Salt-Induced Aggregation of Polyallylamine-Coated Gold Nanoparticles (PAH-AuNP) for Rapid Detection of Methyl Parathion

Deonila A. Basańy1,*, Rey V. Capangpangan1,2, Potsamon Rţţţţvărţnîţ and Werasak Surareunique2

1College of Arts and Sciences, Caraga State University, Butuan City, 8600 Philippines
2Mineral Resources Management Research and Training Center, Caraga State University, Butuan City, 8600 Philippines
3Biochemical Engineering and Pilot Plant Research and Development Unit, National Center for Genetic Engineering and Biotechnology, King Mongkuts University of Technology Thonburi, Bangkok Campus, Bangkok, 10150, Thailand
4School of Biotechnology and Biotechnology, King Mongkuts University of Technology Thonburi, Bangkok Campus, Bangkok, 10150, Thailand

ABSTRACT

Several approaches for the detection of methyl parathion (MPT) have been reported. However, concern about the complexity and complicated instrumentation hampers its application for rapid analysis. Hence, colorimetric mode of detection for rapid analysis of MPT utilizing the unique property of an aggregated gold nanoparticles is reported herein. Polyallylamine-coated gold nanoparticles (PAH-AuNP) were prepared, and recombinant methyl parathion hydrolase (MPH) was used to specifically hydrolyze MPT into parathionhydrolase (PNP). Different experimental conditions, such as the pH of the salt-induced aggregation of AuNP, condition for the immobilization of MPH, the concentration of the MPH enzyme, the duration of incubation, among others were evaluated. Results showed that the prepared AuNP readily recognized the product (PNP) upon salt-induced aggregation when PNP is all converted to its ionic form. Rapid detection was obtained within 5 minutes at a pH greater than 7 at concentrations ranging from 0.1-14 ppm of MPT. The minimum MPT concentration that can be detected using this technique is 0.1 ppm. An ongoing experiment is currently being undertaken to demonstrate the applicability of the method for the detection of similar structure pesticides. Likewise, mechanistic study to further explain the obtained results is also being considered and will be incorporated in the subsequent report.

Keywords: gold nanoparticles, polyallylamine hydrochloride, methyl parathion, methyl parathion hydrolase

* Corresponding Author
Email: mabasnig@gmail.com
1 Introduction

Application of nano-size materials in various fields is the most active areas of research nowadays. Among other nanomaterials being explored, gold nanoparticles have received great interest in the scientific community due to its fascinating properties and versatile surface chemistry. Functionalized gold nanoparticles were extensively studied for various applications such as environmental (Chen, 2011; Wang and Yu, 2013), biomedical (Cai et al., 2008; Pissuwan et al., 2011; Sperling et al., 2008), energy (de Aberasturi et al., 2015) as well as for catalysis application (Daniel and Asrar, 2004). In particular, gold nanoparticles were being utilized for the detection of significant environmental pollutants, including detection of pesticides (Dai et al., 2015, Dakar et al., 2012, Lisha et al., 2009, Liu et al., 2014).

Detection of methyl parathion (MPT) calls for serious consideration due to its massive use as insecticide applied to crops and other agricultural products, particularly in the developing countries. Methyl parathion is an organophosphate pesticide widely used for household and agricultural applications for the chewing and sucking of insects in a wide range of crops. The compound is considered by Environmental Protection Agency (EPA) as a highly toxic insecticide (toxicity class I) and is classified as Ia (extremely hazardous) pesticide by the World Health Organization (WHO) (Yang et al., 2008; WHO, 2004). Concerns about the use of MPT have been raised due to its persistence and potential toxicity in humans and animals. MPT is highly toxic by inhalation and ingestion, and moderately toxic by dermal absorption (Dikshith, 2013). It has been considered as a cholinesterase inhibitor for which uptake via inhalation will lead to a bloody nose, coughing, chest discomfort, and difficulty in breathing (PesticideNews, 1995). On the other hand, extreme cases of exposure of MPT will affect the central nervous system, producing in-coordination, shurred speech, loss of reflexes, weakness, fatigue, and eventual paralysis of the body extremities and respiratory muscles (PesticideNews, 1995). MP has been banned in some countries and misused in developing countries. Thus, there is a need for the detection and regulation of methyl parathion. The conventional method of detecting MPT is the use of highly complex instruments such as gas chromatography (GC), GC/MS, HPLC, Spectrophotometry, TLC or polarography (de Souza Pinheiro et al., 2011; De Lhasera et al., 2009; Tiwari et al., 2013; Castanho et al., 2003). Although these methods offer to be accurate and sensitive, they also suffer the drawbacks such as being expensive, tedious, time-consuming, labor-intensive, and often requires prior sample pre-treatment. Simple and hand-held methods for the detection of MPT have been reported in several papers such as the use of acetylcholinesterase (AChE) inhibition-based amperometric and optical techniques (Anh et al., 2011). A novel method, however, for the fast detection of MPT is highly desirable. Herein, the use of gold-nanoparticle enzyme for the rapid detection of methyl parathion is reported.

Within the last few decades, nano-size metal particles have received a great deal of interest owing to their unique optical and electronic properties (Yu et al., 2003). The noble metals exhibit the strong surface plasmon resonance which allows them to present the intense color in the colloidal solution (Liu et al., 2010). The exact surface plasmon absorption is dependent on several parameters such as shape, size, medium, the distance between particles (Liu et al., 2010). The dispersed gold nanoparticles (AuNPs) around 16 nm diameter have a red color with a surface plasmon absorption band centered at 520 nm. When the interparticle distance decreases to less than the diameter of the particle, the coupling interactions result in a red-shift of the resonance wavelength and also lead to significant aggregation of AuNPs with the distinctive color change from red to blue.

Gold aggregation or clustering can be induced by physical methods such as increasing the ionic strength of the solution (Wang et al., 2001) or the addition of molecules to be able to connect one nanoparticle to another (Gu et al., 2001). This principle can be
used to detect a wide range of analytes from DNA to proteins and metal ions (Zhang et al., 2003). In this study, aggregated gold nanoparticles were utilized for the rapid detection of the target MPT analyte. For the first time, polyallylamine (PAH)-coated gold nanoparticles (PAH-AuNPs) were prepared and applied for the detection of methyl parathion using its hydrolysis product, para-nitrophenol (PNP). To the best of our knowledge, this is the first attempt to use a positively-coated gold nanoparticle for the direct detection of MPT. The recombinant methyl parathion hydrolase (MPH) was utilized to specifically hydrolyze methyl parathion to form the hydrolysis product, para-nitrophenol (Fig. 1). Figure 3 shows the schematic representation of the proposed mechanism for colorimetric detection of MPT thru salt-induced aggregation of gold nanoparticles. As shown, the enzyme will catalyze to produce para-nitrophenol. In an aqueous substrate, the para-nitrophenol will be further dissociated to form a bright yellow product, para-nitrophenolate (Fig. 2). The resulting product will then carry a net negative charge, of which the positively-charged AuNPs will bind into it thru electronic interaction. During the interaction, an inter-linking PAH-AuNPs will be formed, and further nanoparticle aggregation will occur in the presence of the salt. In contrast, conversion of MPT to para-nitrophenol is not possible without the presence of an enzyme, and thus there is no nanoparticle aggregation.

2 Experimental

2.1 Materials

All reagents and chemicals were used as received. Chloroauric acid (H\textsubscript{2}AuCl\textsubscript{4}) and polyallylamine hydrochloride (PAH) (MW 15,000) were purchased from Sigma-Aldrich. Tris(hydroxymethyl)aminomethane (TRIS) from Carlo Erba. Trisodium citrate (C\textsubscript{6}H\textsubscript{5}Na\textsubscript{3}O\textsubscript{7}.H\textsubscript{2}O) was purchased from VWR International Ltd. Hydroxyethyl cellulose (HEC), methyl parathion analytical standard and polyethylene glycol (PEG) were purchased from Sigma-Aldrich. Methyl parathion Hydrolase was provided by Dr. Dau Hung Anh of KMITT. All glasswares used in the preparation of gold nanoparticles were washed with aqua regia (1:3 H\textsubscript{2}SO\textsubscript{4}:HNO\textsubscript{3}) before use to eliminate contamination. Absorbance measurements were taken from SpectraSuite Spectrophotometer in a 300 \(\mu\)L cuvette with an integration time of 4500 \(\mu\)s.

2.2 Method

Synthesis of Citrate-coated Gold Nanoparticles (AuNP)

Citrate-capped gold nanoparticles were synthesized following the published protocol (Daniel and Astruc, 2004). Briefly, ten milliliters (10 mL) of 5 mM (H\textsubscript{2}AuCl\textsubscript{4}) was added with 180 mL ultra pure water (MQ water) and the solution was allowed to boil with constant stirring. Then, ten milliliters of (10 mL) of 0.5% trisodium citrate was added to the mixture, after which the color turns from yellow to colorless. The resulting solution was allowed to boil further until the solution turns from colorless to purple then finally to wine red. Additional heating of about 30 minutes was made, and then the solution was cooled to room temperature with constant stirring. The volume of the solution was adjusted with deionized water to make it to a final volume of 200 mL. The prepared gold nanoparticles have an approximate concentration of 0.25 mM.

Synthesis of Poly(allylamine)-coated Gold Nanoparticles

The polyallylamine-coated gold nanoparticles (PAH-AuNP) were synthesized in smaller volumes to avoid aggregation of samples and to ensure that citrate in gold nanoparticles
can be easily displaced upon mixing with polyallylamine hydrochloride (PAH). In brief, three hundred microliters (300μL) of 10 mg/mL of PAH was added to 1500 μL of citrate-coated AuNPs. The resulting mixture was immediately shaken for about 2 minutes using vortex shaker. The mixture was then continuously shaken for one hour and then centrifuged at 11000 rpm for 30 minutes. The supernatant was discarded, and the nanoparticle pellet was washed with 500 μL deionized water. The mixture was centrifuged for another 30 minutes, and the prepared PAH-coated AuNP was re-constituted with MQ water to a final volume of 750 μL. Several AuNPs were also prepared in a similar way and all of the prepared PAH-AuNP were pooled and homogenized in one solution.

Effect of incubation buffer on Enzyme Immobilization

Immobilization of enzyme, methyl parathion hydrolase (MPH), was made by adsorption onto the nitrocellulose membrane. Two microliters (2 μL) of recombinant MPH was placed on a nitrocellulose membrane (2 × 8 cm). The membrane was then allowed to dry for one hour at 35°C ± 1°C. The immobilized enzyme was then immersed in 700μL 0.1% HEC (or different immobilizing solution) for about 30 seconds until a smooth surface is seen and then cured overnight at 35°C. The immobilized enzyme was then immersed in 1 ppm methyl parathion (MP) (0.1-10 ppm in 10 mM TRIS and hydrolysis reaction was conducted at 30°C ± 1°C for 1 hour. Then, the reaction was stopped and the nitrocellulose membrane was removed from the solution. The hydrolysis products were then used for the aggregation assay.

Induced Aggregation Assay of PAH-AuNP

Two hundred forty microliters (240 μL) of the prepared PAH-AuNP was placed in an Eppendorf tube, and about thirty microliters (30μL) of the buffer (pH 9.1) was added to PAH-AuNP, and another 30μL of the hydrolysis products were added. The aggregation was induced by the addition of 4μL of 0.3 M NaCl. The absorption was measured at 518.5 nm, the λ_max of AuNP (red AuNP), and at 618.1 nm (blue AuNP). The absorbance was recorded at a specified time of aggregation. Aggregation of control solution (a solution derived from an MPT analyte immersed in a nitrocellulose membrane without the immobilized enzyme) was also conducted in a similar manner.

3 Results and Discussion

3.1 Aggregation of PAH-AuNP at Different pH

Aggregation of the prepared polyallylamine-hydrochloride gold nanoparticles (PAH-AuNP) with the substrate MPT was tested at different pH. Evaluation at different pH was conducted to investigate the effect of pH on the degree of aggregation of PHA-AuNPs since the target hydrolysis product para-nitrophenol (PNP) will be ionized at a higher pH. It was observed that aggregation of gold nanoparticles in the presence of MPT itself was very slow. Plausibly, this is because of the effective coating of the AuNPs surface with the high-molecular weight polymer, polyallylamine. It has also been observed that after the coating process, the obtained nanoparticles are well-dispersed in the solution which further justifies the excellent stability of the resulting nanoparticles. Also, complete coating with polyallylamine indicates that the surface of the AuNPs is perfectly stabilized that makes it protected from exposure to the surrounding medium. Thus, aggregation was made by inducing it with sodium chloride solution (6.0 mM NaCl). The extent of aggregation was measured by the ratio of the absorbance at 618.1 nm when PAH-AuNP is aggregating and 518.5 nm when PAH-AuNP is highly dispersed (not aggregated). The
figure shows (Fig. 4) that the prepared PAH-AuNPs will readily aggregate at higher pH (pH > 8). The obtained results corroborate to the fact that polyallylamine hydrochloride (PAH) exists in a deprotonated form in slightly basic solutions and above its pKₐ value (Dorris et al., 2008; Fang et al., 1999; Sartori et al., 2011). As reported, PAH is a weak cationic polyelectrolyte with many ionizable amine groups in its backbone. PAH exists in fully protonated in neutral and acid solutions but partly deprotonated in slightly basic solutions (Sartori et al., 2011; Fang et al., 1999). Likewise, changing the charge density as a function of pH causes the polymer chain to transition between tightly coiled and highly extended conformation (Grunian et al., 2008). PAH is a weak polyelectrolyte at high pH and becomes more extended at low pH as its positive charge density increases. Thus, as pH is increased, PAH loses positive charge density and becomes more likely to form a coiled structure. Conversely, at a higher pH, the polymer (PAH) can be easily attracted to other gold nanoparticles leading to a decrease in the interparticle distance and promoting aggregation. It was observed that the interaction of the derived hydrolysis product of MPT (para-nitrophenol) and the PAH-AuNPs is ubiquitous at higher pH (pH> 8). Most likely, at higher pH, the coiled conformation of the PAH coating predominates that would probably promote nanoparticles to aggregate. In addition, possible interaction of the MPT-derived product (para-nitrophenol) and the gold nanoparticles is most likely to happen via S-Au bond and P-Au interactions and thus causing the nanoparticles to aggregate. However, at a lower pH, all of the amine groups (–NH₃⁺) are protonated and therefore could increase the repulsion between the particles leading to stabilization of AuNPs.

In this experiment, it was observed that at a certain pH range (5-9), both the MPT (substrate) and control solution showed indistinctive aggregation. The distinction on the aggregation behavior of the MPT substrate and the control is apparent only at a pH greater than 8. From the obtained results, it is therefore important to explore on the possible mechanism to enhance the aggregation differentiation caused by both the substrate and the buffer to improve the sensitivity of the technique. In this present investigation, absorbance correction from the buffer was made to eliminate possible interference from the buffer.

3.2 Aggregation of AuNP by the Hydrolysis Products of MPT at Different pH

Methyl parathion (MPT), the substrate, was hydrolyzed first with methyl parathion hydrolase (MPH) to isolate the effect of MPT on AuNP aggregation. The hydrolysis products were then detected with PAH-AuNP. However, to minimize the effect of high pH on gold nanoparticles, aggregation of AuNPs was induced by the addition of salt. Hydrolysis products were made at a low salt concentration, 3.3 mM NaCl solution in AuNP. Aggregation of AuNP with hydrolysis products was studied at a different pH to determine if the ionized form of the target compound, para-nitrophenol (PNP) will affect the aggregation of AuNP. As expected, significant aggregation of AuNP was noted at pH value greater than its pKₐ value (pKₐ 7.15). In this study, it was observed that more significant aggregation was attained at pH 8. This implies that highly ionized para-nitrophenol promotes nanoparticle aggregation via electrostatic interaction with the positively charged AuNPs and through the negative oxygen of the nitro group (–NO₂) and the ionized phenol group (–OH). It is important to note that when the PAH polymer is fully charged, the conformation of the polymer tends to form a rod-like structure because of the electrostatic repulsions. However, when the charge density of the polymer chains is reduced, they adopt a more collapsed globular structure (Burke and Barrett, 2005). Furthermore, the charge density of the chains affects their physical properties.
and thus affects its properties in solution (Burke and Barrett, 2005). In this proposed mode of interaction, the p-nitrophenol acts as a linker between PAH-AuNP which leads to aggregation of the nanoparticles (Fig. 3). Importantly, PAH loses its positive charge density at higher pH and thus becomes more likely to form a coiled structure. This observation supports the obtained results that more gold nanoparticle aggregation was observed at higher pH.

3.3 Effect of Incubation buffer on MPH immobilization

The gold nanoparticle-based colorimetric assay can rely on the assembly of gold nanoparticles in which an enzyme reacts with the free substrate first, and the released products can crosslink the AuNPs which leads to the formation of large aggregates (Liu et al., 2007). In this paper, we report the aggregation of AuNP based on the hydrolysis of methyl parathion to produced para-nitrophenol upon the action of recombinant methyl parathion hydrolase (MPH). However, the presence of the enzyme during the hydrolysis of MPT in gold nanoparticles could result to steric hindrance for the released products, which could possibly amplify the aggregation of AuNPs. Moreover, the MPH could also aggregate the AuNP itself. To circumvent the issue and for the desired application, the MPH is immobilized on the nitrocellulose membrane. Thus, optimization for the immobilization of MPH on nitrocellulose membrane was studied, and different polymers such as polyethylene glycol (PEG) and hydroxyethyl cellulose (HEC) were used to ensure that the MPH does not diffuse into the solution. PEG has been used in some studies (Guarise et al., 2006; Liu et al., 2007) to stabilize the nanoparticles and to prevent the interaction of proteins with gold nanoparticles while hydroxyethyl cellulose (HEC) had been widely used for the encapsulation of some drugs. It has been found out that 0.1% HEC-rinsed MPH in nitrocellulose membrane provides the greatest sensitivity on the aggregation of AuNP without causing the AuNP solution to aggregate even the nanoparticle is left undisturbed (Fig. 6A and 6B). This is because HEC can easily adhere to the surface of nitrocellulose membrane via electrostatic interaction than PEG thus, can easily keep the enzyme in place within the membrane. Hence, HEC solution was used to immobilize MPH on nitrocellulose membrane on the subsequent experiments.

3.4 AuNP Aggregation at Different Volume Ratio of AuNP and Hydrolysis Products

Optimization on the volume ratio of AuNP and hydrolysis products were studied in order to support the hypothesized idea that the ionized PNP serves as a linker between gold nanoparticles. As expected, lowering the volume loading of hydrolysis products on AuNP, the more pronounced is the aggregation because of the increased ratio between AuNP and PNP (Fig. 7). It is believed that the higher the number of PNP interacting on the surface of AuNP, the more crosslinking is provided by the PNP between gold nanoparticles. Apparently, the aggregation of AuNP increases as the volume ratio of AuNP-PNP also increases. However, at a volume ratio of 1:12, aggregation also lessens to some extent. This may be attributed that PNP particles may be provide repulsion to other PNP particles instead of interacting to the positively charged AuNP, and thus lessens the aggregation. In this experiment, the volume ratio of 1:8 was considered to be optimal in aggregating AuNP.

3.5 Enzyme Concentration affects the nanoparticle aggregation

It is known that the higher the amount of enzyme being used, more products can also be obtained. In this experiment, increasing the amount of enzyme immobilized on
nitrocellulose also results in an increase in the amount of products, and thus increasing
the extent of aggregation (Fig. 8A). However, too much loading of enzyme on the
membrane results in an increased protein-protein interaction as there will be less surface
of the membrane for which the enzyme can interact. This result to high loosely bound
protein on the membrane which results to leaking and contribute to aggregation (Fig.
8B). Thus, the amount of enzyme that can be loaded in the membrane ranges from 1-3μL
in the specified dimension of the membrane, and the optimum concentration that gives
highest aggregates (Fig. 8b) without sacrificing the control is at 3μL.

3.6 Effect of MPT Substrate Concentration on the Dynamic
of Nanoparticle Aggregation

Different substrate (MPT) concentration (0.01-14 ppm) was allowed to undergo
hydrolysis in the presence of an immobilized enzyme. For a complete reaction, it is
believed that the product PNP will also have a similar concentration of the initial
substrate concentration (0.01-14 ppm). As expected, the higher the concentration of the
produced product, the greater is the aggregation (Fig. 9A). As the time of aggregation
increases, the extent of aggregation (by monitoring the intensity of the color change)
also increases (Fig. 9A). In fact, the plot of the ratio of absorbance (A618/A518) and
the log concentration of products in ppm gives a good linearity from 0.01 ppm to 14 ppm
(Fig. 9B) with an $R^2$ value of 0.9942 within 5 minutes. As aggregation time increases
to 10 minutes, the extent of aggregation also increases as can be seen on a high A618/A518
value (Fig. 9A).

The $R^2$ value of the absorbance ratio (A618/A518) is plotted versus log ppm concentra-
tion of products. It is noted that the $R^2$ value increases from 0.940, 0.991 and 0.994 for
0.5 minutes, 4 minutes and 9 minutes, respectively (Fig. 10A). The highest $R^2$ value
was obtained within 7 minutes of aggregation (0.997) while it is noted that there is a slight
decrease in linearity at 10 minutes (0.988) (Fig. 10A). This can be ascribed to the fact
that great aggregation of AuNP can lead to the surface interaction of AuNP and great
clustering of gold nanoparticles which may lead to the collapse of the blue color of the
solution and turns into a colorless one. Visual color change for the detection of MPT is
observed even at 5 minutes up to 10 minutes (Fig. 10B). Based on the results obtained,
it was observed that the limit of detection is found to be 0.1 ppm where an apparent
blue color can still be obtained (Fig. 10B). Lower than 0.1 ppm will have slight color
change that is close to that of control (Fig. 10B).

4 Conclusion

Optimization of AuNP for the rapid detection of methyl parathion was conducted at
different conditions. Experiments have shown that methyl parathion substrate and the
buffer did not have an effect on the aggregation of AuNP at a low pH (5-8) but affect at a
higher pH. Detection of methyl parathion using polyallamine-coated gold nanoparticles
was made with the specificity of the hydrolysis products of methyl parathion (MPT) with
methyl parathion hydrolase (MPH). Aggregation of AuNP is more apparent at pH 9, the
pH higher than the pH (pH 8.6) of the functionalized group polyallylamine hydrochloride
on AuNP. Immobilization of methyl parathion hydrolase on nitrocellulose membrane was
enhanced with the hydroxyethylcellulose (HEC) agent. The optimized volume of AuNP
and hydrolysis products for the aggregation of AuNP was also conducted. Aggregation
of AuNP is more apparent when the hydrolysis reaction was increased with an increased
hydrolase concentration and increased substrate (MPT) concentration. The minimum
MPT concentration that can be detected using the salt-induced AuNP is 0.1 ppm. Rapid
detection of methyl parathion through its hydrolysis product can be made at 2–5 minutes, shown by the linearity of aggregation and time, after which the AuNP start to collapse at 10 minutes. At 5 minutes, a good linear relationship of 0.994 is obtained between AuNP aggregation and time for the detection of MPT.

5 Acknowledgment

The authors would like to thank BIOTECH-HRD (Thailand) and Caraga State University (Philippines) for the fellowship grant to conduct the study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


Copyright ©June 2016 D. A. Basnig, R.Y. Capangpangan, P. Rijivanich and W. Surareungchai. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Fig. 1: Hydrolysis reaction of methyl parathion (MPT)

Fig. 2: Dissociation reaction of para-nitrophenol
Fig. 3: Schematic diagram reflecting the aggregation mechanism of PAH-coated AuNPs

Fig. 4: Effect of pH on PHA-AuNPs Aggregation
(Aggregation of PHA-AuNP was made at 1:6 AuNP: MPT volume ratio)
Fig. 5: Effect of pH on the Aggregation of PAH-AuNPs in Hydrolysis Products

Fig. 6: Effect of immobilizing solution on MPH on the aggregation of AuNPs (A) Absorbance measurement after 5 minutes reaction time and (B) Naked eye inspection after 5 minutes reaction time.

Fig. 7: Effect of AuNP-Product Volume Ratio on AuNP Aggregation
Fig. 8: (A) Optimization of AuNP aggregation as a function of MPH concentration on AuNP; (B) Optimization of AuNP aggregation at different MPH concentration (corrected with the control).*

*Absorbance taken after 5 minutes aggregation.

Fig. 9: Absorbance ratio of different MPT concentration; (B) Plot of Absorbance and log concentration of MPT substrate*

*Absorbance taken after 5 minutes aggregation.
Fig. 10: (A) The trend of the plot of $R^2$ value on the linearity of the aggregation of AuNP at various aggregation time; (B) Naked eye visual inspection on the aggregation of AuNP at different MPT concentration*

*Absorbance taken at 5 minutes aggregation.