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Determination of thiamine in solution by UV-visible spectrophotometry: The effect of interactions with gold nanoparticles

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Determination of thiamine in solution by UV-Visible spectrophotometry: the effect of interactions with gold nanoparticles

by

Michael Zielinski

Thesis

Submitted to the Department of Chemistry

Eastern Michigan University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

In

Chemistry

Thesis Committee: Timothy Brewer, PhD, Chair

Lawrence Kolopalo, PhD.

Jose Vites, PhD.

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Table of Contents

Acknowledgements	iv
Abstract	v
Introduction	1
Background and Theory	4
Nanoparticles	4
History.....	5
Properties of Metal Nanoparticles	6
<i>Surface Plasmon Resonance (SPR)</i>	6
<i>Gold Nanoparticles</i>	7
Characterization	8
Thiamine	8
Previous Studies.....	9
Experimental	11
Chemicals Used	11
Synthesis of Gold Nanoparticles.....	11
Preparation of Amino Acid/Gold Nanoparticle Solutions	12
Results and Discussion	13
Gold Nanoparticle Synthesis and Characterization	13
Amino Acids and Thiamine Comparisons	15
Estimation of a Detection Limit.....	20
Absorbance Decrease.....	22
Conclusion	25
References	26

List of Figures

Figure 1. Change in surface-to-volume ratio as the material size changes.	5
Figure 2. Surface plasmon resonance oscillation	7
Figure 3. Molecular structure of thiamine	8
Figure 4 Synthesized gold nanoparticle solutions	12
Figure 5. Absorbance spectra of gold nanoparticles	14
Figure 6. Absorbance spectra of tryptophan and arginine in the presence of gold nanoparticles	15
Figure 7. Absorbance spectra of thiamine and L-glutathione in the presence of gold nanoparticles	16
Figure 8. Structures of amino acids and thiamine	17
Figure 9. Absorbance spectra of a solutions of nanoparticles with thiamine at low concentrations.	20
Figure 10. Primary peak calibration of thiamine and gold nanoparticle complexes	21
Figure 11. Absorbance spectra of thiamine/nanoparticle absorbance decrease (4 μM of thiamine) with relatively higher nanoparticle concentration	22
Figure 12. Absorbance spectra of thiamine/nanoparticle absorbance decrease reproducibility test (4 μM of thiamine) with relatively lower nanoparticle concentration	24

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Abstract

The method presented here provides the foundation for a simple and selective qualitative determination of thiamine in solution. Gold nanoparticles in the presence of thiamine results in the formation of a secondary peak in the absorbance spectrum of the mixture. This peak can be used as an indicator of thiamine, which is useful for the qualitative analysis of solutions, and may provide an alternative to other methods for evaluating thiamine in blood and other biological systems. This method uses gold nanoparticles of a size around 20 to 30 nm and involves their selective interaction with thiamine, compared to selected amino acids. The interaction was measured using UV-VIS spectroscopy. The formation of secondary absorbance peaks was correlated to a change in the shape of the gold nanoparticles. A limit of detection was estimated and the relative selectivity of the method was evaluated. The main challenge in this project was coping with the absorbance decrease of the peaks of the solutions. Further studies are required to find the exact cause of this absorbance decrease. They can provide a further understanding of the usefulness of this method for thiamine detection in solution as well as other applications.

Introduction

Thiamine, also known as vitamin B1, is an essential part of the human diet. It is necessary to fight off diseases such as nephropathy and beriberi, and as such, the detection of thiamine in pharmaceuticals and within the body is an important undertaking. Many methods have been utilized for the detection of thiamine, one of them being UV-VIS spectrophotometry. It has been shown that this method provides a simple and selective way of detecting thiamine in the presence of other vitamins.¹ Gold nanoparticles have been known to affect absorbance spectra in unique ways, making them ideal candidates for improving the detection of compounds, like thiamine in mixtures. Gold nanoparticles influence the absorbance spectra due to the surface plasmon resonance (SPR) that occurs when the electron field around the nanoparticle oscillates due to the light energy being absorbed by the same field. This effect is influenced by the nanoparticle size and shape which will be explained in more detail further into this report. This means that changes in the SPR can be determined and therefore changes in the morphology of the nanoparticle can also be determined.

The use of gold nanoparticles for interaction with organic materials is a widely studied area in chemistry. Interactions between gold nanoparticles and compounds that absorb in the UV-VIS range cause the absorbance spectra to change, both through a shift in wavelength and formation of a secondary peak which can be measured in the UV-VIS region of the spectrum. Tris(2,2'-bipyridine)ruthenium(II) is one of the compounds used extensively as an optical chemical sensor due to its strong absorbance.² As such, mixing tris(2,2'-bipyridine)ruthenium(II) with nanoparticles is a commonly used way to observe and measure absorbance shifts. The presence or absence of various compounds can be determined by measuring the unique absorbance shifts that occur. These shifts are caused by changes in the

environment or by the binding of the nanoparticles to ligands in solution. Secondary peaks can also be observed when the nanoparticle undergoes a shape change. Different shapes and sizes of nanoparticles will give unique absorbance spectra, because each one has a different SPR resulting in different wavelengths being absorbed and scattered under the influence of a UV-VIS beam.

The unique properties of nanoparticles have caused much interest for their use in the detection of biological compounds. Binding of biological compounds to the nanoparticles results in changes to the nanoparticle's SPR, which can be detected by UV-VIS spectrophotometry. These changes can be unique from one compound to another, making the detection of a specific compound possible. This has been used extensively for the detection of different amino acids, such as arginine, in solution.³ Binding of an amino acid, such as arginine, to the surface of the nanoparticle causes an electric dipole to occur. Each nanoparticle with this dipole lines up with others to make longer "nanorods" which form a unique double peak on the absorbance spectra.³ The characteristics of these peaks can be correlated to the presence of a substance in the solution and used to determine its relative concentration.

It is important to know what compounds interact well with gold nanoparticles to determine what compounds can be detected through these interactions. Gold nanoparticles have incomplete valence on their surfaces, due to the fact that the surface atoms are only bound to the internal atoms.⁴ This means that the surface atoms can bind to electron acceptor/donor ligands, however the greater affinity lies in the identity of the acceptor or electron withdrawing species. These would include positively charged ligands, and highly coordinated sulfur and nitrogen groups. This also means that the intermolecular bonds that occur may vary depending on the species binding to the nanoparticles.

It has been observed in previous studies that sulfur heterocycles tend to have stronger interactions with gold.⁵ Thus, thiamine is assumed to act as a good “partner” for gold nanoparticles, due to the cyclic structure containing sulfur in thiamine. The interaction between gold and sulfur moieties can lead to the formation of non-spherical nanoparticle shapes, which can be measured.

While previous methods of thiamine detection using UV-VIS have been shown to be selective, they require detection within the UV range, because thiamine absorbs UV light.¹ Since nanoparticles absorb in the visible spectrum, they allow for an alternative method of detection. Nanoparticles can also allow for a lower limit of detection than previous methods due to their strong interaction with thiamine. This results in a greater likelihood of the formation of nanorods, making the detection of thiamine more likely. Studying this interaction can lead to a greater understanding of nanoparticle interactions with biomolecules. It should also be noted that a method for the detection of thiamine using gold nanoparticles has not been previously published.

In this project, a method of thiamine detection was developed by mixing an aqueous solution of thiamine with a gold nanoparticle solution. The gold nanoparticles were synthesized via a citrate reduction method used in previous studies.⁶ The changes in the absorbance spectrum were measured and the selectivity was estimated by performing the same method of determination on amino acid solutions. A limit of detection was also estimated. The purpose of this study is to determine the presence of thiamine in the test solution using gold nanoparticles as an indicator.

Background and Theory

Nanoparticles

Most applications in chemistry involve the use of materials in bulk quantities; however, there has been a growing interest in micro- and nano-scale applications. In bulk materials the crystal structure of the atoms within the substance are very consistent from one case to the next, and as such, the properties of the bulk material are very similar from one case to the next. However, when nanoscale structures are considered, the size range is outside that of bulk materials and of single atoms. This causes a large change in the properties of the material. Changing the properties of size and shape of the particles has a large influence on the various physical and chemical properties of the materials. One of the main reasons for this is due to the ratio of surface atoms to all atoms within a single structure. In bulk materials, this number is relatively small due to the large amount of atoms within a single structure. However, in nanoparticles this percent is larger, which causes a considerable change in properties, such as an increase in catalytic activity and more opportunities for interactions with other molecules. This means that the percentage of surface atoms in a structure is inversely proportional to the volume of the structure. The change in the surface to volume ratio in a structure of increasing volume can be seen in Figure 1.

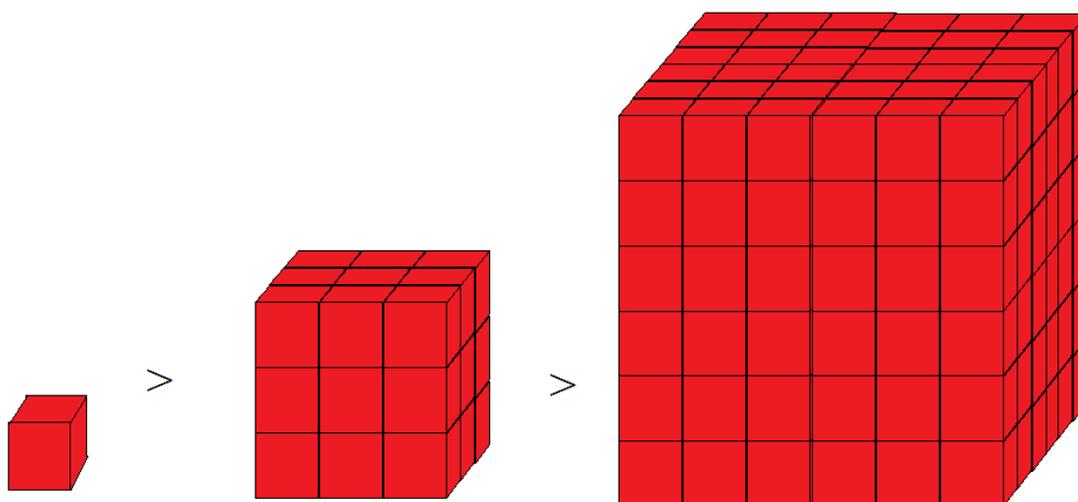


Figure 1. Change in surface-to-volume ratio as the material size changes.

Knowing this, one can argue that the nanoparticles will display an increase in chemical reactivity, as well as a lower melting point.⁷ Nanoparticles can be classified into multiple different categories, however, this research project only looks at metal nanoparticles, which have different properties from other nanoparticles, such as carbon nanotubes. The main difference between metal nanoparticles and other nanoparticles is the presence of surface plasmons, which refers to the electron cloud around the structure, that oscillate under the influence of an outside energy source.⁷ Nanoparticles have multiple applications in biomedicine, such as protein purification, drug delivery and medical imaging,⁸ as well as many properties in manufacturing, environment, and electronics.

History

There has been an increase in the use of nanoparticles in various biomedical and electronic fields, but they were originally used in ancient glass. The optical properties of metal nanoparticles cause them to visually display a wide range of colors, making them useful in many art forms. One of the earliest, and most popular, uses of nanoparticles in glass dates back to the

4th and 5th centuries B.C., in a piece known as the Lycurgus Cup. This piece has a ruby red color in transmitted light and a green color in reflected light.⁹

Nanoparticles were also used during the medieval period frequently in stained glass windows. Copper was used frequently for giving a red coloration¹⁰ and silver as well for providing a range of colors, mostly yellow.¹¹ Studies in the applications of nanoparticles for biomedical purposes started during the 1950s and 1960s when Peter Paul Speiser utilized nanoparticles as a drug delivery system.¹²

Properties of Metal Nanoparticles

Surface Plasmon Resonance (SPR)

The electrons at the surface of nanoparticles have unique properties when they are under the influence of incident radiation, usually light. When the particle size is less than that of the wavelength of the incident light, the electron cloud will oscillate, resulting in an enhancement in both the local and scattered fields around the nanoparticles.¹³ This oscillation is caused by an absorbance of the incident light as shown in Figure 2. This means that the light being transmitted through the nanoparticle solution has a specific wavelength. Thus, a different color of light will appear from the incident light. The SPR also causes some of the incident light to be scattered at a different wavelength than that which was initially introduced into the system. Both the transmitted and scattered light can give separated wavelengths, which is why sometimes the color of the solution changes depending on whether the reflected or transmitted light is being observed. In nanoparticles, the SPR is highly localized. This means that as the size of the nanoparticle changes, the wavelength of scattered light will also change. Different sizes will give specific wavelengths, which can be measured to determine nanoparticle size.

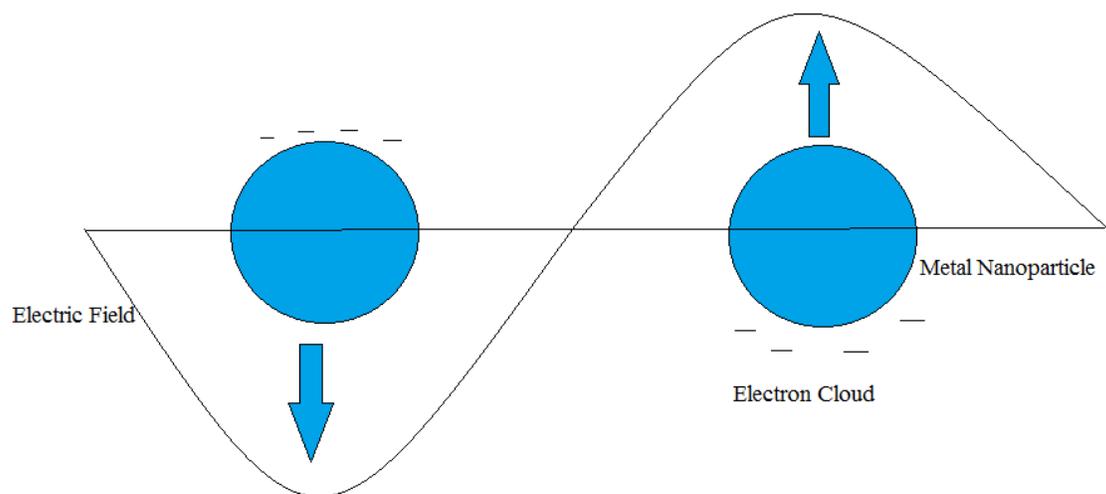


Figure 2. Surface plasmon resonance oscillation

Gold Nanoparticles

Like all metal nanoparticles, the differences in size of gold will influence the optical properties of colloid solutions. By looking at the absorbance spectra of different sizes of particles a correlation between the size and absorbed wavelength can be observed. As the size of the nanoparticles increases, the absorbed wavelength also increases (red-shift), causing the color to appear more blue-green. As the size decreases a blue-shift occurs and the solution appears redder.

Gold nanoparticles also have unique surface properties which allow them to be coated with polymers, small molecules, and biological recognition molecules.¹⁴ Gold nanoparticles also have a high affinity for triple bonding materials such as isocyanides or sulfur-containing compounds.^{15,16} This is due to the incomplete valence of the surface atoms and their affinity for electronegative species like sulfur.

Characterization

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are two of the more direct characterization techniques for nanoparticles. These methods have a high resolution and high imaging speed, making them ideal methods for direct imaging of micro and nanostructures.¹⁷ The direct imaging of nanoparticles is very useful for determining both the size and the shape of the structures. We were unable to perform TEM on our nanoparticle solutions, because we have no access to an instrument.

Another common characterization technique is the use of UV-VIS spectrophotometry. As stated earlier, the change in nanoparticle size causes a shift in the absorbance wavelength. The formation of shapes other than the conventional spheres can also be seen. A non-spherical shape can have multiple orientations within the field polarization, which will result in multiple different absorbance peaks on its UV-VIS spectrum.¹⁸ By observing the characteristic of these multiple peaks, any changes in the nanoparticle shape can be inferred.

Thiamine

Thiamine is a sulfur-containing vitamin more commonly known as vitamin B₁. It is an essential part of diet and is consumed for its neurological benefits.

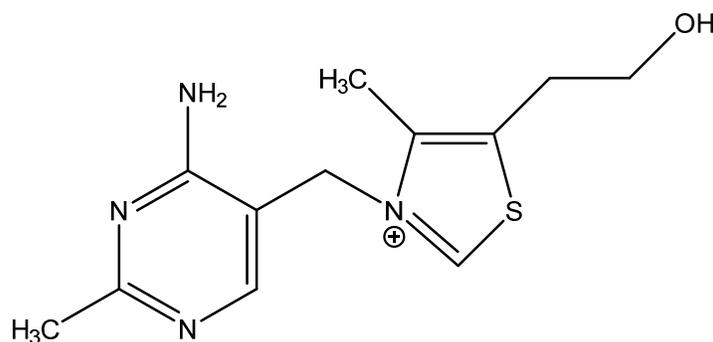


Figure 3. Molecular structure of thiamine

The detection of thiamine is essential due to the conditions which arise because of thiamine deficiency, which include nephropathy,¹⁹ Alzheimer's disease,²⁰ alcoholic brain disease,²¹ and beriberi.²² As shown in Figure 3, thiamine contains sulfur and nitrogen heterocycles. As stated earlier, previous studies have indicated a strong interaction between sulfur heterocycles and gold nanoparticles.⁵ This further supports the potential gold nanoparticles have for thiamine detection, due to this strong interaction.

Previous Studies

The use of gold and silver nanoparticles for the detection of thiamine specifically has not been previously studied. However, there have been similar uses of nanoparticles in detection as well as some published methods for thiamine detection without the use of nanoparticles.

Sehthi and Knecht studied the interaction of gold nanoparticles with amino acids. Time resolved UV-VIS spectrometry and TEM were used to determine the assembly of gold nanoparticles on amino acid chains under various temperature and solvent conditions. The idea behind this study was that as the gold nanoparticles interacted with arginine, an electric dipole occurs that allows the nanoparticles to align into their "rod-like" shapes, which can be seen on the nanoparticle's absorbance spectra. The study involved the use of UV-VIS, TEM, and dynamic light scattering (DLS) were employed as a function of the arginine concentration in solution, the temperature of the assemble process, the solvent dielectric and the solvent ionic strength. These methods were used to determine the reaction kinetics of the gold nanoparticle chain formation.³

A study was performed by Lanterna et al. which involved determining the degree of surface functionalization of gold nanoparticles by sulfur heterocyclic compounds using localized surface plasmon resonance spectroscopy (LSPR). The nanoparticles were functionalized by

three derivative thiones of various chain lengths. It was determined that as the nanoparticle diameter increased, the surface density of the molecules was constant or decreased slightly. Also, as the length of the molecules increased the surface coverage also increased. This is a good method for determining the degree of interaction between the sulfur-containing compound and the nanoparticle surface. However, this would be highly dependent on the concentrations of the nanoparticles and the thiones. The chemical interaction between the thiones and the nanoparticles was demonstrated using surface enhanced Raman scattering and ^1H NMR which gave evidence for LSPR broadening brought on by chemical interference dampening. The resulting complexes were highly stable.⁵

López-de-Alba et al. studied the use of UV-VIS spectrophotometry to determine the presence of multiple molecules within vitamins, one of them being thiamine. The method developed here was used to determine the presence of riboflavin, thiamine, Nicotinamide, and pyridoxine within multivitamin samples. The method required no separation or preconcentration steps. Each sample was measured to find optimum pH conditions. Absorbance spectra were obtained to determine how well the method could simultaneously determine the presence of each substance. Although the method wouldn't be applicable in quantitative determination of each substance, it is still a simple and reliable estimation of the compounds in solution.¹

Experimental

This project has two experimental parts. The first part involves the synthesis of gold and silver nanoparticles using a method that has yielded the best stability through experimentation. The second part of the experiment involves the mixing of the prepared nanoparticles with the amino acids or organic molecules followed by the generation of absorption spectra for the mixtures, observing any peak shifts of absorption changes.

Chemicals Used

Tetrachlorauric acid (HAuCl_4), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and reduced L-glutathione were purchased from Sigma-Aldrich, USA. L(+)-Arginine and L(-)-tryptophan were purchased from Acros Organics, USA. Thiamine HCl was purchased from Nutritional Biochemicals Corporation, USA. All chemicals were of analytical grade. Water was filtered to be ultra-pure with a conductivity less than $18 \mu\text{S}$.

Synthesis of Gold Nanoparticles

A 10.3 mM solution of HAuCl_4 was prepared by dissolving 1 g of the solid compound in 250 mL of ultra-pure water. This solution was then diluted to 1.03 mM before being used in the reaction. A 40.0 mM solution of sodium citrate dihydrate solution was prepared. This was then diluted to 20.0 mM and then to 10.0 mM. An aliquot of 6 mL of the 1.03 mM solution of HAuCl_4 was added to each of the three sodium citrate solutions. The 40.0, 20.0, and 10.0 mM sodium citrate mixtures were heated for 5, 15, and 30 minutes respectively. A deep red color was observed in each instance (Figure 4).



Figure 4. Synthesized gold nanoparticle solutions

Preparation of Amino Acid/Gold Nanoparticle Solutions

Mixtures of the prepared gold nanoparticles and various concentrations of arginine, tryptophan, L-glutathione, and thiamine were prepared in the ultra-pure water solvent. Each solution was prepared with 10% volume of the nanoparticle solution and 160 μM and 120 μM of each amino acid and thiamine tested.

Further testing was done on thiamine samples with concentrations of 0.5, 1, 2, 3, and 4 μM . These samples were stirred until a color change was observed. Absorption spectra were obtained using a Perkin Elmer Lambda 25 UV/VIS Spectrometer, using the ultra-pure water as a blank.

Results and Discussion

Gold Nanoparticle Synthesis and Characterization

The gold nanoparticles were synthesized by reduction of tetrachloroauric acid using trisodium citrate as the reducing agent.



What occurred first in this reaction were the reduction of tetrachloroauric acid to AuCl and the oxidation of trisodium citrate to sodium acetate dicarboxylate (SADC) as shown in equation (1). The AuCl then undergoes a disproportionation reaction to form the metallic gold nanoparticles and AuCl₃ as shown in equation (2). In this reaction gold (III) chloride is the oxidizing agent and is reduced by the citrate ion, the reducing agent.

The stability of the nanoparticles depends on the concentration of the reducing agent used. The reducing agent will act as a stabilizing agent here, surrounding the nanoparticles and in order to prevent aggregation. The amount of reducing agent used was varied to obtain the ideal nanoparticle solution that won't readily undergo aggregation.

Concentrations of 40, 20, and 10 mM trisodium citrate were used as the reducing agent and the nanoparticle solutions made were evaluated one day after synthesis. If any precipitate formed at the bottom of the solution, it was concluded that the nanoparticles had aggregated. The solutions using 20 and 10 mM trisodium citrate stayed in solution for the day span. The 40 mM trisodium citrate solution began to show signs of aggregation after the one-day period, and was observed as a solid formed on the bottom of the sample's container. This is the sign of an unstable nanoparticle solution.

The higher stability of the lower concentrations of the stabilizing agent could result from less competition for interaction of the agent on the surface of the gold nanoparticle. Larger concentrations would result in more competition for the surface binding, resulting in less overall surface coverage. This leads to a higher probability that the nanoparticle will bind with other nanoparticles in solution, increasing the chances for aggregation.

The sizes of the gold nanoparticles synthesized were determined by observing the absorbance spectra of the nanoparticles themselves. Figure 5 shows the absorbance spectra of four gold nanoparticle samples.

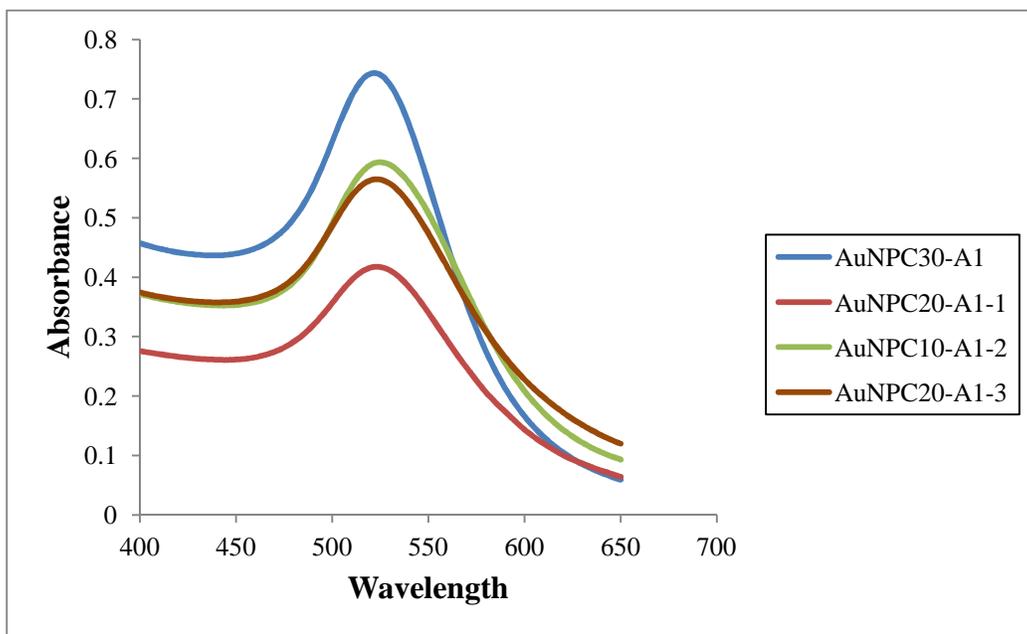


Figure 5. Absorbance spectra of gold nanoparticles. The labels for each nanoparticle indicate the amount of citrate solution used (c10 means 10 mM) and how much of the gold solution was used (A1 means 1 mM).

As can be seen, all of the nanoparticle solutions have peaks at around the same wavelengths, meaning all are about the same size. This shows the consistency of the method for obtaining a single size, but it also means that other methods would need to be employed to obtain

different sizes. The only difference in each sample would be the absorbance at each peak, indicating a difference in concentrations. By comparing Figure 5 to some reference spectra, the size of the nanoparticles can be estimated. The samples made are estimated to be within the 20 to 30 nm range. Another observation to be made is based on the fact that each spectrum only has one peak in the measured range. This means that the nanoparticles made are most likely spherical in shape.

Amino Acids and Thiamine Comparisons

Absorbance spectra were obtained for arginine, tryptophan, L-glutathione, and thiamine. Each biomolecule was measured independently in solution with the gold nanoparticles at 160 and 120 μM . The spectra obtained are shown in Figures 6 and 7.

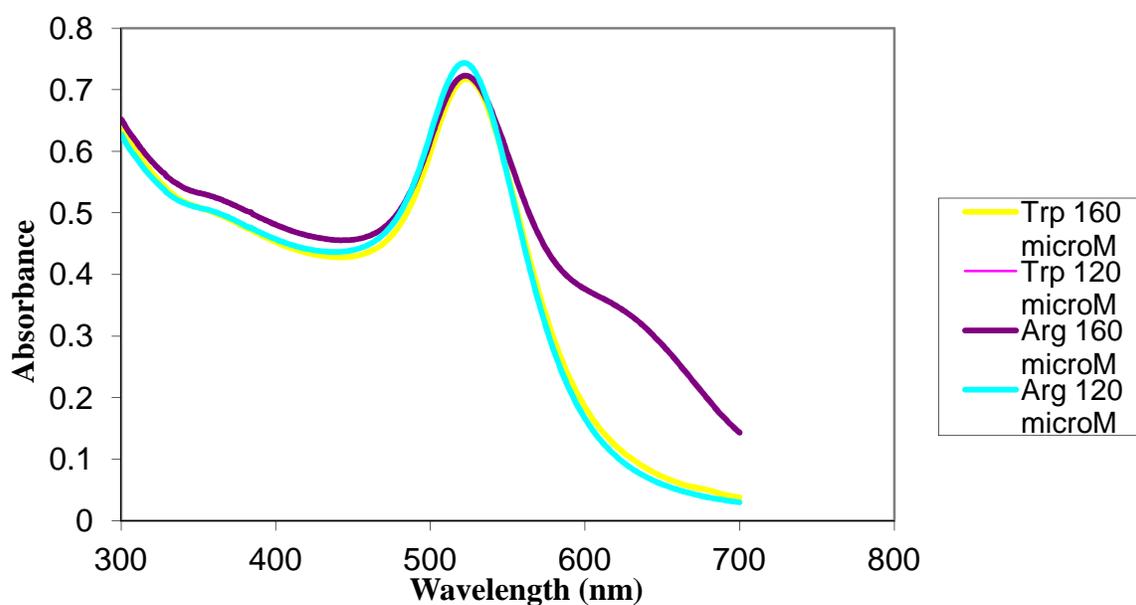


Figure 6. Absorbance spectra of tryptophan and arginine in the presence of gold nanoparticles (both tryptophan curves overlap)

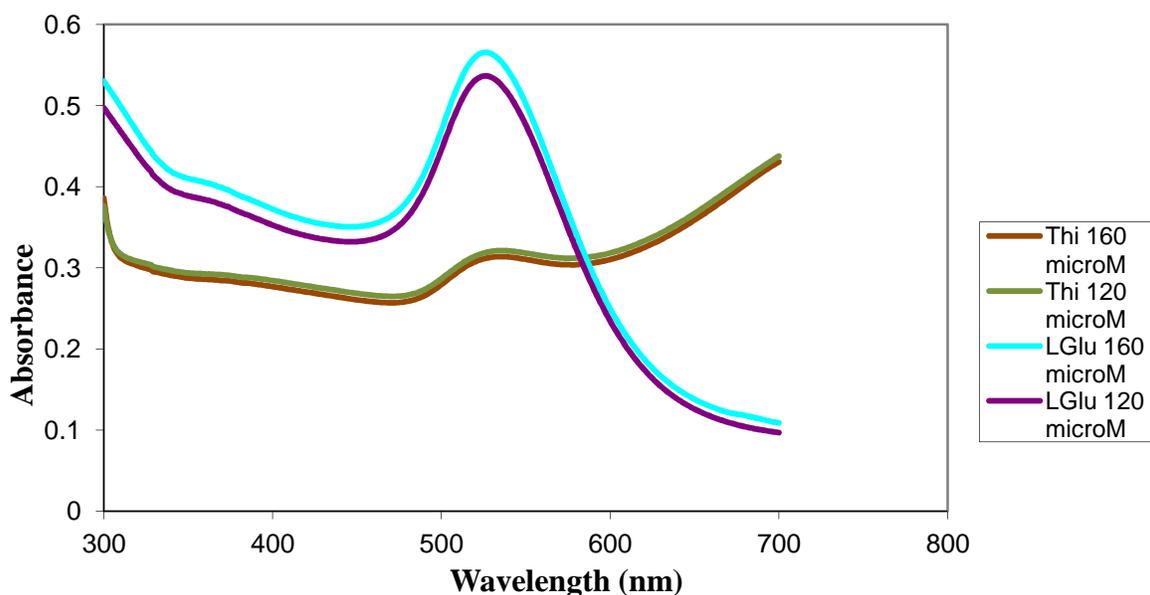


Figure 7. Absorbance spectra of thiamine and L-glutathione in the presence of gold nanoparticles

By comparing the spectrum of the gold nanoparticles alone to that of the gold nanoparticles in the presence of an amino acid or thiamine, one can determine changes from the interactions of the nanoparticles and the biomolecules. The spectra of the mixture of gold nanoparticles and tryptophan or L-glutathione contains the same peak of maximal absorbance as the gold nanoparticle absorbance peaks. This indicates very little, if any, changes in the surface plasmon of the gold nanoparticles, meaning very little shape changes. This means that neither of these two molecules interacted well with the gold nanoparticles. This may be due to the fact that the biomolecules and nanoparticles need to interact to form the dipole needed to form the rod shapes; if there is no indication of rod formation, we can assume that there is no interaction.

A peak broadening is observed at above 600 nm in the spectrum for the solution containing the nanoparticles and 160 μM arginine, indicating some change in the surface plasmon, and hence some change in the nanoparticle shape. However, for the 120 μM arginine solution, this effect disappears, indicating a possible concentration threshold that must be achieved to result in

stronger interaction. This result is promising for UV-VIS spectrophotometry as a method for evaluating the use of gold nanoparticles as an arginine detector; however, the result is not reproducible on further tests. The exact reason for this is unknown; however, previous studies have detected an interaction between gold nanoparticles and arginine,²² meaning this positive result in our experiment may not be as much of an outlier as it would appear to be. Further studies would need to be done to determine the factors that caused a peak broadening within this sample.

The last sample tested was thiamine. Figure 7 shows a very obvious change in the surface plasmon of the gold nanoparticles, indicating a possible shape change upon interaction with thiamine. This spectra not only shows a large decrease in the absorbance of the spherical nanoparticle spectral band (at about 525nm), but also shows a large increase of a secondary peak, which is forming outside of the range of the spectra. The drastic change in the thiamine peak makes it the ideal test compound for detection using the gold nanoparticles. Further testing was done on thiamine to estimate a possible detection limit as well as how the spectra changes over time.

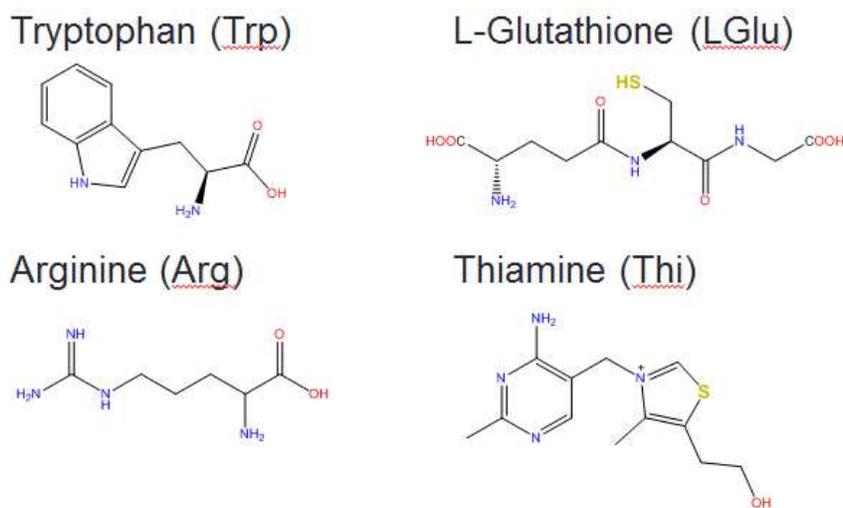


Figure 8. Structures of amino acids and thiamine

The structures for the compounds shown in Figure 8 must be considered to provide an explanation as to why some of the molecules have a greater affinity towards the gold nanoparticles than others. Thiamine contains the sulfur heterocycles which shows signs of high affinity towards the gold nanoparticles. L-glutathione also contains a sulfur group, although it has less affinity than the thiamine. This could most likely be due to a lower polarity in the structure of L-glutathione than in thiamine, meaning the sulfur in L-glutathione will less likely to form a bond with its electrons than the one in thiamine. Neither tryptophan nor arginine has a sulfur group for binding to the gold nanoparticles, however they do contain nitrogen groups, which could bind instead of the sulfur group, although not as well. The reason the arginine showed more signs of interaction with the nanoparticles than the tryptophan, however is not very clear. It may not be due to polarity in this case and more due to the number of sites that could bind to the nanoparticle. Arginine contains a larger number of nitrogen groups than the tryptophan, as well as containing nitrogen groups that are grouped closer together, which could result in more electron density. Another point to make is that thiamine has multiple nitrogen sites grouped closely together, which may provide another binding site for the nanoparticles.

Another aspect of the binding of each molecule to the gold nanoparticles to consider is the pK_a values of each molecule. The gold nanoparticles carry a negative surface charge due to its incomplete valence. This means that it will have a higher affinity toward compounds with a net positive charge. Therefore, species with higher pK_a on the amino and thiol groups will have a higher affinity towards the gold nanoparticles due to the likelihood of the amino and thiol groups being protonated, and therefore becoming more electron withdrawing. The pK_a values of the compounds used here are shown in Table 1.

Substrate	pKa			
Arginine	Carboxyl: 1.82	Amino: 8.99	Guanidino: 12.48	
L-Glutathione	Carboxyl: 2.12	Carboxyl: 3.59	Amino: 8.75	Thiol: 9.65
Tryptophan	Carboxyl: 2.46	Amino: 9.41		
Thiamine	Alcohol: 4.8	Amino: 9.2		

Table 1. pK_a values of substrates²³

A few things can be inferred by looking at Table 1. As stated above, the higher the pK_a value the more likely a protonation is, meaning there will be a greater affinity at that site for the negatively charged gold nanoparticles. It can be seen in arginine that the pK_a of the guanidino (=NH) group has a higher pK_a than any other substrate group. This would explain why the arginine has a stronger interaction seen in the single test where there was a slight broadening in the peak. It also means that the guanido group is more likely to bind to the gold nanoparticles than the amino group. In L-glutathione the thiol group is more likely to bind to the nanoparticles however, the pK_a is close to that of the amino group so there could be some coordination at that site as well. Tryptophan has a pK_a of 9.41 at its amino group so it too could coordinate with the nanoparticles. It is entirely possible, as stated earlier that these amino acids are not coordinating strongly with the nanoparticles at low concentrations. The more interesting observation to be made here is with the thiamine substrate. It seems that the interactions with the thiamine would be less on the amino group than those of the tryptophan, however, no data could be found on the pK_a at the sulfur heterocycles so nothing can be inferred by looking at the pK_a values for thiamine. However, the thiamine does contain a positively charged nitrogen within the heterocycles which could give a stronger interaction with the nanoparticles, especially if one of the groups on the thiamine becomes protonated, i. e. the amino or sulfur groups. This would explain why the thiamine has such a strong interaction, even at such small concentrations as were examined here.

Estimation of a Detection Limit

Spectra were obtained for μM concentrations of thiamine in solution with gold nanoparticles to estimate a possible minimum concentration of detection. The spectra are shown in Figure 9.

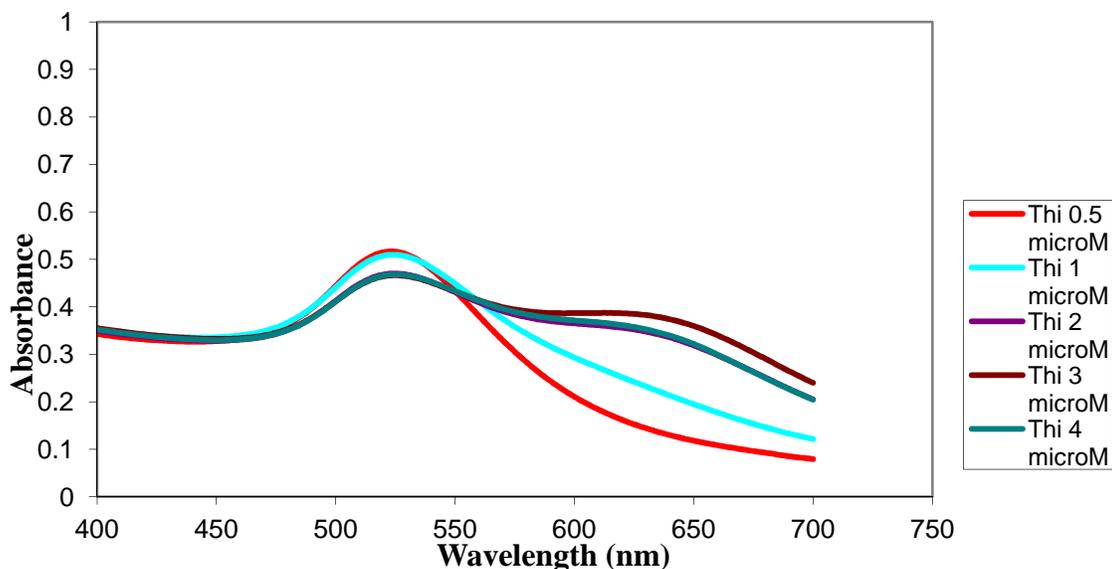


Figure 9. Absorbance spectra of a solution of nanoparticles with thiamine at low concentrations.

This is not a quantitative determination of a detection limit; however, it gives an idea of the concentrations where detection is plausible. As can be seen in Figure 9, concentrations of 4, 3, and 2 μM clearly show the secondary peak indicating a gold nanoparticle thiamine complex. For concentrations as low as 0.5 μM the secondary peak is nonexistent, and only the spherical nanoparticle peak is observed. The 1 μM thiamine solution shows a peak broadening in the spherical peak, indicating the formation of the secondary complex peak. This information shows that for this nanoparticle solution, detection limit of thiamine here could be between 0.5 and 1 μM .

In order for a shape change to occur in the gold nanoparticles, the surface area of the shape must be taken into account. It is possible that at really low concentrations of thiamine, there is little to no surface coverage of the nanoparticles, which results in no formation of an electric dipole and, hence, no rod formation. This means that the absorbance spectrum will only show a single peak, as indicated in Figure 9.

The relationship between the concentration of thiamine and the absorbance of the spectral band at 640 nm is shown in Figure 10.

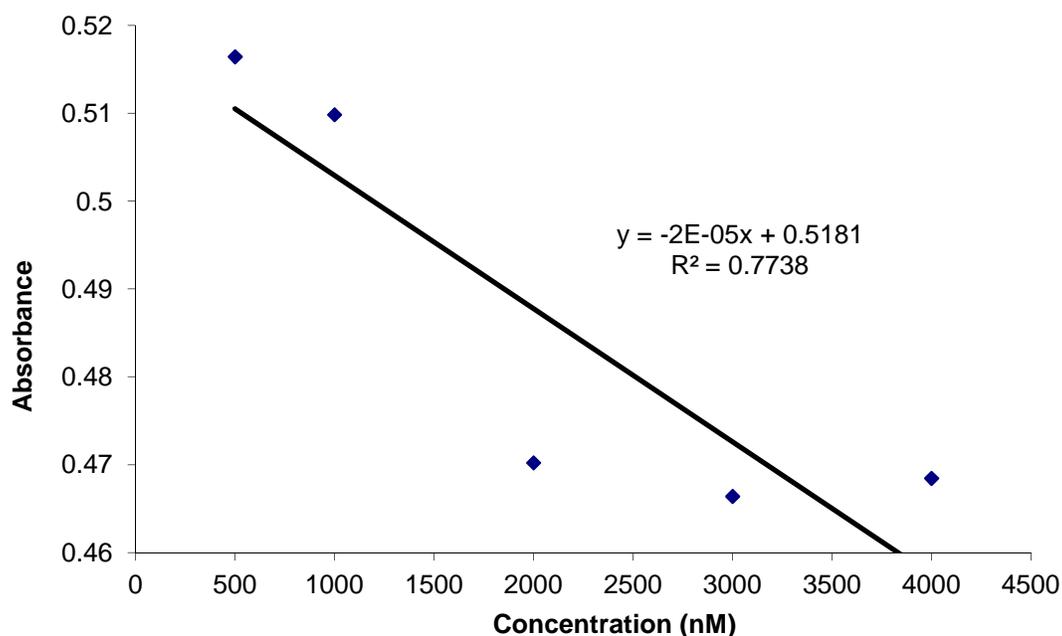


Figure 10. Primary peak calibration of thiamine and gold nanoparticle complexes

As can be seen in Figure 10 the correlation is not very high (0.7738). The main cause of this is most likely the 4000 nM point, it shows a slight increase in absorbance. This means that the rod formation decreased for the largest concentration. This could be due to the effect of competition for the binding sites on the nanoparticle with other thiamine molecules, however, at this low of a concentration it is not very likely. The most likely cause is that the 4000 nM thiamine solution

reacts so quickly with the gold nanoparticles that the absorbance decrease, which will be covered in the next section, is also sped up. This presents a challenge when measuring the spectrum of this complex in that the larger concentrations may still be undergoing reactions or may be aggregating while the spectra are being measured. It is due to this effect that construction of Beer's Law plots are not useful. It is also for this reason that an exact detection limit cannot be determined, it can only be estimated.

Absorbance Decrease

It was observed in each sample that, over time, the nanoparticles would aggregate and the samples would lose some of their color. This indicates a possible decay in the sample over time. Absorbance spectra were obtained at multiple times after the solution first shows signs of color change as observed in Figure 11.

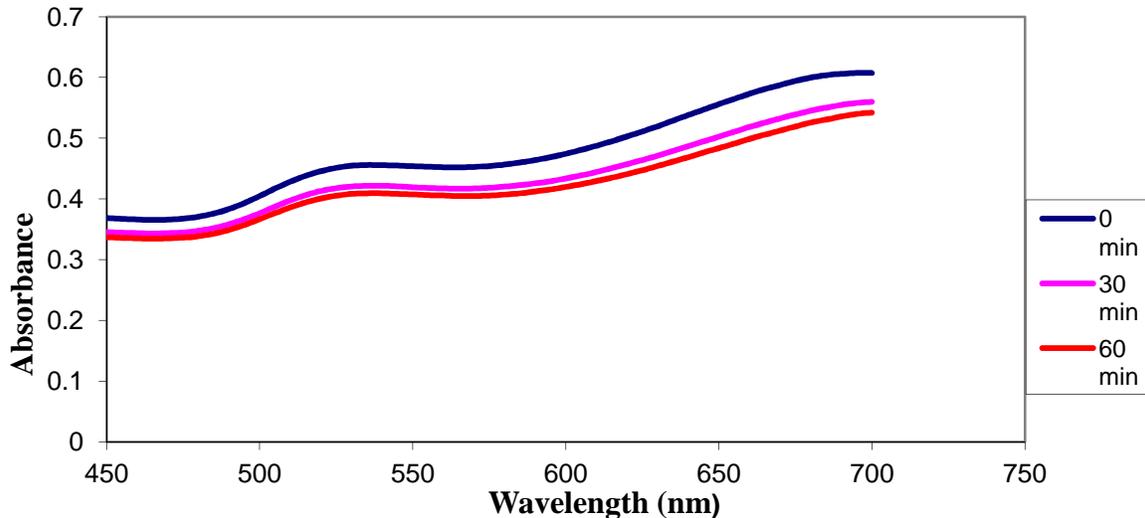


Figure 11. Absorbance spectra of thiamine/nanoparticle absorbance decrease ($4 \mu\text{M}$ of thiamine) with relatively higher nanoparticle concentration

Over a period of 60 minutes, the absorbance of each spectrum decreases fairly consistently. The absorbance decrease of the spectra could be caused by several things. There

could be some secondary interactions occurring between the thiamine nanoparticle complexes that causes aggregation. It is also possible that something in the solution is causing the nanoparticles to reoxidize, or there could be a change in the pH. It is not likely that the pH is a cause of this due to the very low concentrations of thiamine HCl used; the change is only very slight. Another possible reason for this could be an oxidation/reduction of thiamine itself. The re-oxidation of the nanoparticles is the most likely cause for the absorbance decrease here due to the appearance of a precipitate over time, which could be due to a number of things such as the nanoparticle aggregation. There could be any number of reasons why this absorbance decrease occurred and further research will need to be done to provide support for these hypotheses. The re-oxidation of the nanoparticles or the oxidation/reduction of thiamine can be determined by testing the redox capabilities of the system. Other studies can also be done by changing the solvent used and/or the reducing/stabilizing agent used.

The reproducibility of the absorbance decrease test was performed. The measured spectra are shown in Figure 12.

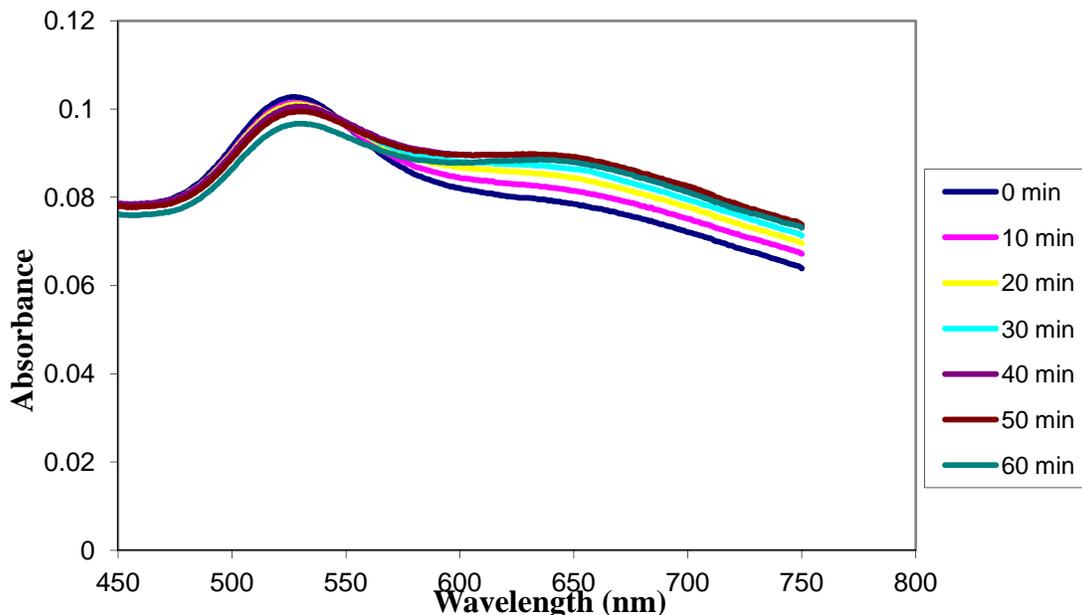


Figure 12. Absorbance spectra of thiamine/nanoparticle absorbance decrease reproducibility test (4 μM of thiamine) with relatively lower nanoparticle concentration

The decrease in absorbance observed in Figure 11 with the larger nanoparticle concentration is not observed at a lower nanoparticle concentration as shown in Figure 12. Instead, the spectra show an increase in the secondary peak and a decrease in the primary peak overtime, which is to be expected as the number of spherical nanoparticles decreases, while the number of non-spherical nanoparticles increase as the thiamine reacts with them. The absence of any sign of an absorbance decrease will require further testing. It is possible that the nanoparticle solution itself is responsible for any absorbance decrease, since, due to limited supply, multiple nanoparticle solutions were in fact used at various concentrations. This can be seen in Figure 12 by a lower absorbance intensity when compared to that observed in Figure 11. This means that there are a much lower concentrations of nanoparticles here, which may be the key to minimizing the absorbance decrease. This will have to be investigated in further detail in a future study.

Conclusion

In this project the use of gold nanoparticles as a means of detecting thiamine in solution was investigated. This was found to be a good method due to its relatively low detection limit and simplicity. The presence of thiamine could be detected visually with a color change in the nanoparticle solution from red to greenish-grey. The limit of detection was estimated to be between 0.5 and 1 μM thiamine. The gold nanoparticles were synthesized in the lab and the sizes were characterized to be in the range of 20 to 30 nm. We also observed absorbance decreases over time and proposed reasons for this. This absorbance decrease could be caused by an unseen reaction causing aggregation of the nanoparticles, or it could be the result of oxidation of the nanoparticles. There also seems to be a correlation between the concentration of the nanoparticle solution and the rate of the absorbance decrease. A more in-depth study into the absorbance decrease of the thiamine/nanoparticle spectra is necessary to determine the cause. Quantitative analysis should also be performed to see how well the method works at determining actual concentrations of thiamine. An actual limit of detection should also be determined quantitatively, rather than estimated as was done here. Overall, we believe this method has the potential to not only be used as a detection method for thiamine, but also to increase our understanding of nanoparticle interaction with biological molecules. This could lead to further advances in the field of medicine and biochemistry, by providing a foundation which can lead to the use of gold nanoparticles in actual disease diagnosis and treatment. This could also lead to further understanding of how nanoparticles interact with inorganic and organic molecules.

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