

2, 4, 6-Trithiol-1, 3, 5-Triazine-Modified Gold Nanoparticles and Its Potential as Formalin Detector

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Abstract. Stabilized gold nanoparticles (AuNP) have been successfully prepared by a modification of ligand 2, 4, 6- trithiol- 1, 3, 5- triazine (TT). TT has three thiol groups and nitrogen atoms on the aromatic ring that can **interact** and stabilize AuNP. TT modified AuNP (AuNP/TT) was characterized using UV-Vis spectrophotometer, particle size analyzer (PSA) and transmission electron microscopy (TEM). The characterization showed that AuNP/TT stable at a maximum wavelength (λ_{maks}) of 537 nm with the particle diameter of 9.41 nm. The increased acidity (pH) causes the protonated thiol groups of TT marked with a visual change of colloidal AuNP/TT from purple to blue, causing AuNP and TT bonds weakened. In this study, the AuNP/TT was reacted with formalin. This interaction shows that AuNP/TT has a potential as an efficient detector of formalin, marked by changes in the diameter of the particle, colloidal color, and maximum wavelength shift.

1 Introduction

The application of nanotechnology in food safety (detection system, biosensor, etc.) has now been widely used as the focus of research because it is closely related to human survival [1-5]. Gold nanoparticles (AuNP) have absorption at a visible wavelength, so it is often used as an ideal method to detect the presence of hazardous chemicals in foods such as borax, formalin, and melamine, one of them through colorimetric sensors. In previous studies, we have studied the interaction between AuNP with melamine as an ideal colorimetric sensor [6]. AuNP detects the presence of melamine through colloidal color changes that occur due to hydrogen bonding between the melamine and acid groups in the modified AuNP. The method offers several advantages over other detection techniques that require complicated sample preparation, expensive cost, and limited detection capabilities. Firstly, this method does not require expensive and complicated instruments, which simplify operations and reduce costs. Secondly, this method allows the detection concentration as low as 2.5 ppb in short enough time [7]. Common methods used for qualitative and quantitative analysis of formalin include titrimetry, photometry, chromatography, NMR ¹³C, HPLC/spectrophotometry [8,9].

On the other hand, it has been well known that nanoparticles are unstable because they have high surface energy [10,11] and cause aggregation [12] and thus need to be stabilized with an appropriate modifier.



Some functional groups, such as cyano (CN), mercapto (SH), and amino (NH₂) are known to have a high affinity for AuNP [13]. A 2, 4, 6-Trithiol-1, 3, 5-triazine (TT) is a ligand with three active thiols (-SH) sites [14] potentially binding to gold nanoparticles. The existence of TT on the AuNP surface is expected to inhibit the aggregation of nanoparticles electrostatically between thiol groups to improve stability. In this study, the preparation of TT ligand modified AuNP ((AuNP/TT) was interacted with formalin. The reducing agent used in the optimization of AuNP synthesis is NaBH₄. AuNP/TT is interacted with formalin to investigate its potential as a fast and efficient foodstuff detector.

2 Experimental

2.1 Materials

HAuCl₄ used in this research was synthesized by dissolving 99.9% Au metal from PT. Antam in aqua regia solvent with HNO₃ and HCl ratio of 1:3. All chemicals were of analytic grade and were used without further purification. Formalin was obtained from Merck and 2, 4, 6-trithiol-1, 3, 5-triazines from Sigma Aldrich. MilliQ water (18.2 Ω cm⁻¹) was used to make aqueous solutions.

2.2 Synthesis of Gold Nanoparticles (AuNP)

Five mL of HAuCl₄ 1.0x10⁻⁴ M solution was added into one mL of NaBH₄ 0.004 M solution then stirred using a magnetic stirrer for 2 min. The same experiment was repeated for NaBH₄ concentrations of 0.007; 0.023; 0.030; and 0.070 M. The characterization was performed using UV-Vis spectrophotometer to find λ_{max} value, and PSA to determine the particle size.

2.3 Modification of AuNP with TT

Optimum AuNP colloid (optimization 2.2) were added to 1.0 mL of TT in various concentrations of 1.33x10⁻⁶; 6.67x10⁻⁶; 1.33x10⁻⁵; and 6.67x10⁻⁵M, while stirring with a magnetic stirrer for 2 min. The formed mixture was characterized using UV-Vis spectrophotometers, PSA, and TEM. Furthermore, the optimum AuNP/TT was tested for stability in pH variations of 1.0-8.0. HCl and NaOH solutions with concentrations of 0.1 M were used by varying the pH of reaction.

2.4 Interaction of AuNP/TT with Formalin

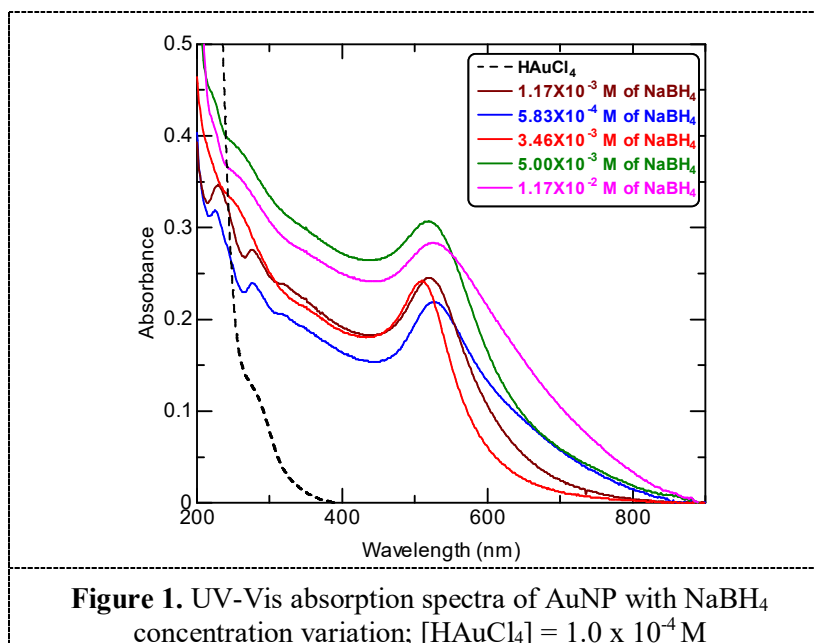
About 0.2 mL of formalin 1.0x10⁻³ M was added into the AuNP-TT colloid under optimum conditions. The mixture was stirred using magnetic stirrer for 3 min. The absorbance of the mixture was observed using UV-Vis spectrophotometer.

3 Results and discussion

3.1 Synthesis of AuNP with various concentration of NaBH₄

AuNP synthesis optimizations were performed by varying the concentration of reducing agent, NaBH₄. The variation of reducing agent concentration aims to control the size of the AuNP formation. The nanoparticle size control will improve the functionalization and selectivity of AuNP [15-17]. Based on previous research, it is known that the parameters affecting the growth, shape, and structure of nanoparticles are the reactants concentration (reductants), type of capping agent or stabilizer, pH of the solution, and reaction time [17]. The formation of AuNP is visually marked by a color change from a colorless (HAuCl₄ solution) to a ruby red colloid [18-21]. Colloidal color changes indicate that the Au³⁺ ion source has been reduced to Au⁰ [22].

Spectroscopic characterization was shown in Figure 1. AuNP formation was characterized by the appearance of λ_{max} spectral peaks in the range 500-530 nm [23-26] from the peak of HAuCl₄ solution spectrum at 200 nm [27].



The graph of NaBH₄ concentration variation in Figure 1 shows that NaBH₄ 3.46 × 10⁻³ M has the smallest λ_{maks} , 509 nm. The λ_{maks} value indicates that the size of AuNP has the smallest size. If the particle size decreases, the Bohr radius distance also decreases, so that electrons will be trapped and led to the band gap energy increases, and λ_{maks} shifted to smaller (higher energy) [31]. In addition to the smallest λ_{maks} value, the use of 3.46 × 10⁻³ M NaBH₄ also yields a considerable absorbance value, that is 0.242. The magnitude of absorbance shows the large number of the formed AuNP. The greater absorbance value, the more amount of light absorbed by AuNP, indicates many AuNP was formed.

The observation using PSA aims to determine the average size distribution of AuNP. Table 1 show Au³⁺ reduction using NaBH₄ 3.46 × 10⁻³ M produced AuNP with the smallest size of 8.64 nm.

Tabel 1. PSA Data: AuNP size with NaBH₄ concentration variation

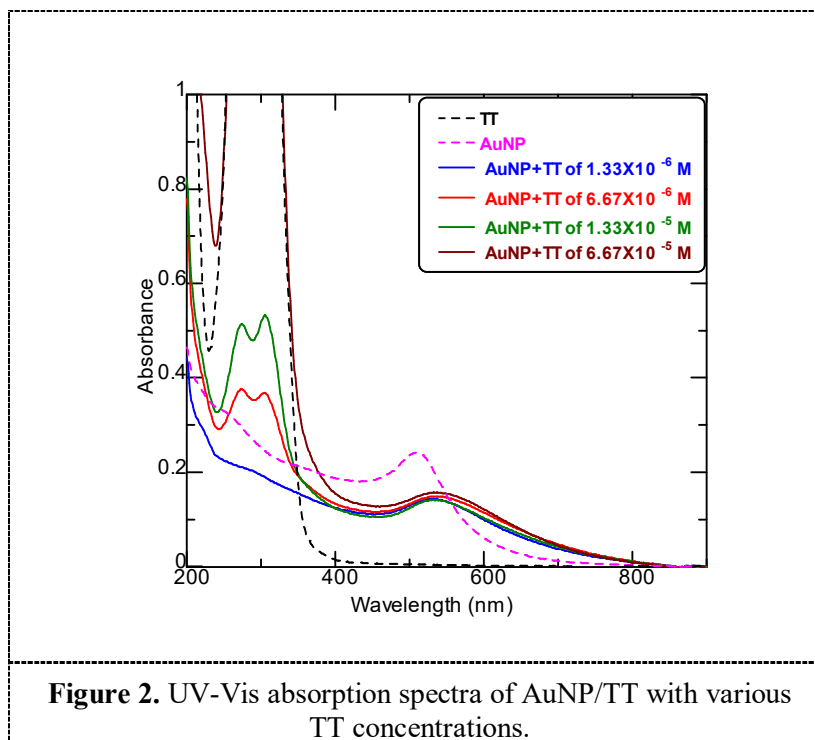
NaBH ₄ Concentration (M)	Size (nm)	% Volume	Peak width (nm)
5.83x10 ⁻⁴	10.89	100.00	6,88
1.17x10 ⁻³	11.54	99.90	7,89
3.46x10 ⁻³	8.64	100.00	8,78
5.00x10 ⁻³	11.68	99.20	4,39
1.17x10 ⁻²	18.02	99.00	4,74

Based on observations using UV-Vis spectrophotometer and PSA were then used 3.46 × 10⁻³ M NaBH₄ for AuNP synthesis in further treatment.

3.2 Modification of AuNP with TT

The TT ligand has three thiols that can stabilize AuNP (AuNP/TT) through Au-S interaction. The optimization of AuNP/TT modification was performed by varying the ligand concentrations at 1.33x10⁻⁶; 6.67x10⁻⁶; 1.33x10⁻⁵; and 6.67x10⁻⁵ M. Figure 2 shows the difference in absorbance and λ_{maks} of AuNP

before and after modified TT ligand. The λ_{maks} shift occurred from 509 to 537 nm, while the absorbance decreased from 0.255 to 0.190.



To facilitate the observation, λ_{max} and absorbance curves against-various TT concentrations were shown in Figure 3.

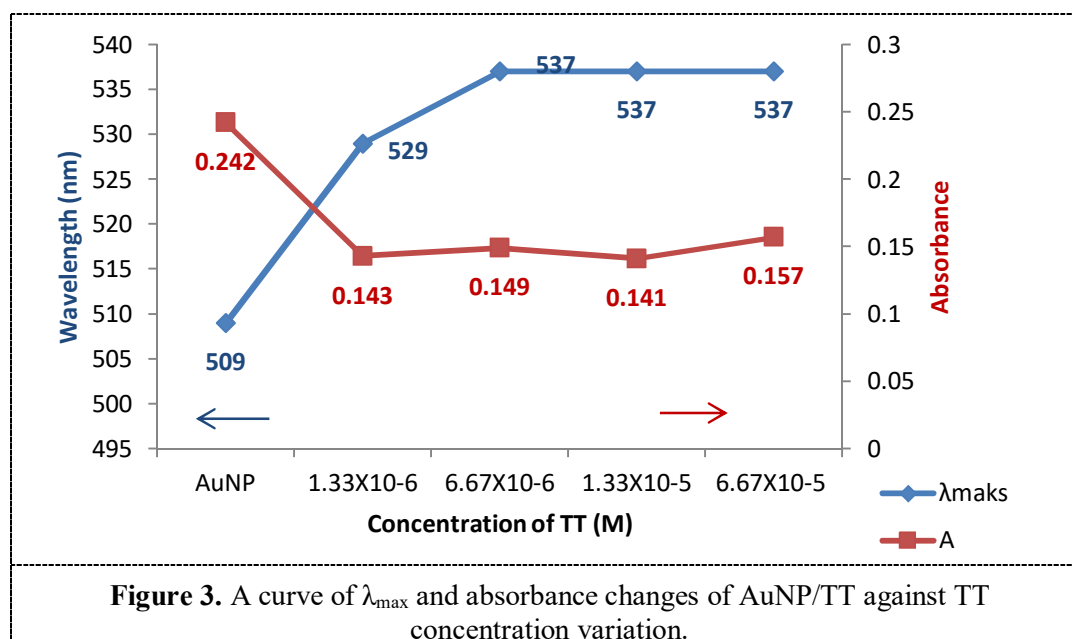
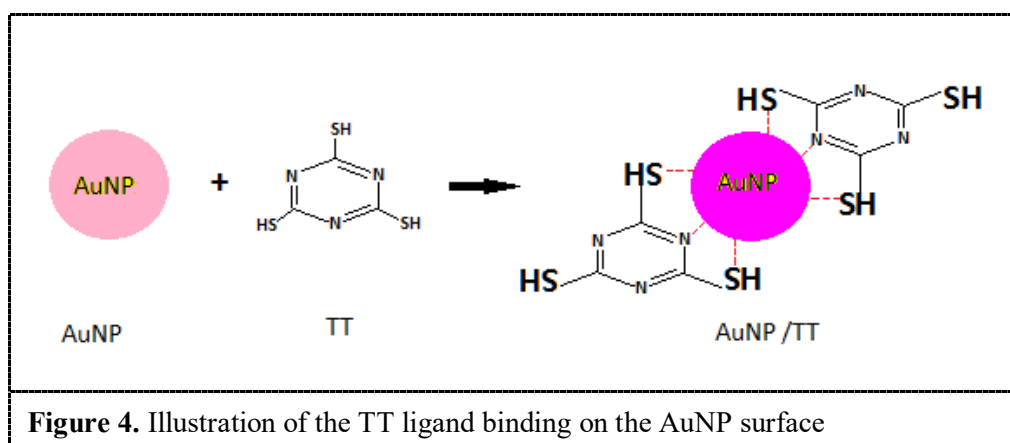
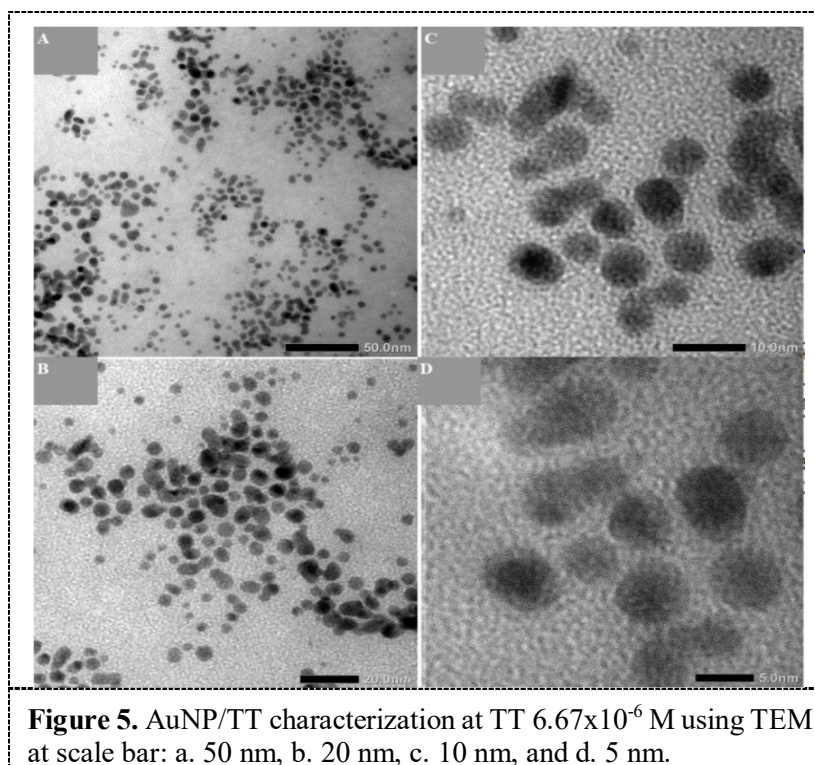


Figure 3 shows that TT 6.67×10^{-6} M was the optimum TT concentration, the λ_{\max} of AuNP/TT peak constantly at 537 nm. The various TT concentrations did not give significant effect to λ_{\max} shift. This suggests that the excess of TT as a modifier does not affect AuNP stability. TT was bound to AuNP surface through interactions between S and N atoms in heterocyclic of TT on AuNP surface. Due to the symmetry of thiol groups and the active site of N atom, the interaction between TT and AuNP involves a tridentate coordination link [28], as illustrated in Figure 4.

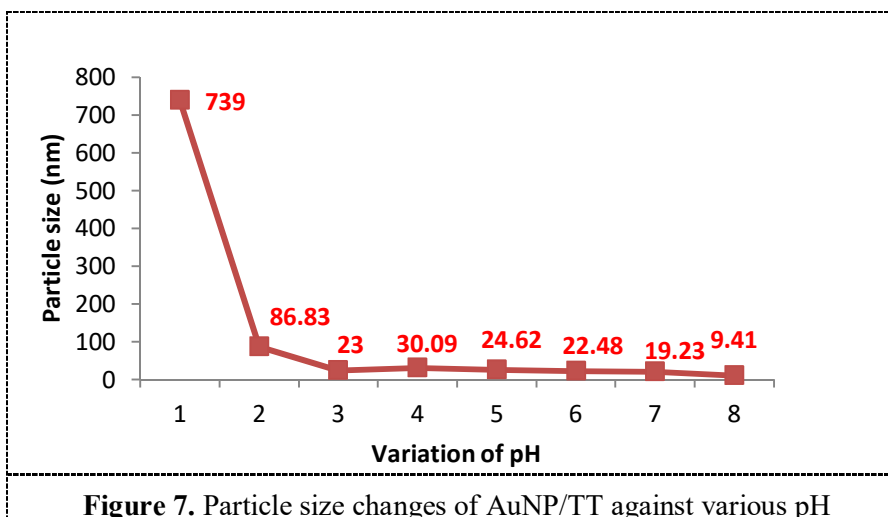
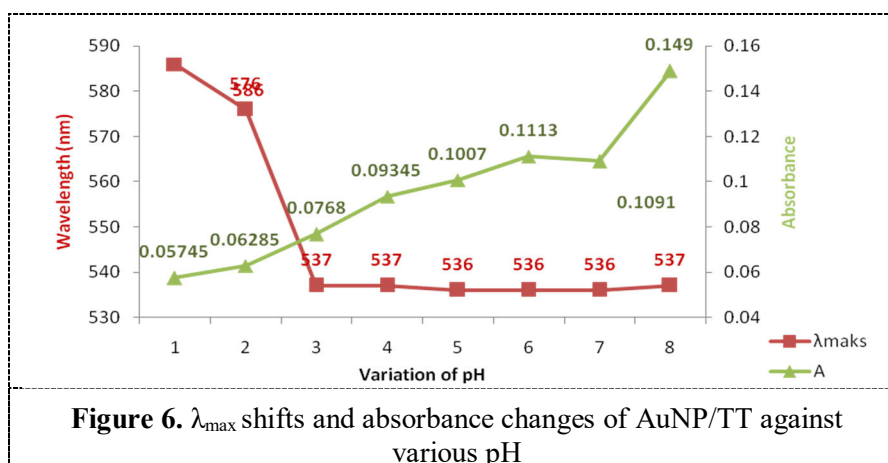


PSA measurements show that the optimum AuNP modified by TT 6.67×10^{-6} M was 9.41 nm in size. The resulted AuNP/TT morphology can be seen using TEM in Figure 5 show nanosphere with 5-10 nm in size .



Furthermore, pH variation of AuNP/TT colloid was started from pH 8.0 then decreased at pH 7.0; 6.0; 5.0; 4.0; 3.0; 2.0 and 1.0. Observations were conducted through λ_{\max} shifts, absorbance changes, and

AuNP/TT size distributions. Increased acidity causes the electrons in thiol group attached stronger to H atom so that electrons are difficult to donate to AuNP. It causes the release of TT from AuNP, as evidenced from the UV-Vis absorption at pH 2.0 and 1.0 (Figure 6) and particle size growth due to agglomeration (Figure 7). The optimum condition of AuNP/TT modification was observed at pH 8.0.



TT was a ligand with three thiol groups and nitrogen atoms in the aromatic ring. The addition of thiol compound to a metal nanoparticle will form a stable compound [29]. Thiol will form a single layer on the surface of the metal nanoparticle (self-assembly monolayer) which means the ligand has modified the surface of nanosphere or three-dimensional space [30]. In this modification, there is an interaction between SH-group with stable metal nanoparticles. The observed results with UV-Vis spectrophotometer were shown in Figure 8.

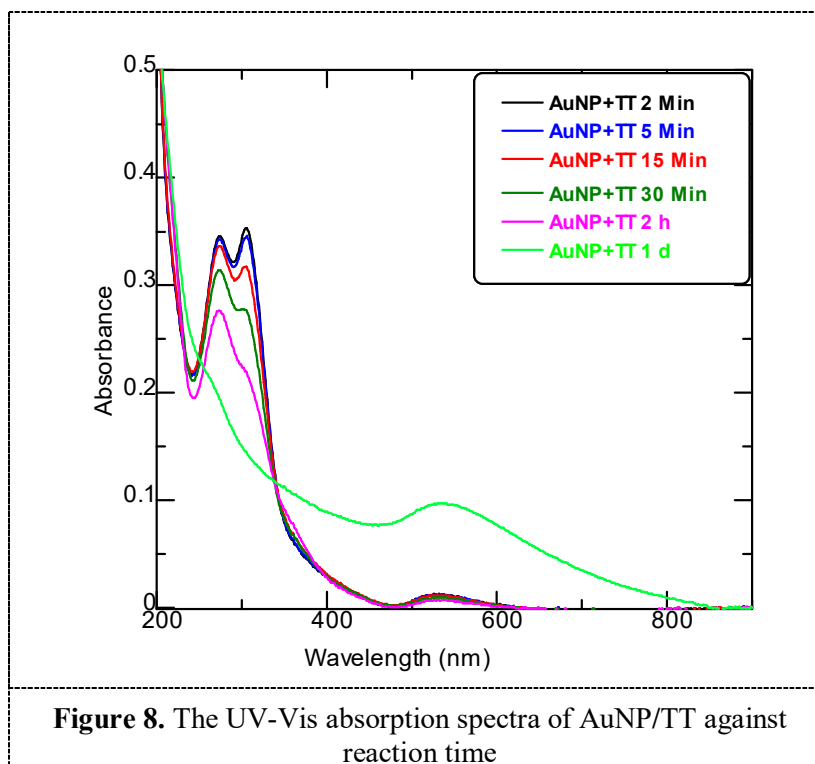
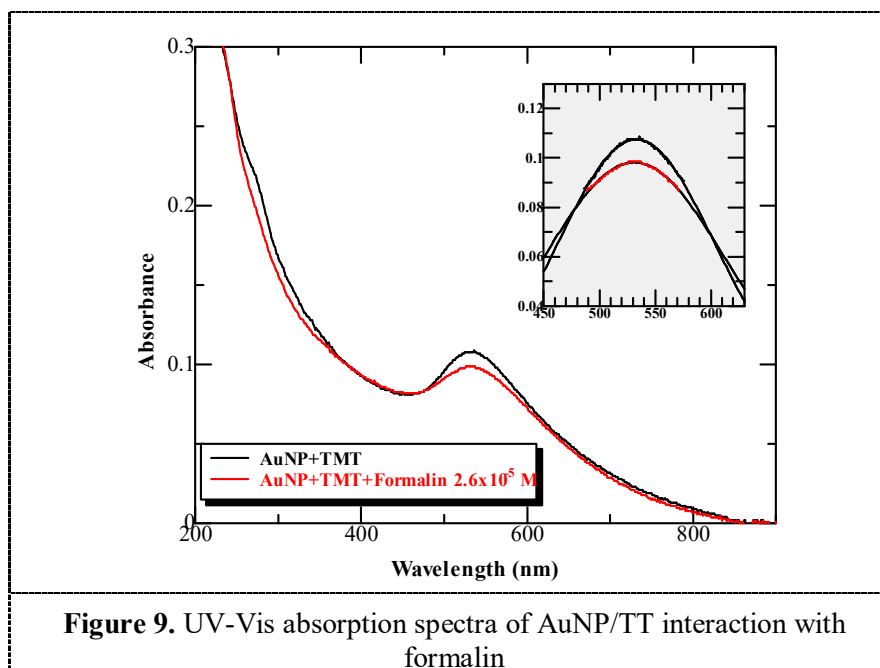


Figure 8 shows the AuNP/TT formation reaction time of 2 min to 2 hours, and no λ_{\max} of AuNP/TT changes at 537 nm, indicating that the modified nanoparticles were stable. The peaks at 280 nm and 320 nm appearing in Figure 8 are peaks of HAuCl_4 and TT, respectively. Increasing reaction time causes both peaks to decrease as they are used in AuNP/TT formation shown in absorption spectra increase at 537 nm. However, from the first day there was a significant increase in absorbance of AuNP/TT to 0.089. The stability of AuNP/TT occurs as a result of electrostatic stabilization of AuNP protected by the self-assembly monolayer of -SH functional group of TT [30]. Electrostatic stabilization can increase colloidal stability so that the size and its distribution of AuNP can be maintained.

3.3 Interaction of AuNP/TT with Formalin

Interactions of AuNP/TT with formalin can be visually observed through colloidal color changes characterized using UV-Vis spectrophotometers. Observations were conducted from the changes of λ_{\max} , absorbance value, and colloidal size.



The UV-Vis absorption spectra (Figure 9) showed a change in λ_{\max} ($\Delta \lambda_{\max} = -1.90$ nm) and a change in absorbance value ($\Delta \text{Abs} = -0.01$) when AuNP/TT was reacted with formalin. Similarly, showed a change in the particle size ($\Delta \text{particle size} = 2.29$ nm) of AuNP/TT before and after reaction with formalin measured using PSA as shown in Table 2. This confirms an interaction between AuNP/TT and formalin in the form of AuNP/TT-formalin complex through hydrogen bond. The thiol group as the active site of TT, in the presence of hydrogen atom in formalin can perform a intermolecular hydrogen bond. Therefore, AuNP/TT has a good enough potential to detect the presence of formalin by a simple method based on AuNP/TT size changes, λ_{\max} shifts, and absorbance value change as a colorimetric detector.

Table 2. Changes in λ_{\max} , absorbance and size of AuNP/TT after interaction with formalin

	λ_{\max}	Abs	Particle Size (nm)	$\Delta \lambda_{\max}$	ΔAbs	$\Delta \text{Particle Size (nm)}$
AuNP/TT	533.03	0.11	9.41			
AuNP/TT-Formalin	531.13	0.10	11.70	-1.90	-0.01	2.29

Abs = Absorbance, ΔAbs = Absorbance change

(Negative $\Delta \lambda_{\max}$ indicate a shift toward the hypochromic)

4 Conclusions

AuNP has been successfully modified with a TT ligand acted as a stabilizer agent to improve AuNP stability. The optimum condition of AuNP/TT modification was conducted at TT concentration of 6.67×10^{-6} M, NaBH_4 concentration of 3.46×10^{-3} M, pH 8.0 with a particle size of 9.41 nm. The release of TT from AuNP occurs at pH 1.0 and 2.0. The formed AuNP/TT exhibits a good interaction with formalin, characterized through changes in particle size, maximum wavelength, and absorbance value. Therefore, AuNP/TT has a potential as formalin detector using colorimetric sensor method.

Acknowledgement

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