Chemiluminescence of luminol catalyzed by silver nanoparticles *Journal of Colloid and Interface Science* **2007** 315 158-163
3. Shevlin, Philip B.; Neufeld, Harold A.; Mechanism of the Ferricyanide-Catalyzed Chemiluminescence of Luminol *J. Org. Chem.* **1970** 35 (7) pg. 2178-2182
4. Elechiguerra, Jose L.; Burt, Justin L.; Morones, Jose R.; Camacho-Bragado, Alejandra; Gao, Xiaoxia; Lara, Humberto H.; Yacaman, Miguel J.; Interaction of silver nanoparticles with HIV-1 *j. nanobiotechnol* **2005** 3 (6) pg. 1-10
5. Chang, Jeong H.; Kang, Ki H.; Choi, Jinsub; Jeong, Young K.; High efficiency protein separation with organosilane assembled silica coated magnetic nanoparticles *Superlattices and Microstructures* **2008** 44 pg. 442-8
6. Schalkhammer, Thomas; Metal Nano Clusters as Transducers for Bioaffinity Interactions *Monatshefte für chemie* **1998** 129 pg. 1067-92
7. Cai, Hong; Xu, Ying; Zhu, Ningning; He, Pingang; Fang, Yuzhi An electrochemical DNA hybridization detection assay based on a silver nanoparticle label *Analyst* **2002** 127 pg. 803-808
8. Solomon, Sally D.; Bahadory, Mozghan; Jeyarajasingam, Aravindan V.; Rutkowsky, Susan A.; Boritz, Charles; Mulfinger, Lorraine; Synthesis and Study of Silver Nanoparticles *J. Chem. Ed.* **2007** 84 (2) 322-325
9. Schneider, Siegfried; Halbig, Peter; Grau, Harald; Nickel, Ulrich; Reproducible Preparation of Silver Sols with Uniform Particle Size for Application in Surface-Enhanced Raman Spectroscopy *Photochemistry and Photobiology* **1994** 60 (6) 605-610
10. Pillai, Zeena S.; Kamat, Prahsnat V.; What Factors Control the Size and Shape of Silver Nanoparticles in the Citrate Ion Reduction Method? *J. Phys. Chem. B* **2004** 108 pg. 945-951
11. A. D. McFarland, C. L. Haynes, C. A. Mirkin, R. P. Van Duyne and H. A. Godwin, Color My Nanoworld *J. Chem. Educ* **2004** 81 544A  
<http://mrsec.wisc.edu/Edetc/nanolab/gold/index.html> accessed 7-23-2010
12. Jana, Nikhil R.; Gearheart, Latha; Murphy, Catherine J.; Seeding Growth for Size Control of 5-40 nm Diameter Gold Nanoparticles *Langmuir* **2001** 17 (22) 6782-6786
13. Duan, Chunfeng; Cui, Hua; Zhang, Zhifeng; Liu, Bo; Guo, Jizhao; Wang, Wei; Size-Dependent Inhibition and Enhancement by Gold Nanoparticles of Luminol-Ferricyanide Chemiluminescence *J. Phys. Chem. C* **2007** 111 4561-4566
14. Xu, Xiao-Hong Nancy; Huang, Shuang; Brownlow, William; Salaita, Khalid; Jeffers, Robert B.; Size and Temperature Dependence of Surface Plasmon Absorption of Gold Nanoparticles Induced by Tris(2,2'-bipyridine)ruthenium(II) *J. Phys. Chem. B* **2004** 108 15543-15551
15. <http://www.nanocomposix.com/knowledge-phys-props/optical-silver-sizes.html>  
Nanocomposix website used for comparing UV-Vis spectra of silver nanoparticles, accessed 7-22-2010

16. Gill, Harindarpal S.; Evaluating the efficacy of tryptophan fluorescence and absorbance as a selection tool for identifying protein crystals *Acta Cryst.* **2010** F66 pg. 364-372
17. Zaheer, Zoya; Malik, Maqsood A.; Al-Nowaiser, F. M.; Khan, Zaheer; Preparation of silver nanoparticles using tryptophan and its formation mechanism *Colloids and Surfaces B* **2010** 81 pg. 587-592
18. Glomm, Wilhelm R.; Moses, Selina J.; Brennaman, Matthew K.; Papnikolas, John M.; Franzen, Stefan; Detection of Adsorption of Ru(II) and Os(II) Polypyridyl complexes on gold and silver nanoparticles by single-photon counting emission measurements *J. Phys. Chem. B* **2005** 109 pg. 804-810
19. <http://www.lookchem.com/cas-143/14323-06-9.html> accessed 10-29-2010
20. Zhang, Zhi-Feng; Cui, Hua; Lai, Chun-Ze; Liu, Li-Juan; Gold Nanoparticle-Catalyzed Luminol Chemiluminescence and Its Analytical Applications *Anal. Chem.* **2005** 77 (10) 3324-3329
21. Tudos, Anna J.; Schasfoort, Richard B. M.; Handbook of Surface Plasmon Resonance **2008** RSC Publishing pg. 1-2
22. <http://www.chm.bris.ac.uk/webprojects2002/fleming/mechanism.htm> accessed on 7-11-2010

## Appendices

# Appendix 1

Enter		Enter		Enter	
m <sup>3</sup>					
Name of sample/batch	Volume of 1 np	Diameter of np in $\mu$ m	Size in m	Total grams of Ag	grams mass of 1 np
Batch 7 6-21	5.23333E-25	10	0.00000001	0.000346263	
Sam 6 6-22	3.34933E-23	40	0.00000004	0.000200953	5.48977E-18
TB large np 5-6-09 NaE	1.1304E-22	60	0.00000006	0.014921725	3.51345E-16
Batch 3 7-7-10	6.54167E-26	5	0.000000005	0.001012137	1.18579E-15
sam 1 7-7	5.23333E-25	10	0.00000001	0.000599727	6.86221E-19
sam 2 7-7	4.18667E-24	20	0.00000002	0.000496624	5.48977E-18
sam 3 7-7	4.18667E-24	20	0.00000002	0.000470849	4.39181E-17
					4.39181E-17
		(m <sup>2</sup> )		Enter	
# of np	# of np/mL	Total surface area	SA/mL	(ml)	Volume of solution g Ag/mL
6.30743E+13	3.50413E+12	0.019805319	0.001100296	18	1.92368E-05
5.71953E+11	42746889722	0.002873494	0.00021476	13.38	1.50189E-05
1.25838E+13	1.67784E+11	0.14224714	0.001896629	75	0.000198956
1.47494E+15	3.68736E+13	0.115783122	0.002894578	40	2.53034E-05
1.09245E+14	3.64148E+12	0.034302776	0.001143426	30	1.99909E-05
1.1308E+13	3.76932E+11	0.01420279	0.000473426	30	1.65541E-05
1.07211E+13	3.57368E+11	0.01346564	0.000448855	30	1.5695E-05

## Appendix 2

### Synthesis of Silver Nanoparticles (np) Lab Name \_\_\_\_\_

**Background** – A nanoparticle is a very small collection of atoms, typically sized between 5 and 100 nanometers. These nanoparticles only contain a small number of atoms and exhibit interesting properties. The particles interact differently with light than larger collections of atoms and are currently being tested as contrasting agents in medical imaging, detecting biomolecules, improving sunscreens and many other topics. Most nanoparticles are made of noble metals, or unreactive metals such as gold, silver and sometimes aluminum, copper or zinc. Most synthesis procedures start with an oxidized salt of the metal such as silver nitrate ( $\text{AgNO}_3$ ) or hydrogen tetrachloroaurate ( $\text{HAuCl}_4$ ) and then reduce the silver or gold in a controlled fashion. If large quantities of chemicals are used, the metal atoms will form large pieces of metal, but with small quantities under controlled conditions, the nanoparticles can be formed and often stabilized by having charged particles attracted to the surface of the nanoparticles.

#### **Materials** Distilled water

Silver nitrate 0.001 M

Sodium borohydride 0.002 M (must be kept in ice and made fresh)

Potassium ferricyanide (0.01 M)

Luminol (0.01 M)

0.3% polyvinyl alcohol solution\*

KOH/ethanol/distilled water solution\*

Stir bar/stir plate, 50 mL beaker, 50 mL graduated cylinder, 10 mL graduated cylinder, Pipettes (8)

**Safety** – Silver nitrate will stain skin and contact should be avoided throughout the experiment and caution taken while cleaning. Potassium hydroxide is caustic and should be handled carefully. Ethanol is highly flammable and should be kept away from oxidizers and ignition sources. Potassium ferricyanide is an oxidizing agent and sodium borohydride is a reducing agent. Wear goggles at all time and gloves are appropriate for use with silver nitrate solutions

#### **Part I – Ag nanoparticle synthesis procedure**

1. All glassware must be thoroughly cleaned with distilled water. Rinse glassware with a solution of ethanol (500 mL), distilled water (fill to 1 L) and potassium hydroxide (60 grams) and then rinse again with distilled water.
2. Measure 30 mL of the .002 M Sodium borohydride solution into the 50 mL graduated cylinder and pour into beaker
3. Measure 10 mL of the .001 M silver nitrate solution into the 10 mL graduated cylinder
4. Add stir bar to beaker and begin stirring the solution
5. Using the pipette, add the 10 mL of .001 M silver nitrate drop by drop into the beaker. The entire 10 mL should take 2-5 minutes to add. After a couple of milliliters a faint yellow color will appear, as you add the rest of the solution the yellow will become much brighter.

\*6. Now add 1 mL of the polyvinyl alcohol solution to stabilize the silver nanoparticles.

\*optional, may improve results but usually not necessary

## Part II – Identifying the size of the nanoparticles

Silver nanoparticles will absorb light between 380 and 440 nanometers. By identifying the peak wavelength we can approximate how large nanoparticles are. This is not completely accurate because the synthesis will produce a range of nanoparticle sizes, but it will be very close to the average size.

### Procedure

1. Turn on the spectrophotometer and allow to warm up for fifteen minutes
2. Set the wavelength to 350 nanometers for absorbance readings.
3. Insert a blank (distilled water) into the spectrophotometer and zero the spectrophotometer
4. Insert a sample of nanoparticles and obtain the absorbance
5. Repeat steps 2-4 for every 5 nanometers up to 450 nanometers
6. Once you have identified the peak wavelength, you can measure the absorbance every one nanometer up to five nanometers below and above this peak to obtain a more accurate measurement of the peak.
7. Use the chart below to determine the size of your nanoparticles

Particle size (nm)	Maximum absorbance wavelength (nm)	PWHM (nm)
10-14	395-405*	50-70
35-50	420	100-110
60-80	438	140-150

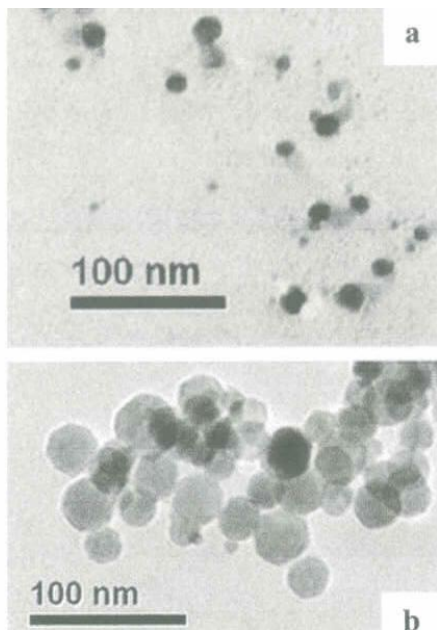
## Part III – Luminol ferricyanide catalysis

1. Obtain six pipettes and make sure to keep them with the appropriate solutions
2. Fill one pipette with luminol solution, one with potassium ferricyanide solution and the third with either the blank (distilled water) or the nanoparticles.
4. Inject all three liquids into the container simultaneously to observe the chemiluminescence reaction
5. Repeat the process this time changing the blank and nanoparticles to compare the reaction with and without nanoparticles.

### Follow up questions

1. How many total atoms of silver were made in this reaction assuming all of the silver nitrate is made into nanoparticles?
2. Based on the size of your nanoparticles, determine approximately how many nanoparticles you made.
3. From the reaction:  $2\text{AgNO}_3 + 2\text{NaBH}_4 \rightarrow \text{H}_2 + 2\text{Ag} + 2\text{NaNO}_3 + \text{B}_2\text{H}_6$  Identify the oxidizing agent and reducing agents.

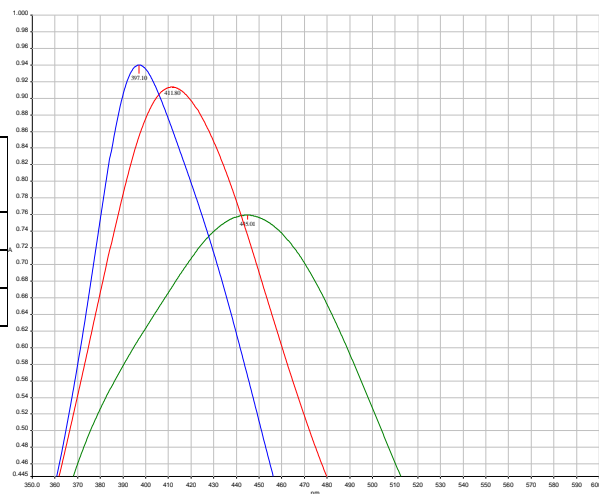




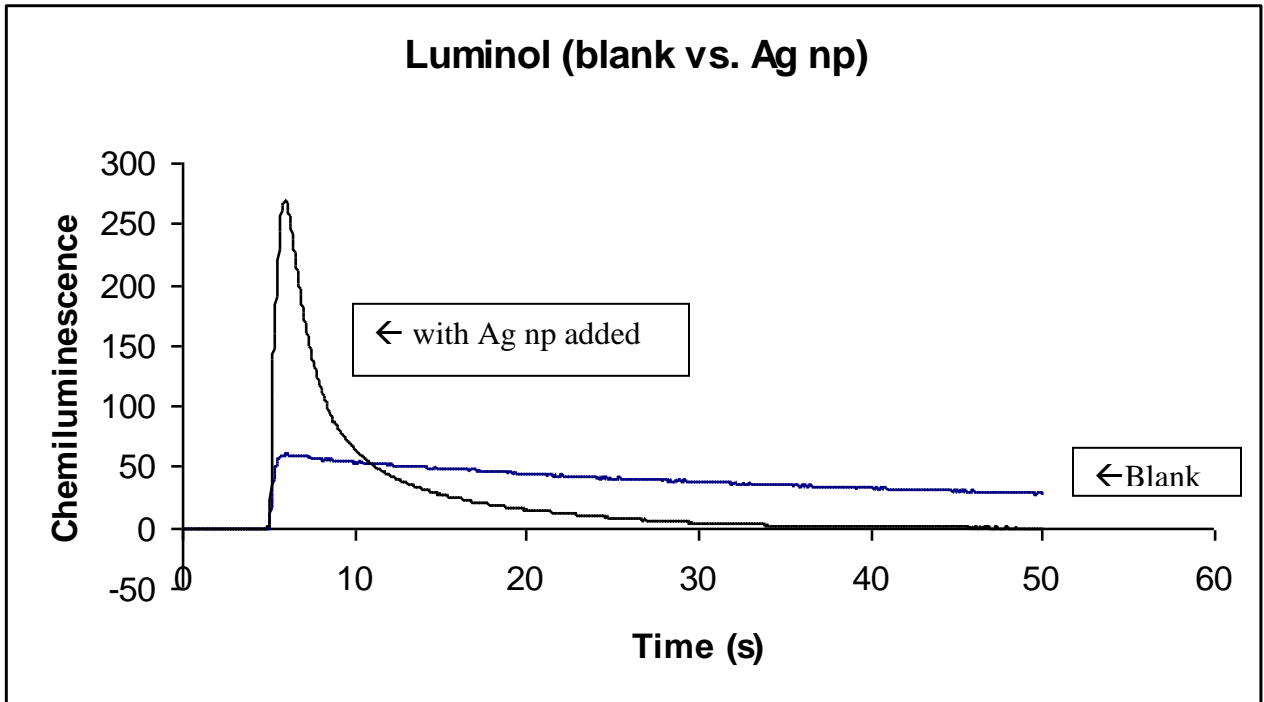
4. Above are images of two batches of silver nanoparticles, the ones you made are similar in size to the ones in figure a. Give a suggestion on how you might be able to make the nanoparticles in figure b instead. IE, what would you change about the procedure?

5. The figure below shows three types of silver nanoparticles, label them as small, medium and large, then determine the approximate size of each nanoparticle using the chart. What size were your nanoparticles?

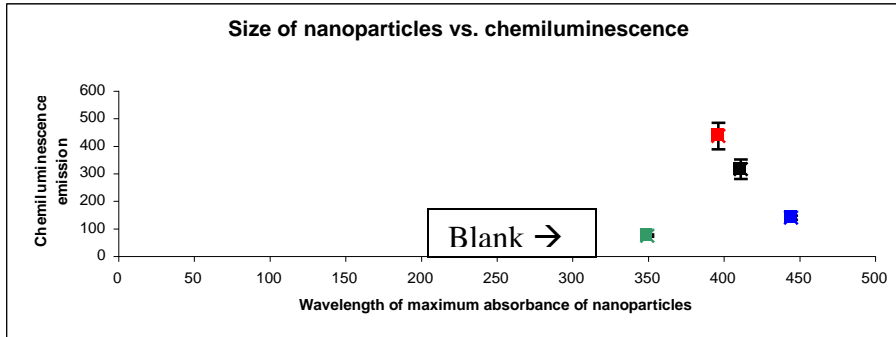
Max. abs. wavelength (nm)	Size of Ag np (nm)
395-405	10-14
420	35-50
438	60-80



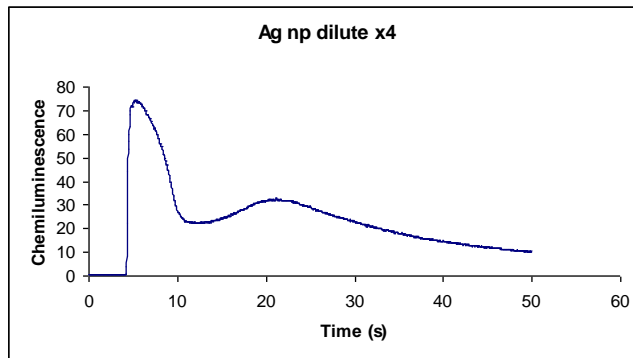
6. Draw a cartoon of the following data comparing the luminol reaction with and without Ag nanoparticles. Use the words very bright, bright, dim and negligible amount of light to describe the two reactions at times (6, 8, 15 and 30). Then describe underneath the images what is happening. <http://www.youtube.com/watch?v=UpuCs0tLHss> (Video of process)



7. On the following chart, label the nanoparticles as small, medium and large. Then describe the effect of changing the size of the nanoparticle has on the peak chemiluminescence value.



8. In one of the experiments, Mr. Milam obtained data that looked like this:



Explain what this would physically look like, or draw pictures. Also, there has been no explanation for what caused this, propose a theory that would explain this data.

Optional Teacher follow-ups:

Metal nanoparticles are a very current chemistry research topic that relates to many high school level chemistry contents. It is advisable that you show some of these during and after this lab.

Possible demonstrations –

1. The nanoparticles are really a colloid, and a laser pointer will easily show the Tyndall effect and prove that this is no longer a solution. The fact that they do not settle out over time shows that this is not a suspension. Shine a laser pointer through one of the student's solutions and ask what the light is reflecting off of? Why doesn't it reflect off of the ions in one of the solutions used to make nanoparticles?

2. Luminol is seen on the TV show CSI. When mixed with potassium ferricyanide it will emit a bright blue glow for a short period of time. Adding nanoparticles to this mixture influences the chemical reaction. Gold nanoparticles can greatly enhance the emission of light if they are of the appropriate size and also quench the light emission if they are very small (5 nm). Silver nanoparticles likewise can enhance or quench this process. They can also affect the time that the reaction lasts. In a darkened room, add 1 mL of .05 M luminol to two separate vials. In one vial add 1 mL of distilled water and in the other add 1 mL of the silver nanoparticles. Then add 1 mL of .01 M potassium ferricyanide and have the students make observations about the brightness and length of the chemiluminescence. Follow this up by passing out two graphs that show the light emission for the reaction with and without nanoparticles added. Have them identify which is which. (This is done in part III, but due to expense of luminol it can be done here as a demonstration)

3. <http://mrsec.wisc.edu/Edetc/nanolab/silver/index.html>

Turn nanoparticles into stained glass pieces using toaster oven

Disposal of chemicals

Silver nanoparticles – boiling or freezing will cause the nanoparticles to clump into larger particles. These particles can be disposed of properly, or recycled using nitric acid to form silver nitrate solution.

Silver nitrate – silver nitrate solution can be stored if it is protected from light. Use light-protected glassware or wrap colorless glassware completely in aluminum foil and store out of direct light. To dispose of silver nitrate solution, add excess sodium chloride, filter the precipitate and place in a cardboard box. Seal the box and dispose in a landfill approved for this particular waste. Be cautious with silver nitrate, as it will stain your skin black if contact is made. Wear gloves and exhibit caution.

**Appendix 3** reading/writing assignment

**Scientific writing analysis**      **Name** \_\_\_\_\_

Analysis of Chemiluminescence of luminol catalyzed by silver nanoparticles \_\_\_\_\_

Chen, Hao; Gao, Feng; He, Rong; Cui, Daxiang; Chemiluminescence of luminol catalyzed by silver nanoparticles *Journal of Colloid and Interface Science* **2007** 315 158-163

**Abstract** – read the abstract and answer the following questions:

1. What is the abbreviation used for silver nanoparticles?
2. What does CL mean?
3. What two chemicals are being reacted in this paper?
4. What is happening to the thioglycolic acid ( $\text{HSCH}_2\text{COOH}$ ) and BSA protein?

**Materials and Method** – read sections 2.2 through 2.4 and answer the following questions:

1. What is section 2.2 describing?
2. What is section 2.3 describing?
3. What is section 2.4 describing?

Figures 1, 4 and 5 – look at figures 1, 4 and 5 and answer the following questions:

1. What can be concluded about Ag nanoparticles and their effects on the luminol reaction with peroxide from these three figures? Specify where you get your information from.

Scheme 1 – Look at the proposed mechanism of reaction, read and answer the following:

1. What do the silver nanoparticles do, to make this reaction go faster?

2. Draw a reaction energy diagram that shows the reaction with and without the Ag nanoparticles added. Highlight the difference and make sure to label which is which.

Conclusions – Read the conclusions and answer the following:

1. What future studies could use the results of this experiment and expand upon them? You may include what the authors state, or something else that you think of.

2. Look back through the entire paper and find 3 words that you do not know what they are, and two abbreviations that you do not know what they are.

#### **Appendix 4 – Standards for silver nanoparticle lab (appendix 2)**

The following standards can be taught or assessed using this lab with slight or no modifications.

##### **Inquiry standards**

C1.1A – Generate new questions that can be investigated in the laboratory or field.

C1.1B – Evaluate the uncertainties or validity of scientific conclusions using an understanding of sources of measurement error, the challenges of controlling variables, accuracy of data analysis, logic of argument, logic of experimental design, and/or the dependence on underlying assumptions.

C1.1C – conduct scientific investigations using appropriate tools and techniques (e.g., selecting an instrument that measures the desired quantity-length, volume, weight, time interval, temperature-with the appropriate level of precision).

C1.1D – Identify patterns in data and relate them to theoretical models.

C1.1E – Describe a reason for a given conclusion using evidence from an investigation.

C1.1f – predict what would happen if the variables, methods, or timing of an investigation were changed.

C1.1i – Distinguish between scientific explanations that are regarded as current scientific consensus and the emerging questions that active researchers investigate.

##### **Enthalpy and Entropy**

C3.4e – Predict if a chemical reaction is spontaneous given the enthalpy and entropy changes for the reaction using Gibb's Free Energy.

##### **Elements, Compounds and Mixtures**

P4.p2C – Separate mixtures based on the differences in physical properties of the individual components

##### **Solids**

C4.3e – Predict whether the forces of attraction in a solid are primarily metallic, covalent, network covalent, or ionic based upon the elements' location on the periodic table.

C4.3h – Explain properties of various solids such as malleability, conductivity, and melting point in terms of the solid's structure and bonding.

##### **Periodic Table**

C4.9A – Identify elements with similar chemical and physical properties using the periodic table

C 4.9b – Identify metals, non-metals, and metalloids using the periodic table.

##### **Rates of Reactions**

C5.r1a – Predict how the rate of a chemical reaction will be influenced by changes in concentration, temperature and pressure.

C5.r1b – Explain how the rate of a reaction will depend on concentration, temperature, pressure and nature of reactant.

### Balancing equations

C5.2A – Balance simple chemical equations applying the conservation of matter.

C5.2B – Distinguish between chemical and physical changes in terms of the properties of the reactants and products.

C5.2e – Identify the limiting reagent when given the masses of more than one reactant.

C5.2g – Calculate the number of atoms present in a given mass of element.

### Reduction/Oxidation Reactions

C5.6a – Balance half-reactions and describe them as oxidations or reductions

### Notes

This experiment assesses the topics of data analysis, stoichiometry and redox chemistry. This experiment can be used in an AP chemistry course to displace the organic synthesis lab, or for the lab involving using a spectrophotometer.



**Appendix 5:** Explanation of calculations performed in Appendix 1.

When using the excel file to determine constant concentrations of nanoparticles, surface area or silver, there are always four pieces of information that need to be entered. The first is a name to keep track of the sample, the second is the diameter of the nanoparticle which is typically found using UV-Vis spectroscopy, scanning electron microscopy or is given by the company that synthesized the nanoparticles. The total mass of silver must be determined and it is noted here that this is different than the mass of silver nitrate. Lastly the total volume of solution must be entered.

Once these four items are put into the excel file everything else will automatically be calculated for you. The volume of the nanoparticle is calculated using the formula  $V = 1/6\pi(\text{diameter})^3$  and this is used to determine the mass of a single nanoparticle by assuming the packing of the atoms leads to the density of bulk silver (10.49 g/mL). The number of nanoparticles is then determined by dividing the total mass of silver used by the mass of silver in a single nanoparticle. This is divided by the volume of solution used so that different volumes of solutions can be compared. The total surface area of all of the nanoparticles is then calculated by using the formula  $\text{Surface Area} = \pi(\text{diameter})^2$  and multiplying the result by the total number of nanoparticles. The surface area of all nanoparticles is then divided by the volume of the solution. The last column is simply the mass of the silver used divided by the volume of solution.