Gold Nanoparticles as Thiophenol Detection Probes

by
Emily Pond

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Bioengineering
(Honors Scholar)

Presented December 6, 2023
Commencement June 2024

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Vincent Remcho

The development of simple, rapid, and sensitive methods for thiophenol detection is of great importance in various fields, including environmental monitoring and food quality. Here, we present a novel approach for the colorimetric detection of thiophenols using gold nanoparticles (AuNPs) as the sensing platform. AuNPs aggregate in the presence of thiophenols. The aggregation of the AuNPs causes a bathochromic shift which is qualitatively detected by color change and quantitatively measured using UV-VIS spectroscopy. This AuNP-based colorimetric assay offers a simple and effective method for the detection of thiophenols and has the potential to be further developed for other applications in environmental and biological sensing.

Keywords: Gold nanoparticles, thiophenols, colorimetric assay

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Honors Baccalaureate of Science in Bioengineering project of Emily Pond presented on December 6, 2023.

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Table of Contents

List of Figures 1
Introduction 4
Materials and Experimental Methods 10
Reagents and Materials 10
Instruments 10
Gold Nanoparticle Synthesis 11
Beaker 11
Reflux and filtering method 12
AuNP and Thiophenol Assays 13
Varying Thiophenol Concentration Assay 13
AuNP and ppb Thiophenol Assay 14
Varying Thiophenol Concentration with different sized AuNP 15
Results and Discussion 17
Gold Nanoparticle Synthesis 17
Beaker 17
Reflux and Filtering AuNP Synthesis 18
AuNP and Thiophenol Assay 20
Varying Thiophenol Assay 20
AuNP and ppb Thiophenol Assay 27
Varying Thiophenol Concentration with Different Sized AuNP 28
Conclusions 35
Future Work 36
References: 37

List of Figures

Figure 1. Chemical structures of 3-methylbenzenethiol (m-toluenethiol), 4-methylbenzenethiol (p-toluenethiol), 2-methylbenzenethiol (o-toluenethiol), and 2-methoxybenzenethiol. The four main thiophenols (4-methylbenzenethiol being the most prominent) were selected as assay development targets for the work described in this thesis.....6

Figure 2. Photons hitting a gold nanoparticle, causing the AuNP's electrons to oscillate. [7].....7

Figure 3. Absorption spectra of dispersed and aggregated AuNPs. 8

Figure 4. SEM image depicting aggregated AuNPs and non-aggregated AuNPs. 8

Figure 5. Beaker method for synthesizing AuNPs. 11

Figure 6. Reflux AuNP synthesis set up. 13

Figure 7. Synthesis of gold nanoparticles. a) 20mL of 1mM HAuCl4 was brought to a boil. b) immediately following the addition of 3mL of 1% (w/w) sodium citrate solution. The combined solution became a deep blue color. c) after boiling, the solution turned a wine-red color within 1 minute. This is a solution of gold nanoparticles. 17

Figure 8. UV-Vis Spectrum of AuNP made on 5/16/22 with the beaker method. 18

Figure 9. Absorbance spectra of AuNP A, B, C, and D. 19

Figure 10. Thiophenols and AuNPs assay. The thiophenols from left to right are p-toluenethiol, o-toluenethiol, m-toluenethiol, and 2-methoxybenzenethiol. All thiophenols at all concentrations appear deep blue. 21

Figure 11. p-toluenethiol absorbance spectra. 5 ppm and 10 ppm are aggregated. 50 ppm and 100 ppm are partially aggregated. 22

Figure 12. 10 ppm and 5 ppm are aggregated, and 100 ppm and 50 ppm are slightly aggregated for m-toluenethiol. 23

Figure 13. AuNP and o-toluenethiol absorbance spectra. 5 and 10 ppm induced aggregation while 50 and 100 ppm induced only slight aggregation.....24

Figure 14. AuNP and 2-methobenzenethiol Absorbance Spectra, 5, 10, and 50 ppm are aggregated. 100 ppm is less aggregated. 25

Figure 15. The absorbance at 520 nm of the 4 thiophenols at varying concentrations. Lower absorbance than that of AuNP indicates aggregation or partial aggregation. 26

Figure 16. The absorbance at 680 nm of the 4 thiophenols at varying concentrations. Higher absorbance than that of AuNP indicates aggregation or partial aggregation. 26

Figure 17. Low concentration thiophenols and AuNPs assay. A mix of all 4 thiophenols were used. 1000 ppb and 500 ppb are deep blue, 250 ppb is purple, and the rest are a similar wine red as the control. 27

Figure 18. The UV-Vis absorbance spectra of the low concentration thiophenols and AuNPs assay. 1000 ppb and 500 ppb are aggregated, and 250 ppb is partially aggregated 28

Figure 19. AuNP A (13 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows B-F. Control AuNP A is in row A. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 100 ppm and 50 ppm are purple. 5 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original red. C) 20 minutes after thiophenol addition. Colors are similar to Figure 25 B. 29

Figure 20. AuNP B (16 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP B is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 100 ppm, 50 ppm, and 5 ppm are purple. 500 ppb is blue, indicating aggregation. 50 ppb is still the original red. 30

Figure 21. AuNP C (47 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP C is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 100 ppm and 50 ppm are purple. 5 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original red. C) 24 hours after thiophenol addition, all wells are aggregated. 30

Figure 22. AuNP D (81 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP D is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 50 ppm and 5 ppm are purple. 100 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original redish color. C) 24 hours after thiophenol addition, wells A, B, and D appear aggregated. 31

Figure 23. Absorbance of AuNP A (13 nm) 10 minutes after thiophenol addition. 32

Figure 24. Absorbance of AuNP B (16 nm)10 minutes after thiophenol addition 32

Figure 25. Absorbance of AuNP C (47 nm) 10 minutes after thiophenol addition. 32

FIGURE 26. ABSORBANCE OF AUNP D (81 NM) 10 MINUTES AFTER THIOPHENOL ADDITION.....33

FIGURE 27. ABSORBANCES AT 710 NM FOR EACH AUNP. BLUE INDICATES COMPLETE AGGREGATION, PURPLE INDICATES PARTIAL AGGREGATION, AND RED INDICATES NO AGGREGATION. 34

Introduction

Thiophenols are toxic chemicals harmful to the environment and to human health.[1,2,3] Interestingly, they are also partly responsible for the smoky, ashy flavor of smoke taint in wine.[4] Although it was initially thought that phenols were the sole contributors of smoky flavor to wine, Tomasino et al. recently reported in *Food Chemistry Advances* that thiophenols are also a contributor to the smoky, ashy flavor of smoke-tainted wine.[4]

Thiophenols are an organosulfur compound present in grapes after smoke exposure. When grape juice is made from smoke-exposed grapes, the characteristic ashy flavor from thiophenols is not present. Those ashy flavors emerge after the fermentation process, yielding smoky wine that is undesirable to consumers and difficult to market.[5] In recent years, this has cost the wine industry significant financial loss.[5]

Thiophenols can be analyzed using gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS), but these instruments are expensive and require expertise.[5] Fluorescent probes are an alternative tool for thiophenol detection. However, fluorescent probes require specific environmental conditions for their function, limiting their use. In addition, naked eye detection of fluorescence is challenging. These methods can have slow detection times and some fluorescent probes are toxic and dangerous to handle, making alternative approaches appealing.[3]

These issues necessitate an inexpensive and simple assay for the rapid detection of thiophenols in grapes. In this work, the chief research focus is on gold nanoparticles (AuNPs) as probes for thiophenols in solution. AuNPS have unique chemical and optical properties that enable their use in colorimetric detection.[6,7] In this work, thiophenol detection using AuNPs will be compared to detection using a fluorescent probe. This fluorescent probe, 2,4-dinitro-N-(7-nitrobenzo[c] [1,2,5] oxadiazol-4-yl) benzenesulfonamide, is unique in that it can preferentially detect aromatic thiols as opposed to aliphatic thiols.[8]

When evaluating detection methods, the application of smoke taint determination was the primary consideration, but the detection methods developed and described in this thesis could certainly be applied to other areas, such as environmental testing and monitoring.

Some thiophenols that present as significant components in smoke tainted wine include: 3-methylbenzenethiol (m-toluenethiol), 4-methylbenzenethiol (p-toluenethiol), 2-methylbenzenethiol (o-toluenethiol), and 2-methoxybenzenethiol (Figure 1).[4] Optimally, the proposed detection methods would be able to detect all four. These thiophenols are present in grapes in parts per trillion (ppt), thus, samples must be pre-concentrated to facilitate detection.

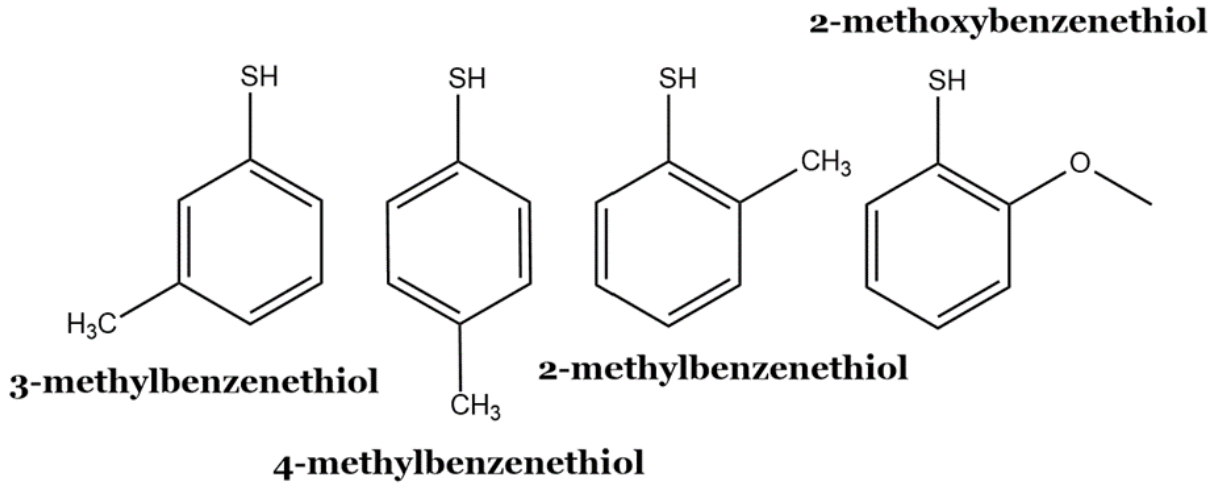

Figure 1. Chemical structures of 3-methylbenzenethiol (m-toluenethiol), 4-methylbenzenethiol (p-toluenethiol), 2-methylbenzenethiol (o-toluenethiol), and 2-methoxybenzenethiol. The four main thiophenols (4-methylbenzenethiol being the most prominent) were selected as assay development targets for the work described in this thesis.

Gold nanoparticles commonly employed in nanomaterials experiments range in diameter from 10 - 100 nm. AuNPs are used in many applications, ranging from drug delivery to sensing.[7, 10] AuNPs have unique properties related to conductivity, redox behavior, and surface plasmon resonance. [7] Specific to detection as envisioned for this thesis work is surface plasmon resonance. Surface plasmon resonance occurs when a photon hits a gold nanoparticle of appropriate size and stimulates oscillation of its electrons (Figure 2). To the naked eye, this appears as a red coloration of the solution, and in a spectrophotometer this manifests as a characteristic absorption peak in the range of 500 nm - 550 nm. The wavelength at which surface plasmon resonance presents is dependent on the shape and size of the AuNP.

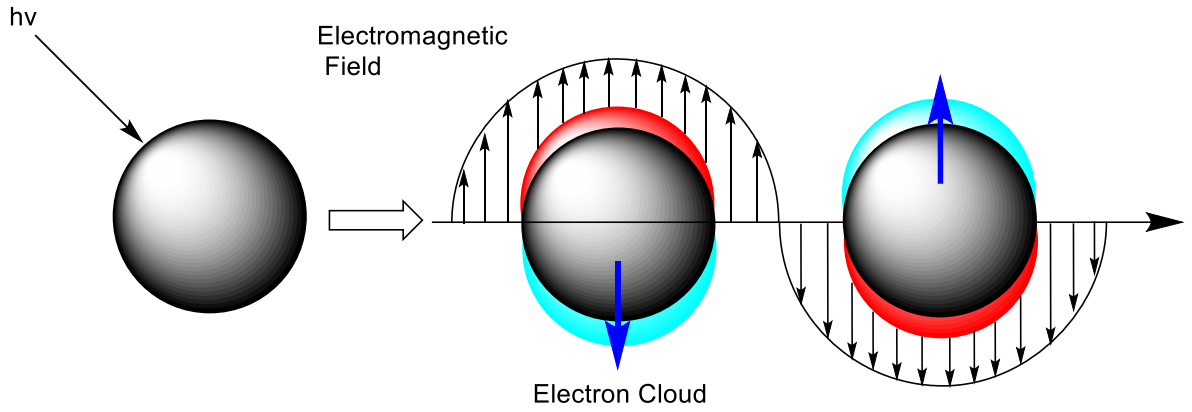

Figure 2. Photons hitting a gold nanoparticle, causing the AuNP's electrons to oscillate. [7]

AuNPs can be suspended in solution, giving the solution a red or blue color depending on the AuNP's aggregation state. [7] AuNPs aggregate in the presence of some chemicals and conditions, thiol-containing amino acid can aggregate AuNPs.[9] The aggregation of AuNPs results in a bathochromic shift, a shift from shorter to longer wavelengths (from blue to red), of the absorbance spectrum (Figure 3). This is represented in the absorbance spectrum by a decrease in absorbance at the red surface plasmon resonance peak. It was hypothesized that thiophenols will induce AuNP aggregation and different diameters of AuNPs may influence aggregation.

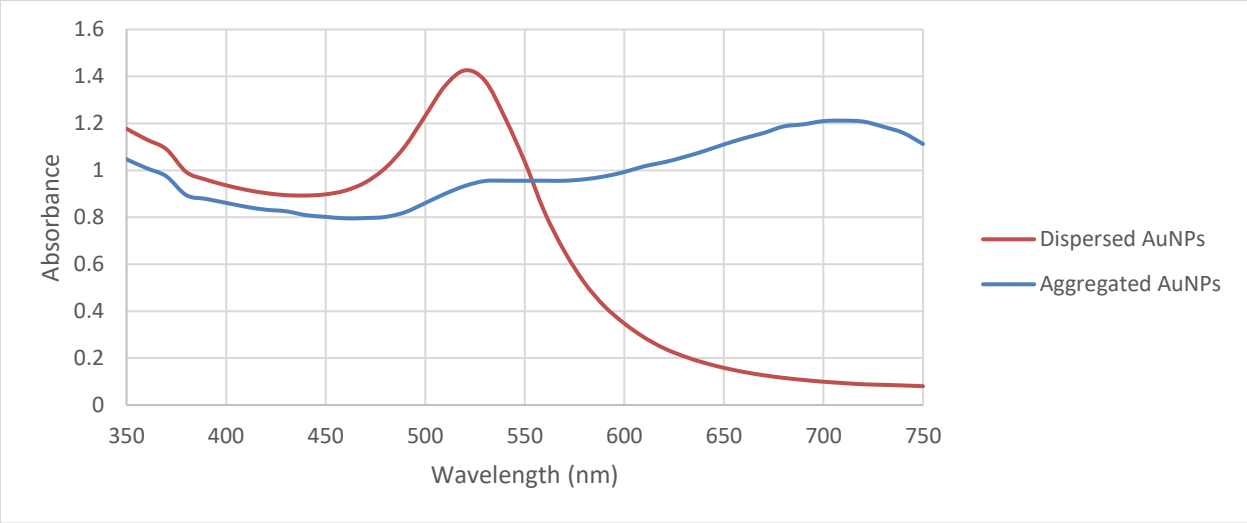

Figure 3. Absorption spectra of dispersed and aggregated AuNPs.

Aggregated AuNP are clustered together and dispersed AuNP are separate from each other (Figure 4).

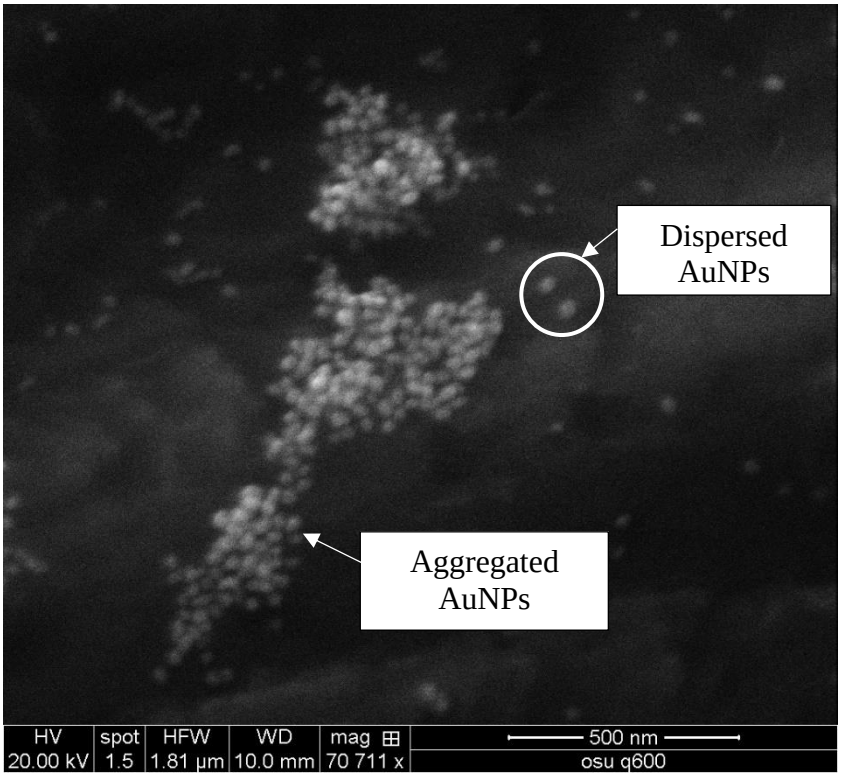

Figure 4. SEM image depicting aggregated AuNPs and non-aggregated AuNPs.

Although interactions between gold and thiols are well characterized, interactions between AuNPs and thiols are not well understood. Despite being the same chemically, the properties of gold nanoparticles (particularly steric repulsion) make the surface chemistry very different from a bulk gold surface. [11] On gold surfaces, thiols adsorb to the surface forming thiol-gold dative bonds that are not as strong as covalent bonds. [10] Au-S has a bond strength of 298 ± 2 kJ/mol; covalent bonds are much stronger with a range from 400 kJ/mol to 1000 kJ/mol. [10] Thiols can be used to cap gold nanoparticles, but specific steps must be taken, otherwise the thiols cause AuNP aggregation.[12] The mechanism for this aggregation has not been elucidated, although it's hypothesized that

aggregation is associated with a loss of surface charge which may be associated with thiophenols adsorbing onto the surface. [12]

Materials and Experimental Methods

Reagents and Materials

Chloroauric acid was purchased from Company (Location). Sodium citrate dihydrate was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). 2-methoxybenzenethiol, 4-methylbenzenethiol, m-Toluenethiol, and o-Toluenethiol were purchased from TCI (Portland, OR). Methanol was purchased from VWR Chemical (Radnor, PA). Hydrochloric acid was purchased from Fisher Scientific (Hampton, NH). Nitric acid and acetone were purchased from EMD Biosciences Inc. (Gibbstown, NJ). Filter paper cartridge (0.2um PTFE) was purchased from VWR Chemical (Radnor, PA).

Deionized water was obtained from a Milli-Q water purification system (Millipore Milli-Q Advantage A10) from EMD Millipore (Burlington, MA, USA) and used for all solutions unless otherwise specified. Chloroauric acid solution was prepared by diluting chloroauric acid to 10^{-2}% by weight. Citrate solution was prepared with 1% sodium citrate by weight. Stock solutions of 1000 ppm, 100 ppm, 5 ppm, and 1 ppm were made for each thiophenol in methanol. A 1000 ppm thiophenol mix solution was made with equal parts of m-toluenethiol, p-toluenethiol, o-toluenethiol, and 2-methoxybenzenethiol in methanol.

Instruments

Well-plates were read with a SpectraMax 190 Microplate Reader purchased from Molecular Devices (Sunnyvale, CA, USA). AuNP images were taken with FEI Quanta 600F Field Emission Environmental SEM purchased from FEI (Hillsboro, OR).

Gold Nanoparticle Synthesis

Beaker

Gold nanoparticles were synthesized using Turkevich and Fren's method.[13,14] 50mL of chloroauric acid solution was heated in a 100 mL or 150mL beaker with a stir bar on a hot plate until boiling with a watch glass over the top to prevent evaporation (Figure 5). All glassware was rinsed with aqua regia and then washed.

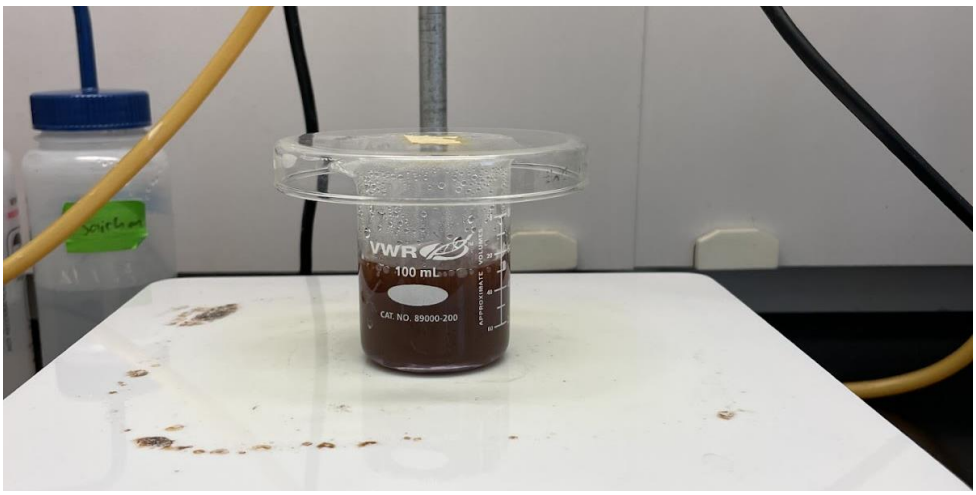

Figure 5. Beaker method for synthesizing AuNPs.

Citrate solution was added to the boiling chloroauric acid solution. Different volumes were added to make different diameters of AuNPs (Table 1).[13]

Table 1. Volume of sodium citrate solution added and its predicted effect on AuNP diameter.

Volume of sodium citrate solution (μL)	AuNP Diameter (nm)
1000	16
750	24.5
500	41.0

300	71.5
210	97.5
160	147.0

After the sodium citrate solution was added, the combined solution was boiled and stirred until the solution changed color from yellow to blue to wine red (or in the case of larger AuNPs, slightly purple). The bluish color after adding the sodium citrate solution indicates the formation of gold nuclei.[15] The solution's red color indicates the formation of nanoparticles. The AuNPs were stored in amber containers to protect the AuNPs from degradation due to light. They were stored in the refrigerator.

The diameters of the AuNPs were experimentally determined by measuring the absorbance at the surface plasmon resonance (A_{spr} the absorbance peak) with the spectrophotometer and then plugging A_{spr} into Equation 10 and 11 from Haiss's paper.[16]

Reflux and filtering method

Due to inconsistencies with the beaker method, the reflux method was used. The set-up is pictured below in Figure 6. All glassware was washed and then rinsed with aqua regia before use. A 500mL Florence flask was set on a hot plate with a stir bar inside. A glass adapter was put on top of the flask and then a condenser was put on top of that. The condenser had cold water flowing from bottom to top through it. This method does not allow vapor to escape during the boiling process, ideally giving more consistent results than the beaker method where vapor could escape.

Figure 6. Reflux AuNP synthesis set up.

The chloroauric acid solution was boiled and the sodium citrate solution was added as before. After the combined solution had boiled and changed color to a red solution, it was filtered with a 0.2 μm PTFE syringe filter into its storage container.

AuNP and Thiophenol Assays

Varying Thiophenol Concentration Assay

Assay performed with 4 different thiophenols (p-toluenethiol, o-toluenethiol, m-toluenethiol, and 2-methoxybenzenethiol) at concentrations of 100 ppm, 50 ppm, 10 ppm, and 5 ppm. The microwell plate loading scheme is shown in Table 2. AuNPs were also in each well.

Table 2. AuNP and varying thiophenol assay set-up. A, B, C, and D are rows and 1-4 are columns.

	1 p-toluenethiol	2 o-toluenethiol	3 m-toluenethiol	4 2-methoxybenzenethiol
A -100 ppm				
B - 50 ppm				
C - 10 ppm				
D - 5 ppm				

AuNP and ppb Thiophenol Assay

Assays were performed using an equimolar mix of the four target thiophenols (p-toluenethiol, o-toluenethiol, m-toluenethiol, and 2-methoxybenzenethiol) at total concentrations of 5ppb to 1000 ppb. The microwell plate loading scheme is shown in Table 3.

Table 3. Thiophenol assay format.

Microwell Plate Row	AuNP concentration, M	Thiophenol concentration, ppb
A	$7.5 * 10^{-9}$	1000
B	$7.5 * 10^{-9}$	500
C	$7.5 * 10^{-9}$	250
D	$7.5 * 10^{-9}$	100
E	$7.5 * 10^{-9}$	50
F	$7.5 * 10^{-9}$	10
G	$7.5 * 10^{-9}$	5
H	$7.5 * 10^{-9}$	0 (blank)

Varying Thiophenol Concentration with different sized AuNP

This assay analyzed the effect of thiophenol concentration on detection with AuNP. A solution of p-toluenethiol was used. Certain volumes of AuNP and thiophenol (Table 6) were put into microwell plates (Table 4 and Table 5) and then a UV-Vis spectrum was collected in the wavelength range of 350 nm – 750 nm, using a SpectraMax 190 Microplate Reader. The total volume put into each microwell was 200 μL.

Table 4. Loading Scheme for AuNP A Experiment

Microwell Plate Row	AuNP concentration, M	Thiophenol concentration
A	$1.17 * 10^{-9}$	0 (blank)
B	$1.17 * 10^{-9}$	100 ppm
C	$1.17 * 10^{-9}$	50 ppm
D	$1.17 * 10^{-9}$	5 ppm
E	$1.17 * 10^{-9}$	500 ppb
F	$1.17 * 10^{-9}$	50 ppb

Table 5. Loading Scheme for AuNP B, C, and D Experiments

Microwell Plate Row	AuNP B Concentration, M	AuNP C Concentration, M	AuNP D Concentration, M	Thiophenol concentration
A	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	100 ppm
B	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	50 ppm
C	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	5 ppm
D	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	500 ppb
E	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	50 ppb
H	$7.71 * 10^{-10}$	$2.12 * 10^{-11}$	$3.45 * 10^{-12}$	0 (blank)

Table 6. Volumes of constituents for AuNP and thiophenol assay

Microwell Contents		AuNP (μL)	1000 ppm p-toluenethiol (μL)	100 ppm p-toluenethiol (μL)	5 ppm p-toluenethiol (μL)	1 ppm p-toluenethiol (μL)	Methanol (μL)
AuNP A		125					75
AuNP A and 100 ppm thiophenol		125	20				55
AuNP A and 50 ppm thiophenol		125	10				65
AuNP A and 5 ppm thiophenol		125		10			65
AuNP A and 500 ppb thiophenol		125			20		55
AuNP A and 50 ppb thiophenol		125				10	65

Results and Discussion

Gold Nanoparticle Synthesis

Beaker

Gold nanoparticles were synthesized following the beaker synthesis method. Figure 7 shows images of the synthetic results for AuNPs that were produced on 5/16/22.

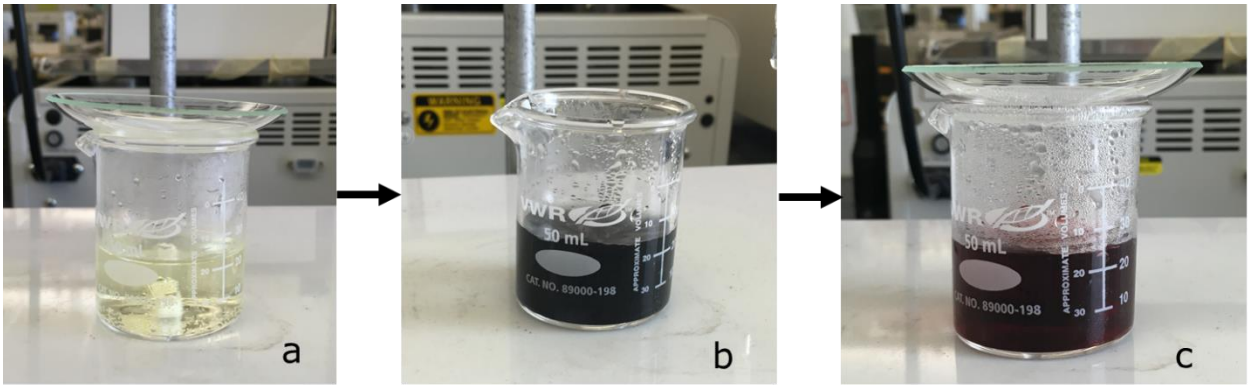

Figure 7. Synthesis of gold nanoparticles. a) 20mL of 1mM HAuCl4 was brought to a boil. b) immediately following the addition of 3mL of 1% (w/w) sodium citrate solution. The combined solution became a deep blue color. c) after boiling, the solution turned a wine-red color within 1 minute. This is a solution of gold nanoparticles.

The size of the AuNPs was determined by collecting a UV-Vis spectrum for each of the solutions (Figure 8), identifying the surface plasmon resonance (spr) peak (the maximum absorbance), and using this value for A_{spr} in Equation 10 or Equation 11 to yield the AuNP diameter. [16]

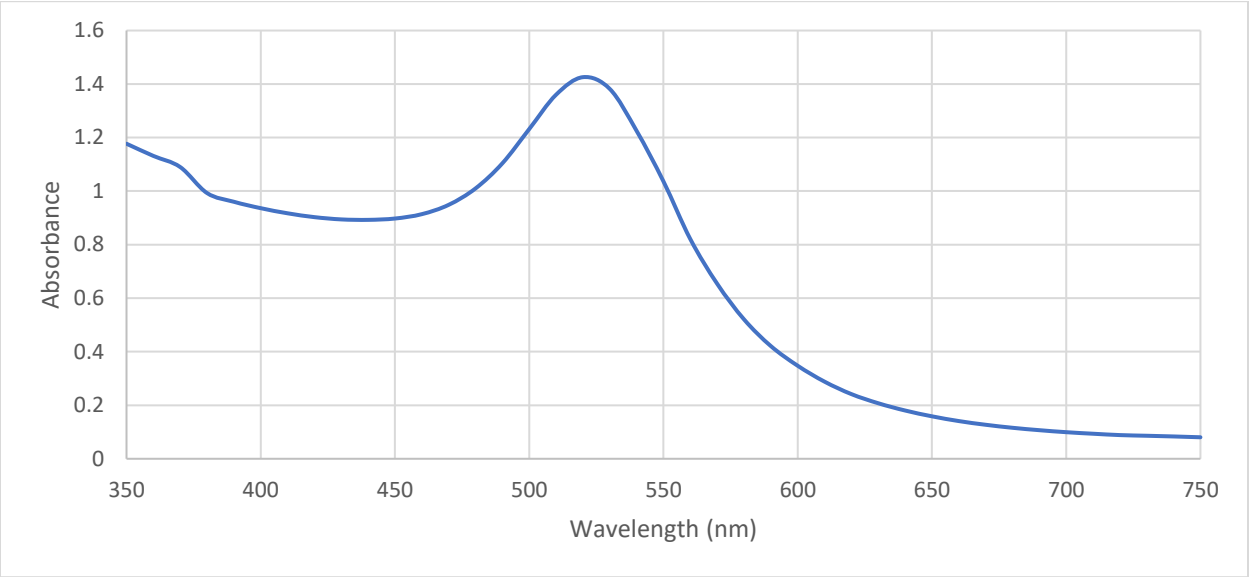

Figure 8. UV-Vis Spectrum of AuNP made on 5/16/22 with the beaker method.

The surface plasmon resonance peak occurs at 520 nm with 1.4257 absorbance. The absorbance at 450 nm is 0.8976. Using Equation 11 from Haiss, the diameter of the AuNP is about 13 nm. [16]

Reflux and Filtering AuNP Synthesis

Gold nanoparticles were synthesized following the reflux and filtering method mentioned above. Four different sized AuNPs were made by adding different volumes of sodium citrate solution (1000 µL, 750 µL, 500 µL, 300 µL) designed to make 16 nm, 24.5 nm, 41 nm, and 71.5nm AuNPs, respectively. The amount of citrate solution necessary to generate the various diameters of AuNPs was determined using the method described by Frens et al (Table 1). [14] After synthesis, a UV-Vis spectrum was collected in the wavelength range of 450 nm – 750 nm, using a SpectraMax 190 Microplate Reader for each of the four AuNP solutions, A, B, C, and D, which are designed to be 16 nm, 24.5 nm, 41 nm, and 71.5nm diameter, respectively (Figure 9).

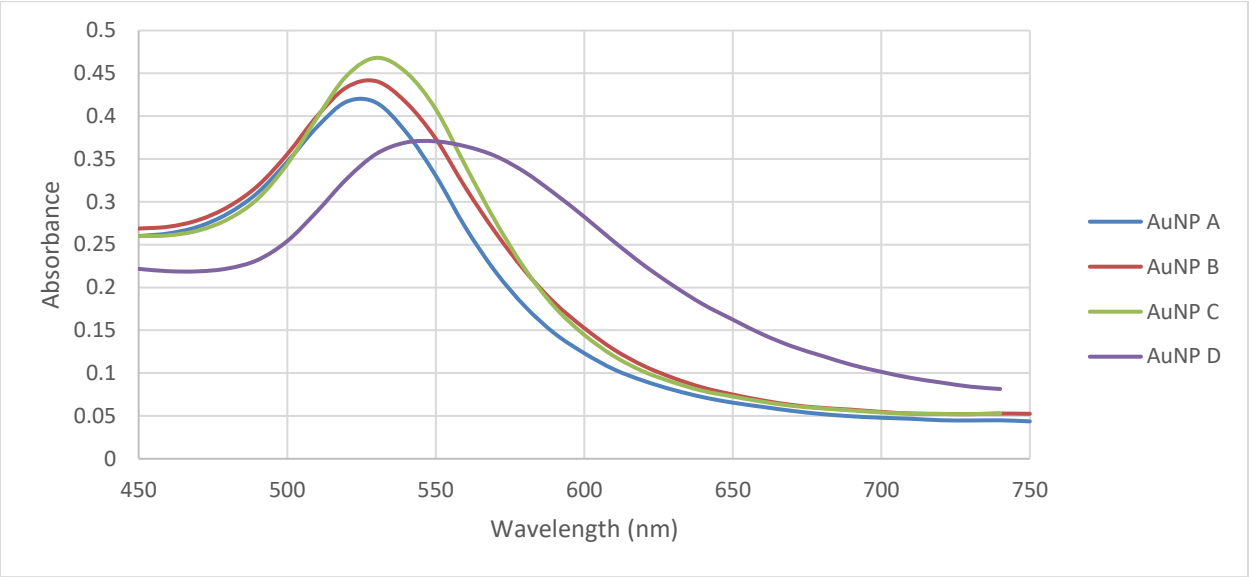

Figure 9. Absorbance spectra of AuNP A, B, C, and D.

A UV-Vis spectrum was collected in the wavelength range of 450 nm – 750 nm, using a SpectraMax 190 Microplate Reader. The A_{spr} and A_{450} were extracted from the scan and used to find the calculated diameter of the AuNPs (Table 7).

Table 7. $A_{spr,}$ $A_{450,}$ expected diameter, and calculated diameter of AuNPs.

	Lambda (nm)	Absorbance	Expected diameter (nm)	Eq 10 diameter (nm) Valid 35 – 100 nm	Eq 11 diameter (nm) Valid 5 – 80 nm	Eq 12 diameter (nm) Valid 5 – 50 nm	Estimated Diameter (nm)
A	520	0.4169	16	9.4	13.6	13.6	13.6
	450	0.26					
B	530	0.4408	24.5	46.9	15.2	16.3	15.7
	450	0.2688					
C	530	0.4681	41	46.9	24.6	19.7	46.9
	450	0.2599					
D	550	0.3706	71.5	81.5	16.7	9.4	81.5
	450	0.2217					

Equation 10 and 11 have certain ranges of diameters that they are valid for. Even in the ranges that they are both valid for, they provide different results. The estimated diameters for the synthesized AuNPs A, B, C, and D are 13 nm, 16 nm, 47 nm, and 81 nm, respectively. This was estimated by averaging the results from Equation 11 and Equation 12 for AuNP A, B, and C; and taking the result from Equation 10 for AuNP D.

AuNP and Thiophenol Assay

Varying Thiophenol Assay

The assay was performed with 4 different thiophenols (p-toluenethiol, o-toluenethiol, m-toluenethiol, and 2-methoxybenzenethiol at concentrations of 100 ppm, 50 ppm, 10 ppm, and 5 ppm (Figure 10). This assay tested how AuNPs with a 13 nm diameter aggregated for the 4

different thiophenols at varying concentrations. Before running the assay, it was hypothesized that AuNP would aggregate best for the thiophenols based on steric hinderance. So, p-toluenethiol would aggregate best followed by m-toluenethiol, o-toluenethiol, and 2-methoxybenzenethiol. It was also hypothesized that the AuNPs would aggregate more for the higher concentrations, which turned out to be incorrect. The AuNPs turned visibly blue almost immediately for all thiophenols and at all concentrations as seen in figure 10. A UV-Vis spectrum was collected in the wavelength range of 350 nm – 750 nm, using a SpectraMax 190 Microplate Reader 10 minutes after thiophenol addition.

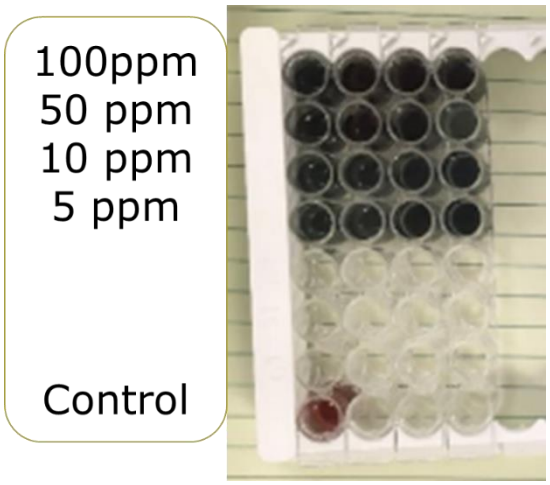

Figure 10. Thiophenols and AuNPs assay. The thiophenols from left to right are p-toluenethiol, o-toluenethiol, m-toluenethiol, and 2-methoxybenzenethiol. All thiophenols at all concentrations appear deep blue.

On the UV-Vis spectrum, the AuNPs aggregated for 5 ppm and 10 ppm concentrations of p-toluenethiol (Figure 11). For 50 ppm and 100 ppm of p-toluenethiol, the UV-Vis spectrum are significantly shifted to longer wavelengths, meaning that they are partially aggregated, but not to the extent that 5 ppm and 10 ppm of p-toluenethiol are. For p-toluenethiol, the lower concentrations were further along in the AuNP aggregation process than the higher concentrations. For AuNPs to

be considered aggregated, the absorbance at 520nm (AuNP's surface plasmon resonance) needs to drop and the absorbance at a wavelength associated with aggregation needs to increase. For p-toluenethiol, even though the absorbance of 50 ppm and 100 ppm is the same as the lower concentrations at 710 nm, 50 ppm and 100 ppm have the same absorbance as AuNPs at 520 nm, meaning that they are only partially aggregated.

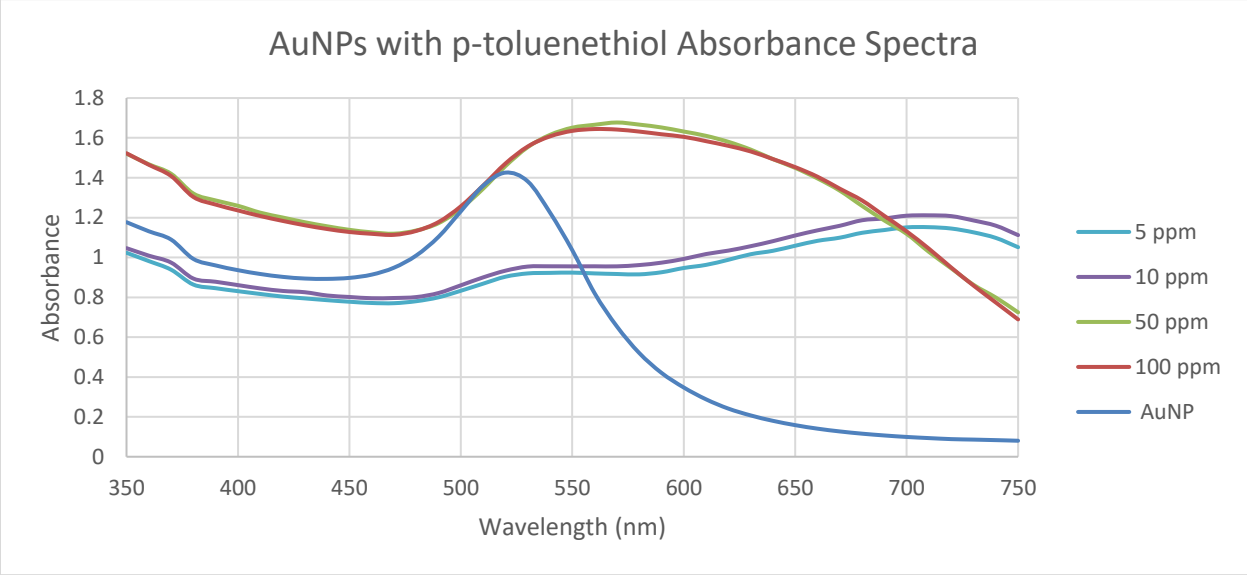

Figure 11. p-toluenethiol absorbance spectra. 5 ppm and 10 ppm are aggregated. 50 ppm and 100 ppm are partially aggregated.

The AuNPs aggregated for 5 ppm and 10 ppm concentrations of m-toluenethiol (Figure 12). For 50 ppm and 100 ppm of m-toluenethiol, the UV-Vis spectrum are significantly shifted to longer wavelengths, meaning that they are partially aggregated, but not to the extent that 5 ppm and 10 ppm of m-toluenethiol are. The 50 ppm spectra shifted a little more than the 100 ppm spectra, meaning that AuNPs with 50ppm m-toluenethiol are more aggregated than 100 ppm m-toluenethiol.

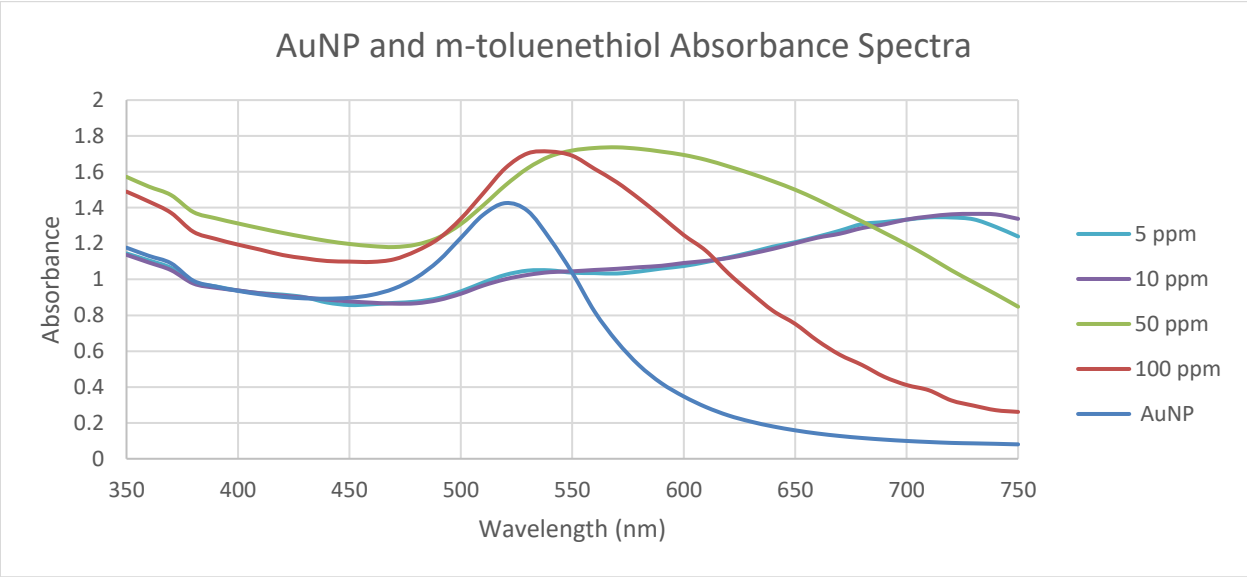

Figure 12. 10 ppm and 5 ppm are aggregated, and 100 ppm and 50 ppm are slightly aggregated for m-toluenethiol.

The AuNPs aggregated for 5 ppm and 10 ppm concentrations of o-toluenethiol (Figure 13). For 50 ppm and 100 ppm of o-toluenethiol, the UV-Vis spectrum are slightly shifted to longer wavelengths, meaning that they are partially aggregated, but not to the extent that 5 ppm and 10 ppm of o-toluenethiol are.

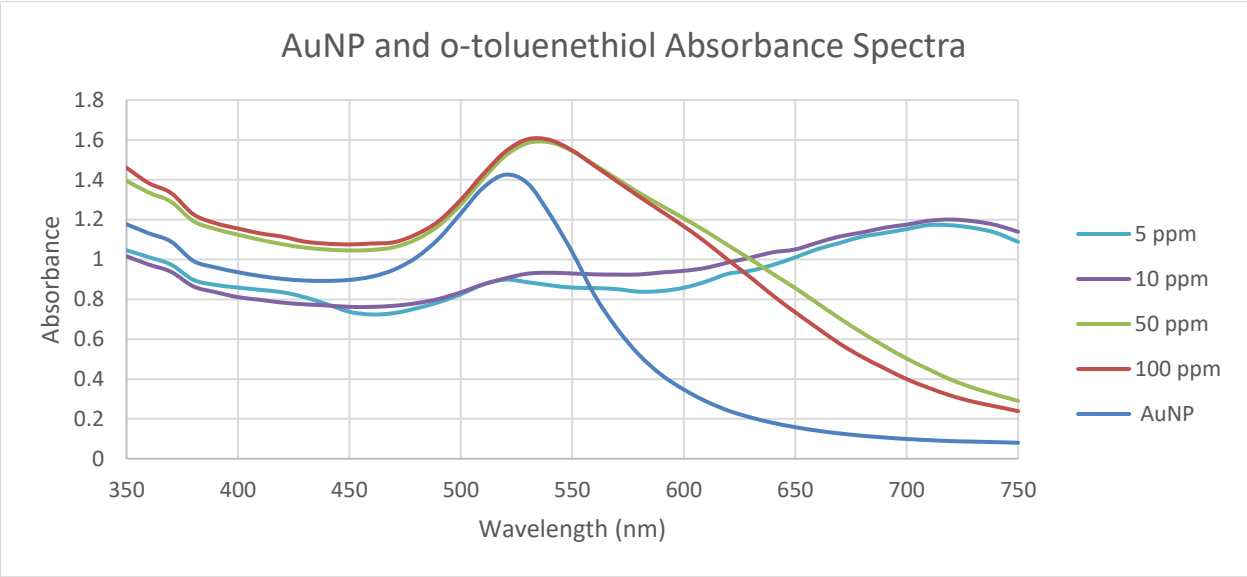

Figure 13. AuNP and o-toluenethiol absorbance spectra. 5 and 10 ppm induced aggregation while 50 and 100 ppm induced only slight aggregation.

The AuNPs aggregated for 5 ppm, 10 ppm and 50 ppm concentrations of 2-methobenzenethiol (Figure 14). For 100 ppm of 2-methoxbenzenethiol, the spectra is slightly shifted to longer wavelengths, meaning that it is partially aggregated. At 680nm, all the concentrations have an increased absorbance compared to AuNP. At 520 nm, only 100 ppm has a similar absorbance to AuNPs.

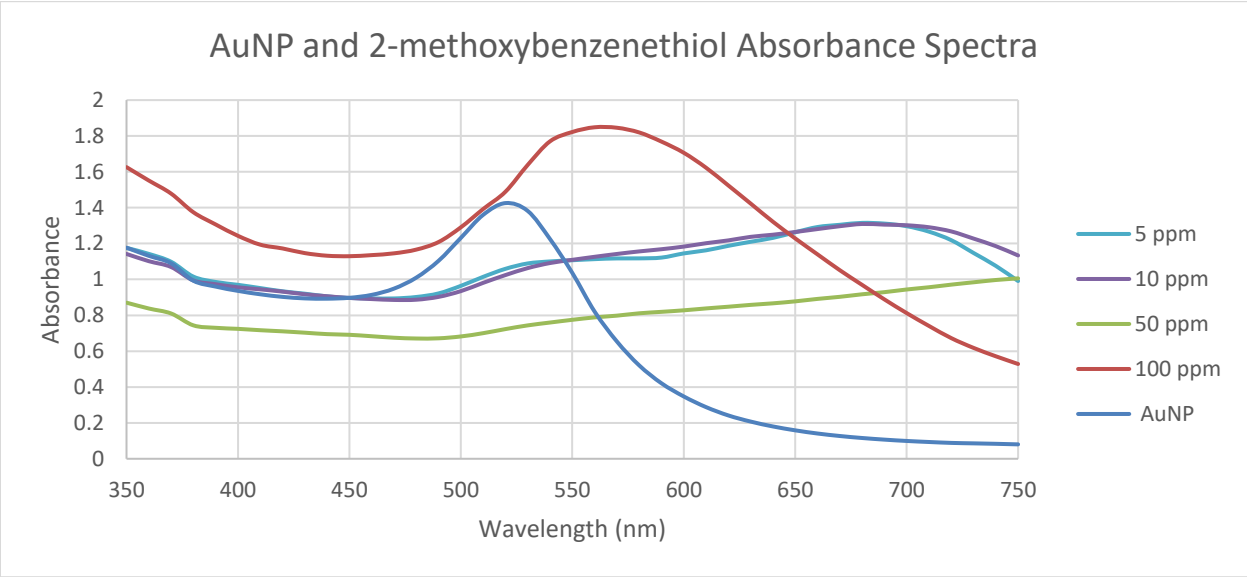

Figure 14. AuNP and 2-methobenzenethiol Absorbance Spectra, 5, 10, and 50 ppm are aggregated. 100 ppm is less aggregated.

The goal of this assay was to compare how different thiophenols caused the aggregation of the AuNPs. P-toluenethiol was expected to cause the most aggregation of AuNPs compared to the other thiophenols because it does not have as much steric hinderance as the other molecules. AuNPs with p-toluenethiol aggregated for 5 ppm and 10 ppm and partially aggregated for 50 ppm and 100 ppm. AuNPs with p-toluenethiol aggregated more than with m-toluenethiol or o-toluenethiol (Figure 15, 16). AuNPs with 2 – methoxybenzenethiol also aggregated for concentrations of 50 ppm.

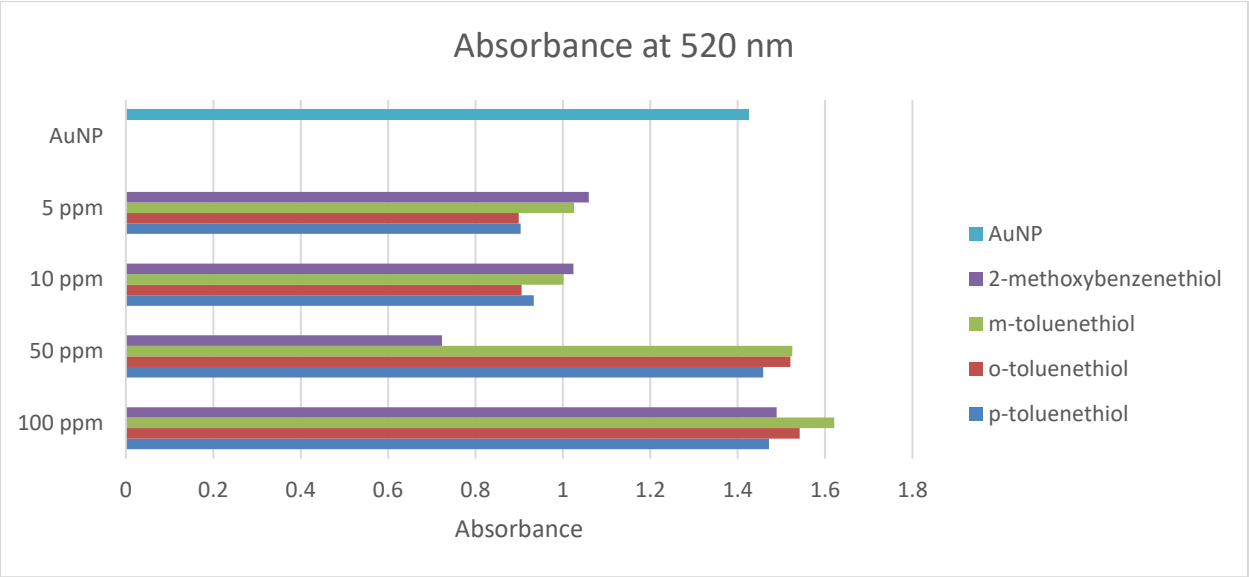

Figure 15. The absorbance at 520 nm of the 4 thiophenols at varying concentrations. Lower absorbance than that of AuNP indicates aggregation or partial aggregation.

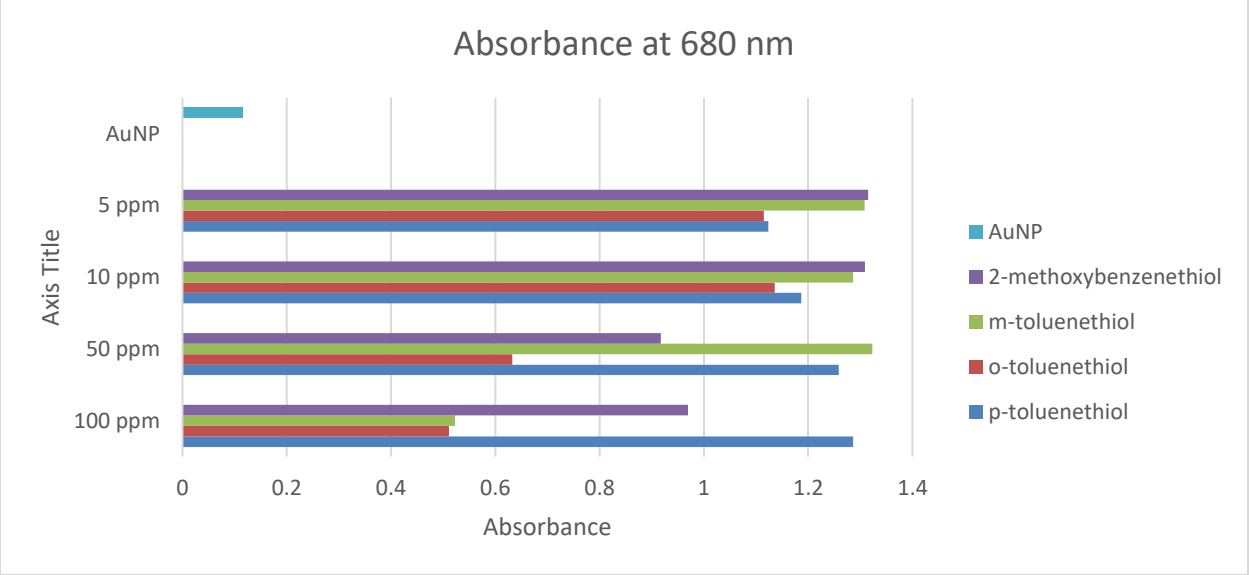

Figure 16. The absorbance at 680 nm of the 4 thiophenols at varying concentrations. Higher absorbance than that of AuNP indicates aggregation or partial aggregation.

Interestingly, with all thiophenols tested in this assay, lower concentrations of thiophenols induced stronger aggregation. The reason for this is unclear, but the results are consistent. It could be because at higher thiophenol concentrations, the thiophenols begin to act as a stabilizing ligand, coating the surface, instead of a destabilizing the citrate cap and changing the surface charge. Regardless of the reason, this is good news for detection because the goal is to detect lower concentrations.

AuNP and ppb Thiophenol Assay

This assay was performed with a mix of the 4 thiophenols with concentrations from 1000 ppb to 5 ppb to see how AuNPs with a diameter of 13 nm aggregated (Figure 17).

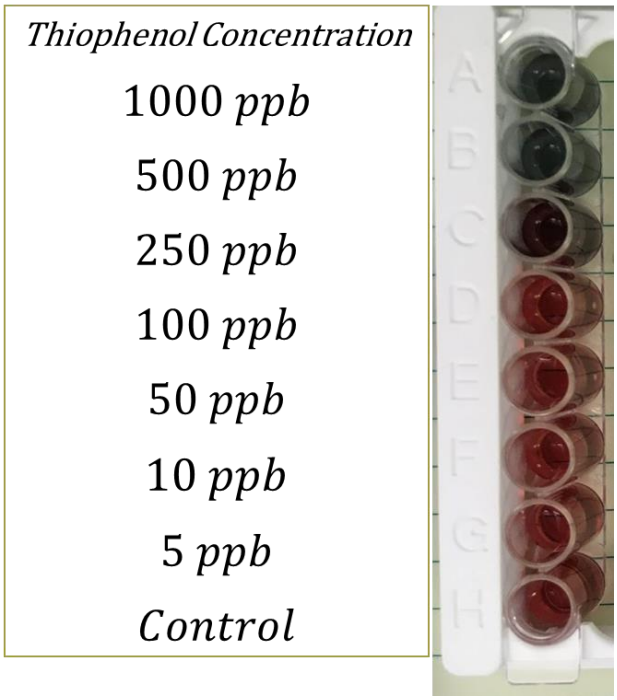

Figure 17. Low concentration thiophenols and AuNPs assay. A mix of all 4 thiophenols were used. 1000 ppb and 500 ppb are deep blue, 250 ppb is purple, and the rest are a similar wine red as the control.

The UV-Vis absorbance spectra of the low concentration thiophenols and AuNPs assay confirms what is seen visually (Figure 18). 1000 ppb and 500 ppb are aggregated, and 250 ppb is partially aggregated. All the other concentrations have very similar absorbance spectra to the AuNP control, indicating that they are not aggregated. AuNPs will aggregate for 500 ppb thiophenols.

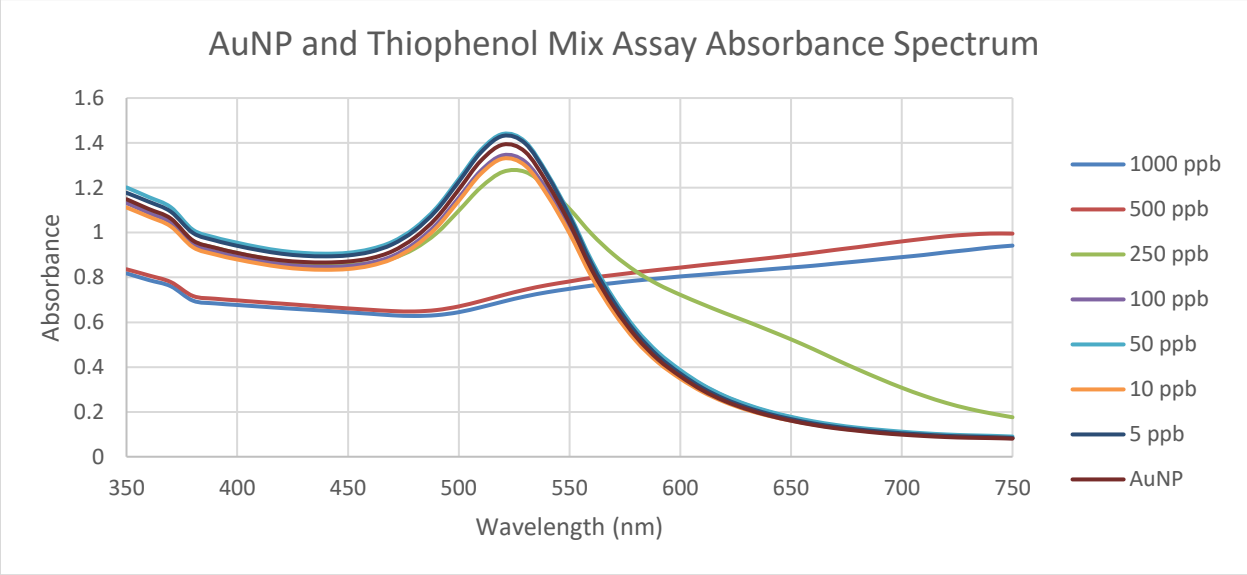

Figure 18. The UV-Vis absorbance spectra of the low concentration thiophenols and AuNPs assay. 1000 ppb and 500 ppb are aggregated, and 250 ppb is partially aggregated.

Varying Thiophenol Concentration with Different Sized AuNP

For these assays, four sizes of AuNPs are used to determine what effect AuNP diameter has on aggregation due to thiophenols. They are estimated to be 13, 16, 47, and 81 nm in diameter, labeled AuNP A, AuNP B, AuNP C, and AuNP D, respectively. Thiophenol was added to each well in concentrations varying from 50 ppb to 100 ppm. The assay pictures at different times relative to thiophenol addition are shown in figures 19, 20, 21, and 22. The absorbance spectra of AuNP A, AuNP B, AuNP C, and AuNP D are shown in figures 23, 24, 25 , and 26, respectively. AuNP A

aggregated for 5ppm and 500ppb. AuNP B aggregated for only 500 ppb. AuNP C aggregated for 5ppm and 500 ppb. AuNP D aggregated for 500 ppb and 100 ppm. AuNPs A and C performed better by aggregating for the most thiophenol concentrations.

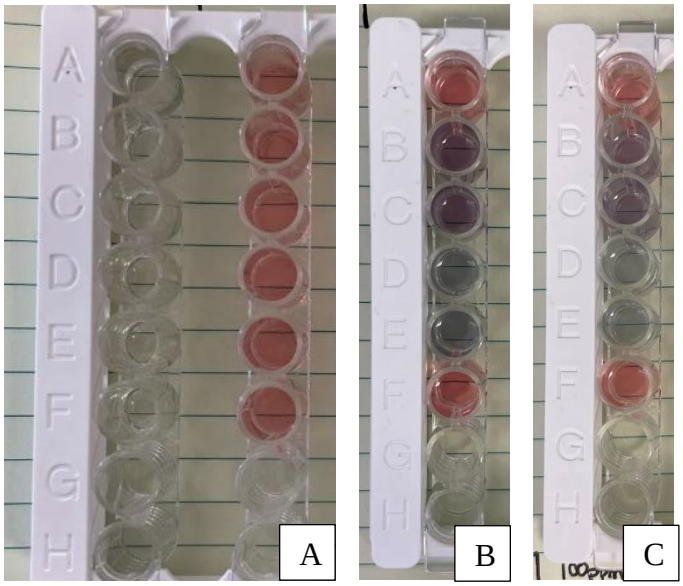

Figure 19. AuNP A (13 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows B-F. Control AuNP A is in row A. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 100 ppm and 50 ppm are purple. 5 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original red. C) 20 minutes after thiophenol addition. Colors are similar to Figure 25 B.

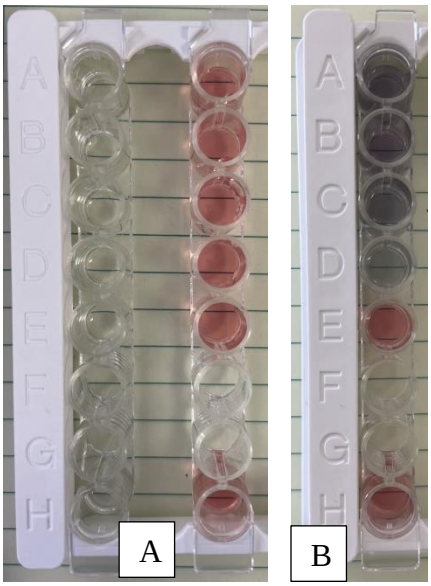

Figure 20. AuNP B (16 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP B is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 100 ppm, 50 ppm, and 5 ppm are purple. 500 ppb is blue, indicating aggregation. 50 ppb is still the original red.

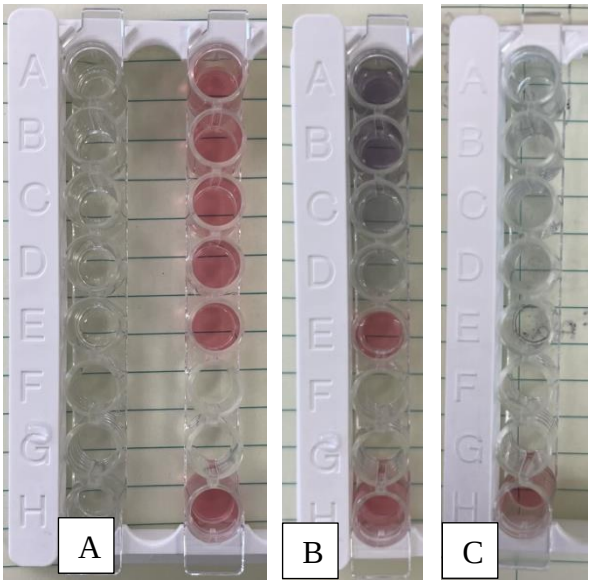

Figure 21. AuNP C (47 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP C is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition.

100 ppm and 50 ppm are purple. 5 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original red. C) 24 hours after thiophenol addition, all wells are aggregated.

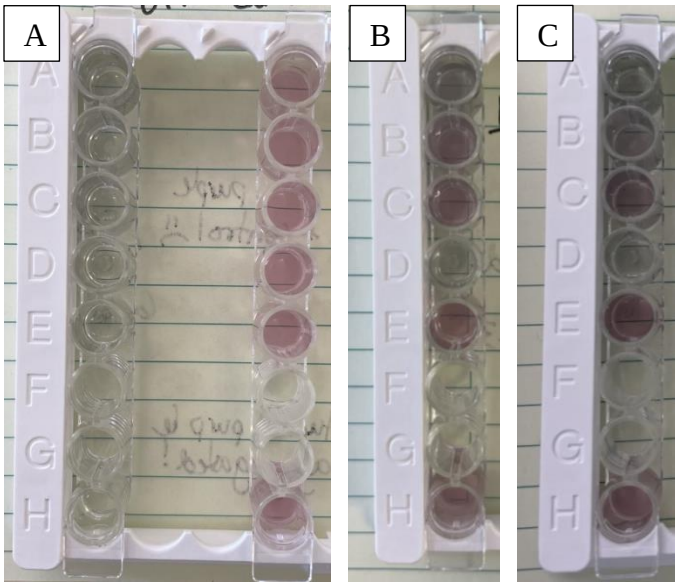

Figure 22. AuNP D (81 nm) with 100 ppm, 50 ppm, 5 ppm, 500 ppb, 50 ppb thiophenol in rows A-E. Control AuNP D is in row H. A) Before adding thiophenol. B) 10 minutes after thiophenol addition. 50 ppm and 5 ppm are purple. 100 ppm and 500 ppb are blue, indicating aggregation. 50 ppb is still the original redish color. C) 24 hours after thiophenol addition, wells A, B, and D appear aggregated.

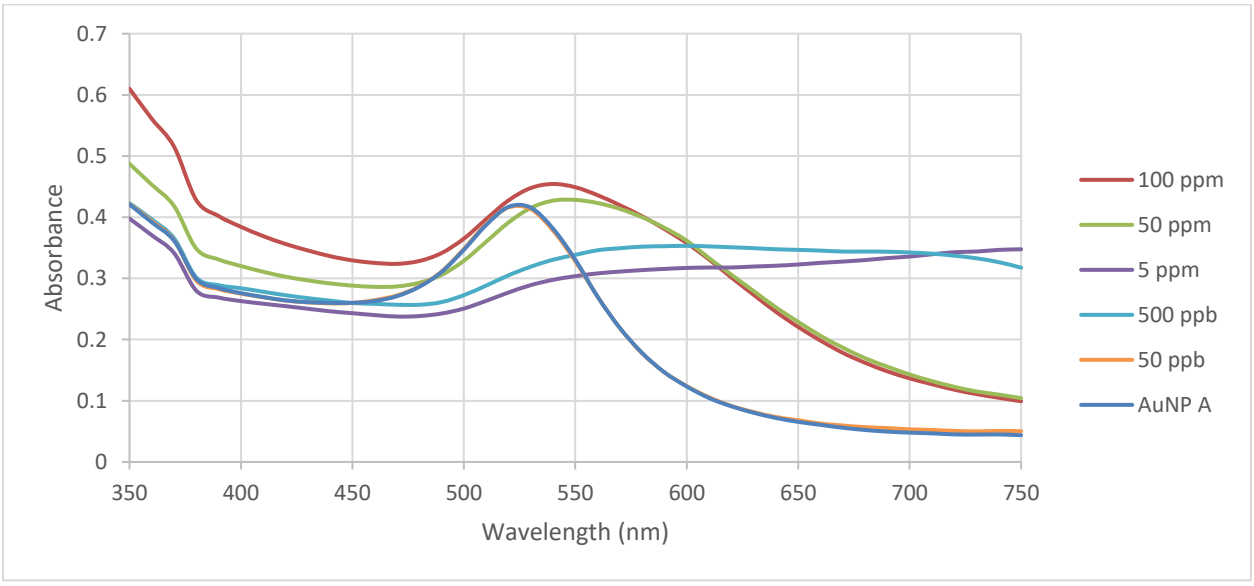

Figure 23. Absorbance of AuNP A (13 nm) 10 minutes after thiophenol addition.

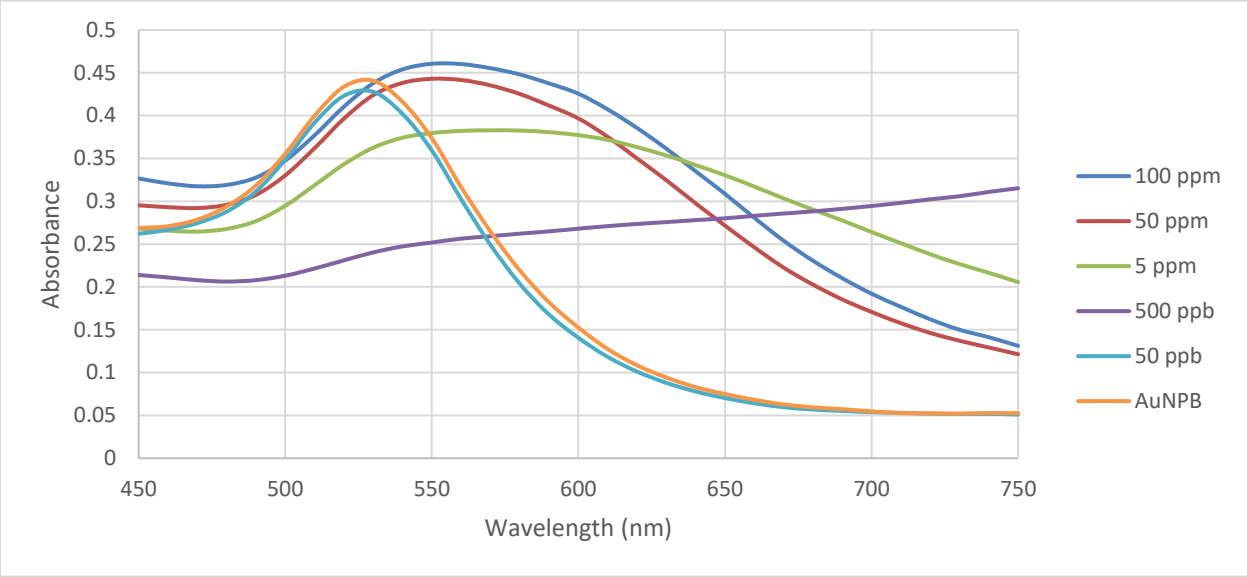

Figure 24. Absorbance of AuNP B (16 nm)10 minutes after thiophenol addition.

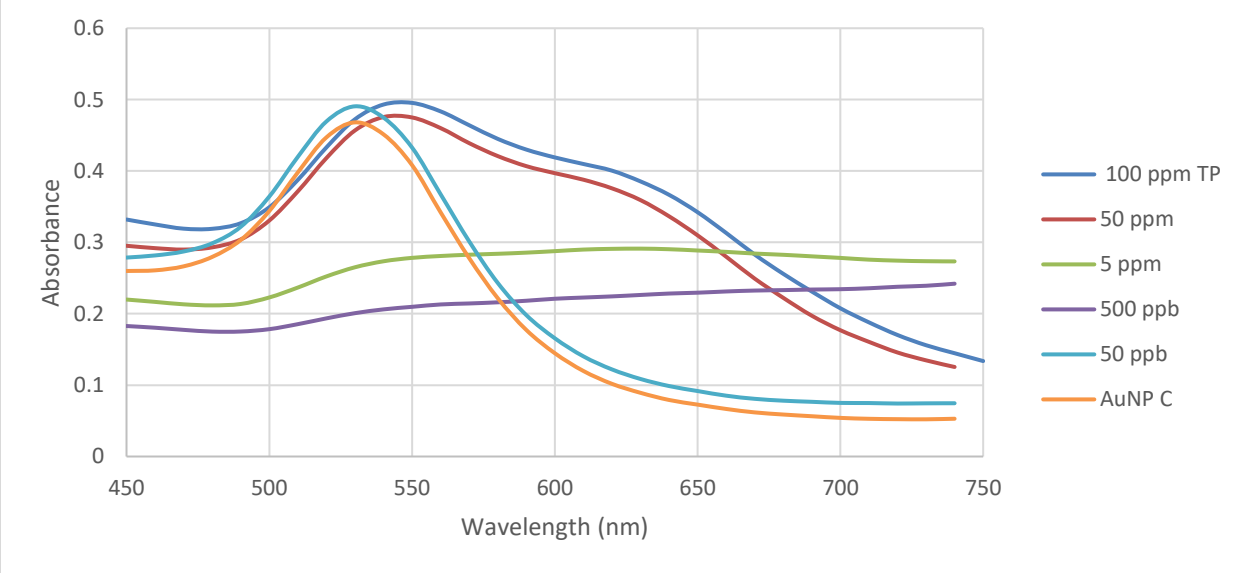

Figure 25. Absorbance of AuNP C (47 nm) 10 minutes after thiophenol addition.

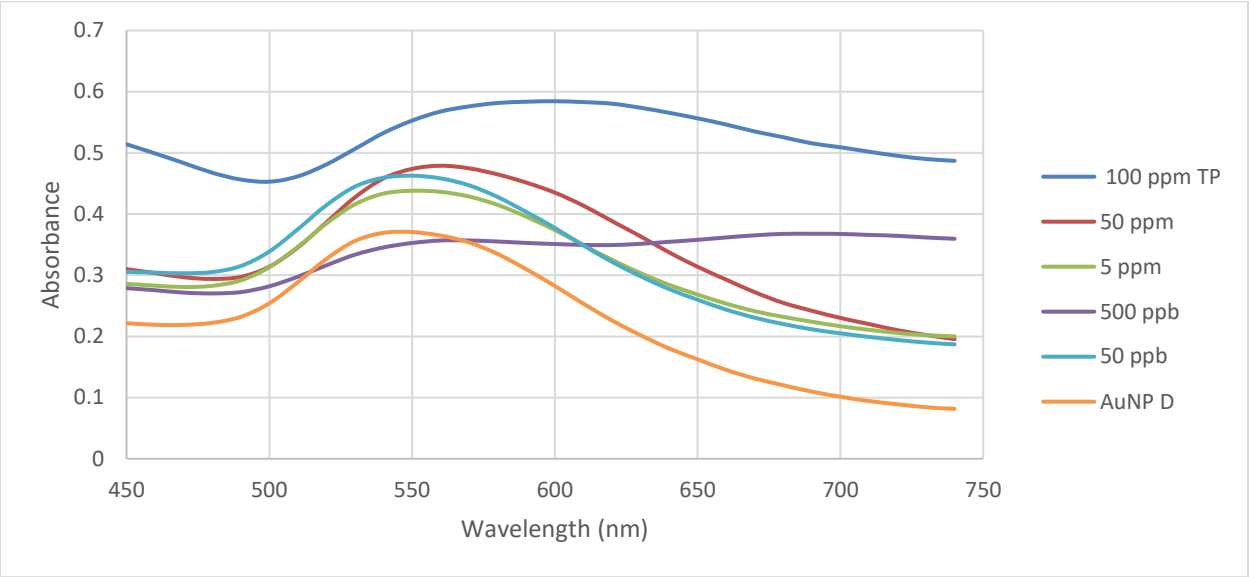

Figure 26. Absorbance of AuNP D (81 nm) 10 minutes after thiophenol addition.

The absorbance at 710 nm is a good indicator for aggregation. Absorbance values more than the AuNP absorbance are either partially aggregated or aggregated. Figure 27 compares these four AuNPs in one figure. Larger difference in absorbance from AuNP absorbance, represented by the black dashed line, indicate more complete aggregation. The colors of each bar represent the aggregation state of each sample. Blue indicates complete aggregation, purple indicates partial aggregation, and red indicates no aggregation. 13 nm, 47 nm, and 81nm AuNPs aggregated for two different concentrations. Looking at Figure 26 and Figure 22 suggest that 81 nm is not the best diameter AuNP to work with because of the faintness of its colors visually. Both 13 nm and 47 nm AuNPs aggregated for 5 ppm and 500 ppb concentrations of p-toluenethiol. The UV-Vis spectrum scan (Figure 25) of 47 nm diameter AuNPs shows that 5 ppm and 500 ppb are more shifted towards aggregated than 13 nm diameter AuNPs of the same concentrations. Therefore, 47 nm diameter AuNPs offers the best aggregation induced by thiophenols out of the options tested.

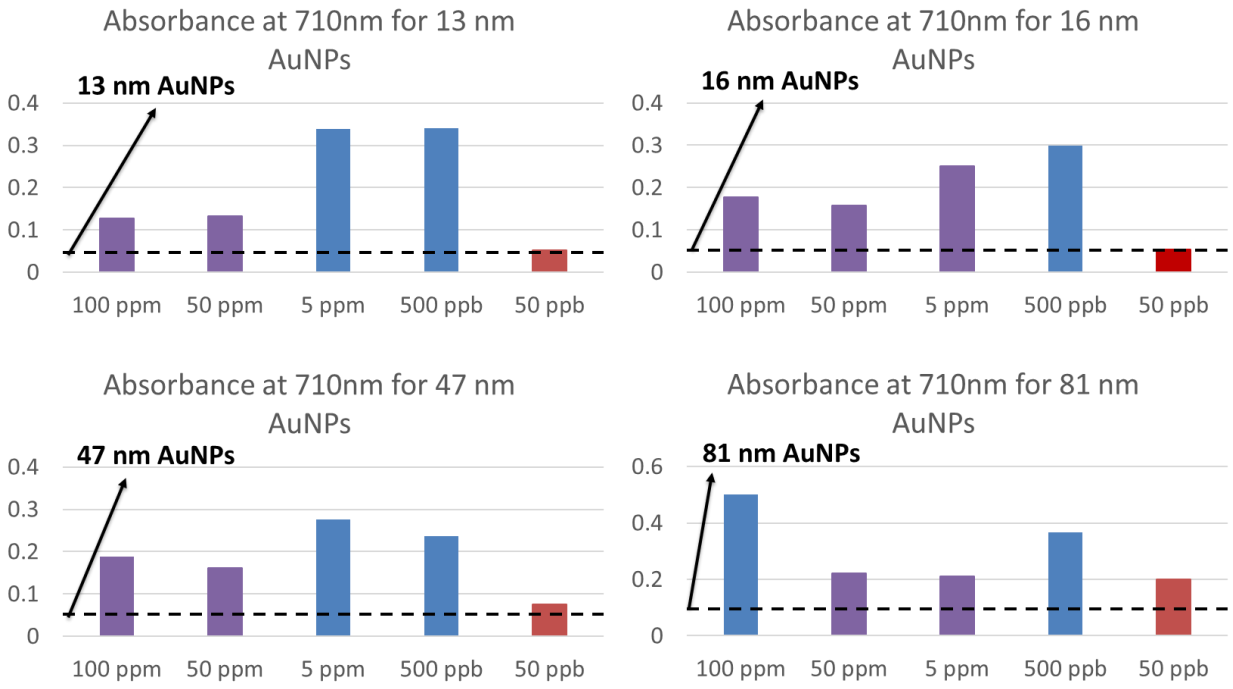

Figure 27. Absorbances at 710 nm for each AuNP. Blue indicates complete aggregation, purple indicates partial aggregation, and red indicates no aggregation.

Conclusions

AuNPs do aggregate in the presence of thiophenols. AuNP aggregation depends on both thiophenol concentration and AuNP diameter. AuNPs aggregate best when the thiophenol concentration is between 50 ppm and 500 ppb. 47 nm diameter AuNPs aggregated the best in the presence of thiophenols. AuNPs aggregated for thiophenols down to a concentration of 500 ppb. The proposed AuNP-based colorimetric assay offers a rapid, simple, and effective method for the detection of thiophenols and has the potential to be further developed for other applications in environmental and biological sensing.

Future Work

Gold nanoparticles have the advantage of being rapid and colorimetric for detection. However, they are not specific to thiophenols, and the results are qualitative not quantitative. It would be prudent to experiment with methods that have specificity for thiophenols with quantitative potential. Erin and I worked together to make a fluorescent probe that is specific to thiophenols and Erin performed experiments showing that it has a high sensitivity and specificity for thiophenols. Future work for that could be conjugating the probe to a cellulose surface and running the detection experiments with thiophenol-spiked grape juice. Another detection method to try would be using gold nanoparticles conjugated with DSP-GSSG. [17] This method is specific to thiols. The conjugated AuNPs are aggregated together and in the presence of thiols unaggregated.

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