CHAPTER 3

SYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES AND ITS SENSORY DERIVATIVE

3.1 Experimental

3.1.1 Materials

Production of AuNP mainly used gold chloride and trisodium citrate dihydrate (sodium citrate) sourced from Sigma Aldrich, USA along with hydrochloric acid (HCl) and nitric acid (HNO_3) in precautionary steps in making AuNP. Meanwhile, introduction of disulfide linkage on AuNP used 3,3-dithiodipropionic acid (3,3-DTDPA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC HCl), N-hydroxysuccinimide (NHS), and glutathione disulfide (GSSG) also sourced from Sigma Aldrich, USA.

3.1.2 Methods

3.1.2.1 Precautionary steps

AuNP synthesis process demands the glassware used to be clean from stray impurities on the walls of the glassware. Cleaning the glassware prevented accidental nucleation and growth of AuNP. The glassware used were cleaned using aqua regia, mixture of HCl and HNO_3 with 4:1 volumetric ratio.

3.1.2.2 Synthesis of AuNP and its sensory derivative

AuNP in this research was produced using an adapted and modified reported protocol from Turkevich et al. (1951) with lower temperature and lower concentration of gold chloride and sodium citrate. In order to synthesize AuNP, 20 ml of 1 mM gold chloride solution was stirred and heated at 100°C. While stirring, 2 ml of 1% sodium citrate solution was added into the reaction mixture and continued on stirring at constant speed. The pale yellow colour of the reaction mixture turned into brilliant red indicating successful synthesis of AuNP. Stirring was stopped when the colour of reaction mixture no longer showing any changes. The produced AuNP was then stored at 4°C until further usage. The procedure was triplicated and repeated using 2 mM and 3 mM gold chloride solution, 2% and 3% sodium citrate solution, and heating at 80°C and 60°C as described in Table 3.1. The flow process is shown in Figure 3.1.

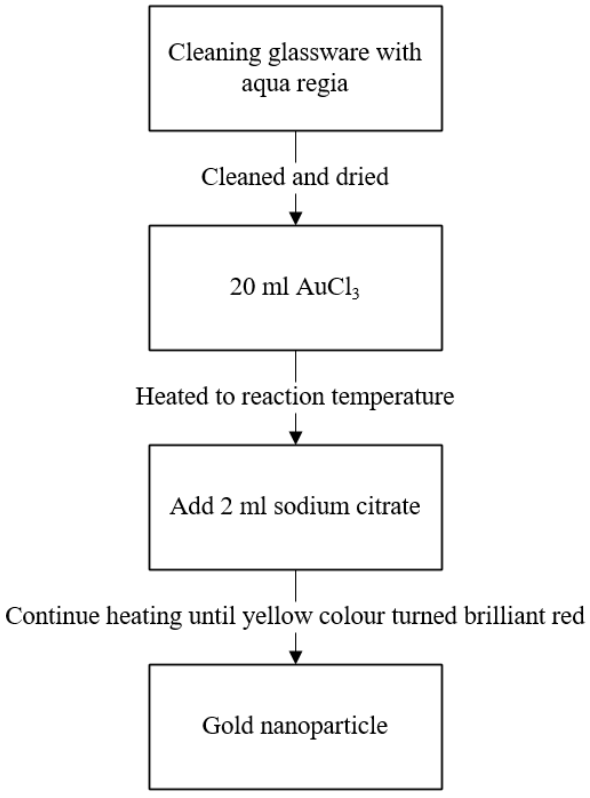

Figure 3.1: Flow process of synthesizing AuNPs

Table 3.1: Repetitions done for variables studied

Reaction temperature (°C)	Concentration of gold chloride (mM)	Concentration of sodium citrate (%)	Repetitions
100	1	1	3
80	1	1	3
60	1	1	3
100	2	1	3
100	3	1	3
100	1	2	3
100	1	3	3

Successively, surface functionalization of AuNP with glutathione disulfide (GSSG) was done using an altered combination of reported protocols from Durocher et al. (2009) and Dutse et al. (2017). 3 ml of prepared AuNP sample (1 mM $AuCl_3$, 1% sodium citrate, 100°C) was mixed with 1 ml of 0.1 ppm 3,3-DTDPA and shaken for 1 hour at 30°C. Then, a mixture of 1 ml of 0.3 ppm EDC HCl and 1 ml of 0.3 ppm NHS were added into the reaction mixture. The mixture was continued on shaking for another hour at 30°C. Upon completion, 1 ml of 0.1 ppm GSSG added into the reaction mixture and incubated for another 1 hour at 30°C.

3.1.2.3 Characterization of synthesized AuNP and its sensory derivative

The prepared AuNPs was characterized using FTIR spectroscopy, UV-Vis spectroscopy, and dynamic light scattering (DLS) analysis. Citrate capping of AuNP surface was confirmed *via* FTIR spectroscopy using Varian 3100 Excalibur FTIR spectrometer with wavenumber ranging between 650 to 4500 cm^{-1}. SPR band of AuNP was observed *via* UV-Vis spectroscopy using Perkin-Elmer Cary 50 UV-Vis spectrophotometer in single beam mode with wavelength ranging from 300 to 700 nm. In order to quantify the particle size from the resulting UV-Vis spectrum, a formula

derived by Haiss et al. (2007) as shown in Equation 1, was used and compared against the hydrodynamic size of AuNP.

$$d(nm) = e^{3.55 \frac{A_{SPR}}{A_{450\ nm}} - 3.11} \quad \text{Eq. 1}$$

where; d = particle size of AuNP in nanometres,

A_{SPR} = maximum absorbance at SPR band,

$A_{450\ nm}$ = absorbance at 450 nm.

Meanwhile, the hydrodynamic size of AuNP was measured *via* DLS analysis using Micromeritics NanoPlus 3 zetasizer with one-minute runtime, 25°C operating temperature, and scattering angle at 90°.

3.2 Results and discussions

3.2.1 UV-Vis spectroscopy analysis of AuNP

UV-Vis spectroscopy analysis shown peak maxima of SPR band (500 – 550 nm) changed as the synthesis variables altered as shown in Figure 3.2. The decrease in peak maxima of SPR band clearly depicted in the UV-Vis spectra when the reaction utilized lower reaction temperature and higher gold chloride concentration. However, increasing sodium citrate concentration reduced the absorption at 518 nm.

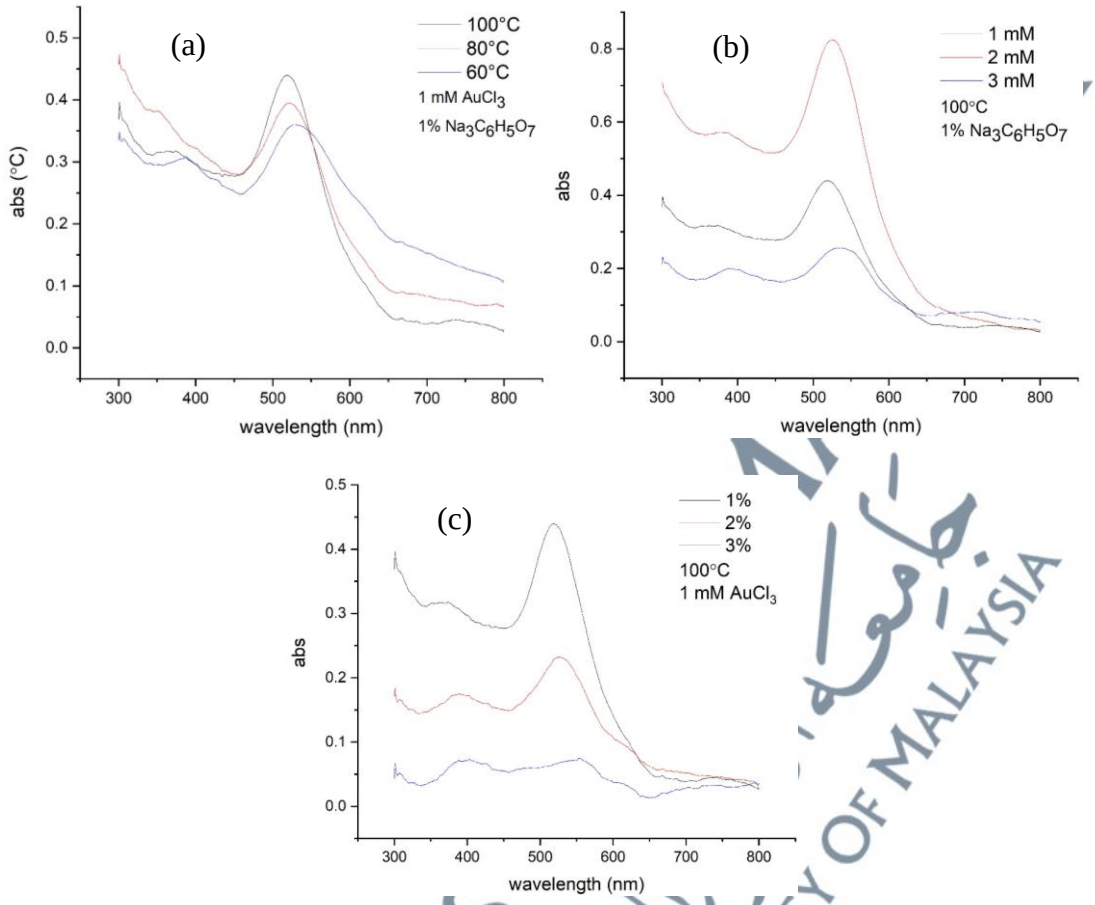

Figure 3.2: UV-Vis spectra of synthesized AuNP using different synthesis parameters: (a) reaction temperature; (b) gold chloride concentration; (c) sodium citrate concentration.

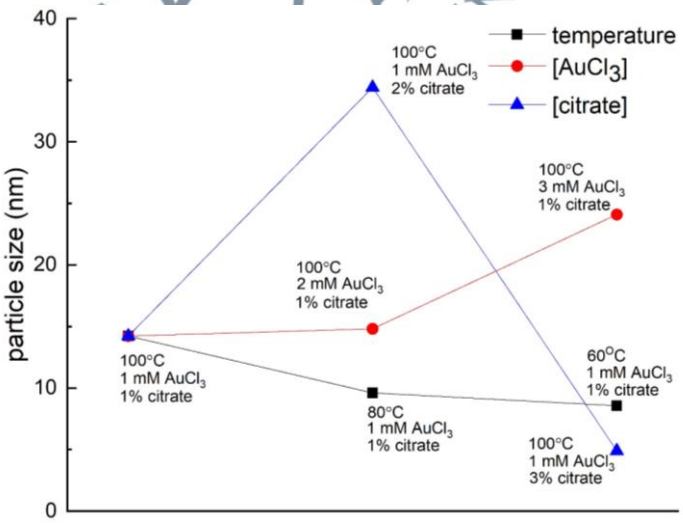

Figure 3.3: Calculated particle size of synthesized AuNPs using formula by Haiss et al. (2007) from the UV-Vis spectra.

Figure 3.2 shows the UV-Vis spectra of synthesized AuNPs using different synthesis parameters, while Figure 3.3 shows the calculated particle size of synthesized AuNPs using the formula derived by Haiss et al. (2007) from the UV-Vis spectra. Kiroula et al. (2016) and Tran et al. (2016) findings agreed on the maximum absorbance reduced insignificantly when the reaction temperature used is lowered. Reduction of maximum absorbance of the SPR band shown AuNPs with large size able to reduce the peak maxima.

The calculated particle size of AuNPs produced using varying concentration of gold chloride solution from 1.048 mM to 3.096 mM showed significant increase from 14.219 nm to 24.080 nm as shown in Figure 3.3. The increase in particle size was due to increase in the number of gold atoms available from 1.230×10^6 to 3.632×10^6 as nanoparticle synthesis precursor as shown in Table 3.2.

Table 3.2: Calculated number of gold atoms available in reaction solution

Mass of gold chloride used (mg)	Concentration of gold chloride solution (mM)	Mole of gold chloride used in 20 ml solution (mole)	Calculated number of gold atoms available in reaction solution*	Particle size (nm)
31.8	1.048	2.096×10^{-5}	1.230×10^6	14.219
65.5	2.159	4.318×10^{-5}	2.533×10^6	14.808
93.9	3.096	6.192×10^{-5}	3.632×10^6	24.080

*Number of gold atom = Mole of gold in solution × Avogadro constant (6.22×10^{23})

Meanwhile, varying the concentration of sodium citrate solution from 1.013 w/v% to 3.000 w/v% also caused the particle size of AuNPs to increase and decrease due to the amount of acting capping agent available to stabilize the formation of AuNPs, and the changing pH level of the reaction solution. Increasing the concentration of sodium citrate also raised the amount of citrate ions acting as the essential capping agent

for stabilization of AuNPs. Tran et al. (2016) previously discussed on the effects of molar ratio of sodium citrate to gold chloride on the resulting particle size of the synthesized AuNPs, commented on the fluctuations of resulting particle sizes occurred when the molar ratio was increased. Table 3.3 below shows the molar ratio of sodium citrate to gold chloride, calculated from the concentration of sodium citrate solution.

Table 3.3: Molar ratio of gold chloride to sodium citrate

Mass of sodium citrate used (g)	Concentration of sodium citrate solution (w/v %)	Concentration of sodium citrate solution (M)	Mole of citrate ion available in 2 ml solution (mole)	Molar ratio of sodium citrate to gold chloride[b]	Particle size (nm)
1.0127	1.013	0.0392	7.84×10^{-5}	3.7411	14.219
2.0046	2.005	0.0777	1.55×10^{-4}	7.3964	34.404
7.4999	3.000[a]	0.1163	2.33×10^{-4}	11.1111	4.891

[a]per 250 ml distilled water
[b]Molar ratio = moles of 2 ml of used concentration of sodium citrate to 20 ml of 1 mM of $AuCl_3$

As shown in Table 3.3, the molar ratio of sodium citrate to gold chloride shown to be increased from 3.7411 to 11.1111 along with increasing concentration of sodium citrate. Comparing to the calculation results of particle sizes as shown in Figure 3.3, the particle size reduced significantly as the molar ratio increased. High amount of capping agent available in reaction solution was able to stabilize the growth of AuNPs. However, as observed in Figure 3.3, using 2% sodium citrate solution as the reducing agent, the resulting particle size spiked from 14.2 nm to 34.4 nm, contradicted the aforementioned statement of higher citrate concentration yielded lower particle size. The occurrence of

large particle size using 2% sodium citrate solution can be explained using the findings by Kumar et al. (2007) in the equations below.

Oxidation of citrate to dicarboxy acetone;

$$3C_6H_5O_7^{3-} \overset{[O]}{\rightleftharpoons} 3C_5H_4O_5^{2-} + 3CO_2 + 3H^+ + 6e^- \quad \text{Eq. 2}$$

Reduction of Au (III) to Au (I);

$$3AuCl_3 + 6e^- \rightleftharpoons 3AuCl + 6Cl^- \quad \text{Eq. 3}$$

Disproportionation of Au(I) to elemental Au;

$$3AuCl \rightleftharpoons 2Au + AuCl_3 \quad \text{Eq. 4}$$

Overall reaction;

$$2AuCl_3 + 3C_6H_5O_7^{3-} \rightleftharpoons 2Au + 3C_5H_4O_5^{2-} + 3CO_2 + 3H^+ + 6Cl^- \quad \text{Eq. 5}$$

It was well understood that formation of AuNPs were due to disproportionation of gold (I) complex to metallic elemental gold as depicted in Equation 4. However, the gold (I) complex were generated from reduction of gold (III) complex as shown in Equation 3. Reduction of gold (III) complex was highly pH dependent as gold (III) complex are stable in high pH level, making the reduction rate to be reduced. As sodium citrate is known by its alkaline nature, increasing its concentration also raised the pH level thus making the gold (III) complex more stable and harder to reduce to gold (I) complex.

3.2.2 FTIR spectroscopy analysis of AuNP

FTIR spectroscopy of AuNPs can be used to determine any functional group changes by observing the changes in the IR spectrum of both reactant and product. In this study, FTIR spectroscopy was used to observe and confirm the citrate capping of the AuNPs surface. Confirming the surface citrate capping is essential for the intended sensory moiety to be functionalized onto the surface of AuNPs as can be observed in Figure 3.4.

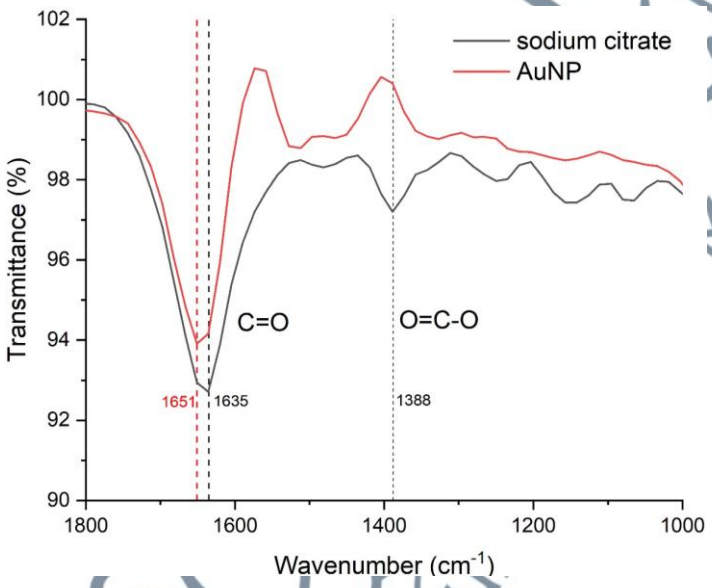

Figure 3.4: IR spectra of the synthesized AuNPs compared to sodium citrate solution in distilled water

Figure 3.4 shows the IR spectra of the synthesized AuNPs compared to sodium citrate solution in distilled water. The IR spectra shown peak diminishing on 1388 cm^{-1} and slight peak shifting at 1635 cm^{-1} to 1651 cm^{-1}. The results obtained was aligned with the findings of Wulandari et al. (2008, 2015) that characterized the interaction of AuNPs with citrate ions. Ionic bonding between sodium and citrate ion exhibited with a higher intensity peak at 1388 cm^{-1}. The peak intensity of AuNPs at 1388 cm^{-1} was significantly reduced, showing the formation of monodentate bonding of AuNP with

citrate ion. Furthermore, the peak at 1635 cm^{-1} slightly blue shifted to 1651 cm^{-1} further proved the monodentate bonding of citrate ion to AuNP as reported by Wulandari et al. (2008). Previous reports claimed MO calculations done using the experimental FTIR spectroscopy results shown citrate ions bonded to AuNPs *via* covalent monodentate bonding. Evidently, as the experimental results was aligned with the previous reports, AuNPs was successfully characterized and differentiated from its initial reactant using FTIR spectroscopy.

3.2.3 DLS measurement of AuNP

DLS is one of spectroscopic methods utilizing Brownian motion of particles in matrix solution to determine the size distributions of miniature particles, in this case, AuNPs. It uses light scattering, complemented with Stokes-Einstein equation to calculate the average hydrodynamic size of the particles in the analyte solution. In this study, DLS was used to render the intensity-weighted size distribution of the AuNPs as shown in Figure 3.5.

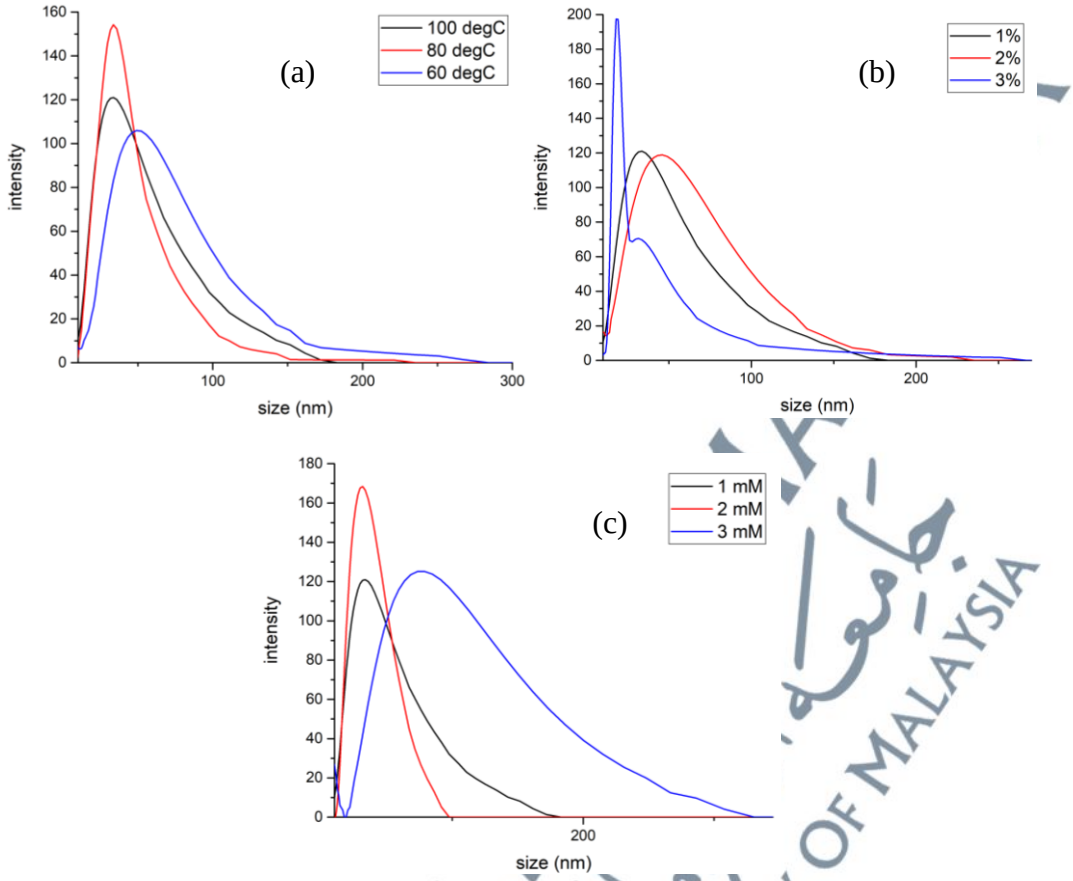

Figure 3.5: Intensity-weighted size distributions of synthesized AuNP using different synthesis parameters: (a) reaction temperature, (b) sodium citrate concentration, (c) gold chloride concentration

Table 3.4: Average hydrodynamic size of synthesized AuNPs along with its respective polydispersity index

Reaction temperature (°C)	Gold chloride concentration (mM)	Sodium citrate concentration (%)	Polydispersity index, PdI	Average hydrodynamic size (nm)
100	1	1	0.346	33
80	1	1	0.331	33
60	1	1	0.369	50
100	2	1	0.350	30
100	3	1	0.377	80
100	1	2	0.695	46
100	1	3	0.615	18, 30*

*two average values obtained from bimodal size distribution

Figure 3.5 shows the intensity-weighted size distribution of the synthesized AuNPs using different synthesis parameters. Meanwhile, Table 3.4 shows the average hydrodynamic sizes of synthesized AuNPs along with its polydispersity indices. Analysing the synthesized AuNPs *via* DLS yielded unimodal distribution curves, having a singular value of average hydrodynamic size while using 3% sodium citrate solution yielded bimodal distribution curve, possessed two average hydrodynamic sizes as summarized in Table 3.4. The broadness of the curves indicated the degree in deviations of individual particle sizes and can be explained by the polydispersity index of each synthesis.

As production of AuNPs yielded polydisperse nanoparticles as opposed to monodispersed nanoparticles produced by previous reported experimental procedures, the variations in individual particle sizes were quantified as polydispersity index. The polydispersity indices of the synthesized AuNPs were stated in Table 3.4 and ranged from 0.331 to 0.695, indicating all synthesis processes yielded AuNPs with adequately dispersed particle sizes. High polydispersity indices (PdI > 0.5) were recorded as the concentration of sodium citrate solution was increased from 1% to 3% in the synthesis process. In order to dictate the determining factor of influencing the polydispersity index, further study and analysis were necessary as Cavalli et al. (2009) mentioned multitudes of variable in the study affected the polydispersity index significantly.

3.2.4 Formation of disulfide-linked AuNPs as AuNPs-based sensor

In this study, functionalization of AuNPs was done *via* conjugation of AuNPs with GSSG compared to earlier procedure of Durocher et al. (2009) that uses Lomant's reagent as a crosslinking agent. The usage of 3,3-DTDPA and NHS, accompanied with

EDC HCl can be a lower-cost crosslinking alternative as suggested by Dutse et al. (2017). The alternative functioned the same as the Lomant's reagent used by Durocher et al. (2009) as 3,3-DTDPA, EDC HCl and NHS worked successively as its low cost replacement. Figure 3.6 shows a synthesis flow of AuNP-GSSG using the Lomant's reagent alternative.

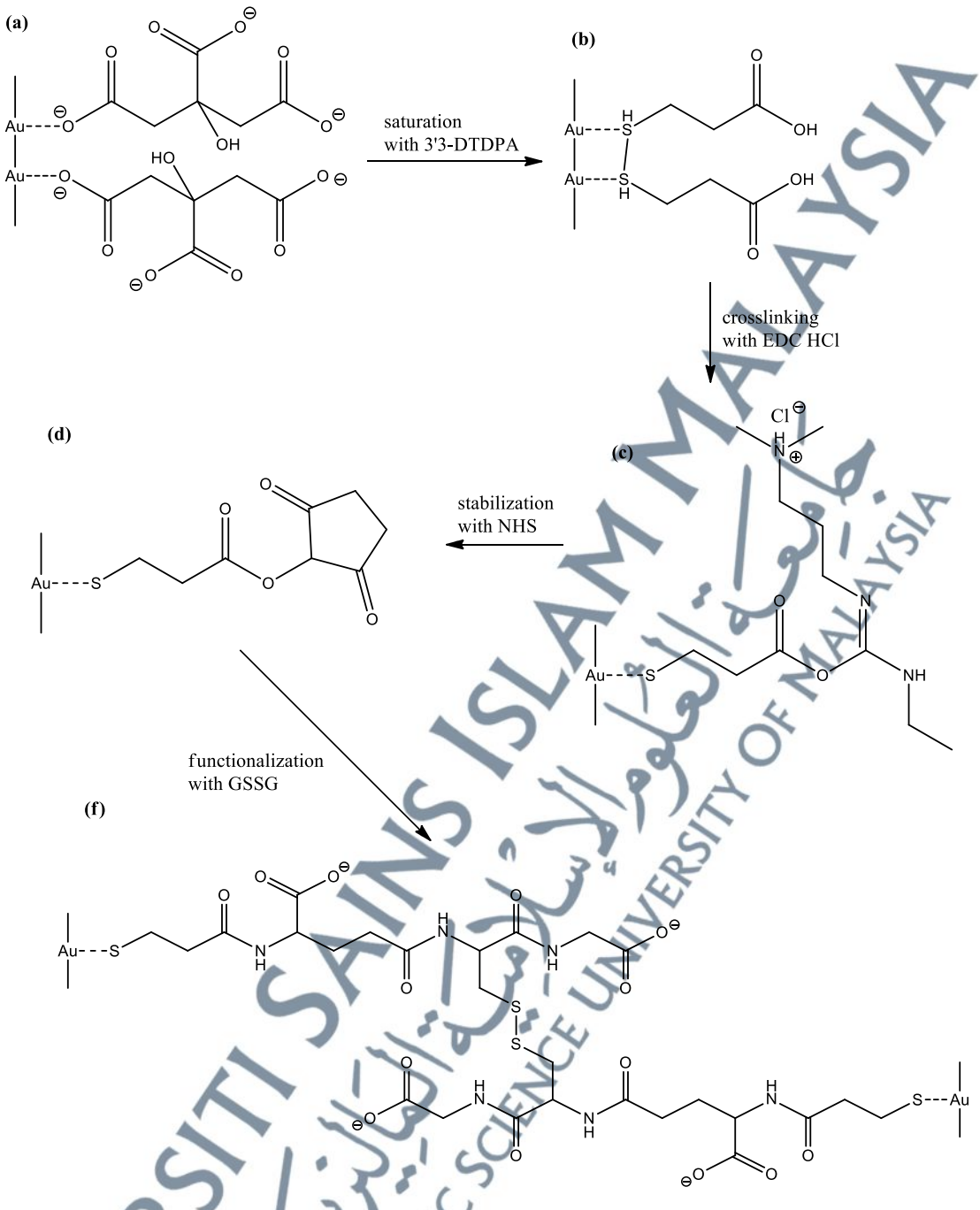

Figure 3.6: Synthesis of AuNP-GSSG using 3,3-DTDPA, EDC HCl and NHS to replace Lomant's reagent

Figure 3.6 describes the synthesis of AuNP-GSSG using 3,3-DTDPA, EDC HCl and NHS as a replacement for Lomant's reagent. The produced citrate-capped AuNPs (structure (a)) was first saturated with 3,3-DTDPA to form thiol capping and saturate the surface in the same time. As described by Fischer (2010), following usage of EDC

HCl functioned to form an active intermediate of O-acylisourea (structure (c)) after reacting with the carboxyl group at both ends of 3,3-DTDPA. However, due to its low stability in aqueous media, NHS introduced into the solution at the same time with EDC HCl to avoid the O-acylisourea to regenerate the carboxyl group and unable to functionalize with GSSG. Addition of NHS alongside EDC HCl formed an amine-reactive NHS ester (structure (d)), ready and available to be functionalized with GSSG to form structure (f).

Due to limitation of FTIR spectrometer to detect any changes in molecular structure of the functionalized AuNPs, UV-Vis spectroscopy was used to determine the functionalization of AuNPs as done by Durocher et al. (2009) and Rutherford et al. (2015). Successful functionalization with the sensing moiety will result in peak shifting that can be observed in the UV-Vis spectrum (Figure 3.7).

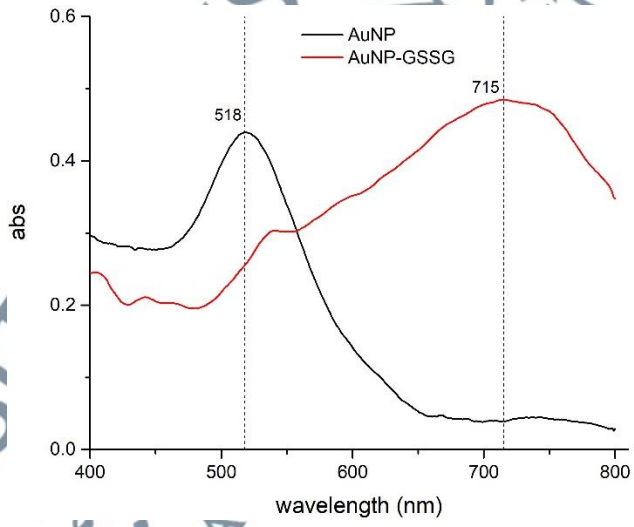

Figure 3.7: UV-Vis spectra of the synthesized AuNPs and the functionalized AuNPs with GSSG

Figure 3.7 shows the UV-Vis spectra of the synthesized AuNPs and the functionalized AuNPs as AuNP-GSSG. As shown in the UV-Vis spectra, the peak

maxima of AuNP-GSSG was red shifted because of successful conjugation with GSSG (518 – 715 nm). The red shifting occurred was caused by massive changes in the aggregation states of the AuNPs itself. The findings discussed by Mohd Yusri et al. (2019) stated that high aggregation state of AuNPs gave out similar effect to large resulting particle size, influencing the SPR band to be shifted or diminished. Apart from the changes of aggregation states, verifying successful conjugation of sensing moiety also discussed by Durocher et al. (2009) and Rutherford et al. (2015). The results showed altercations in form of peak shifting can be observed due to changes on the surface of AuNPs. Therefore, it can be concluded that the conjugation of GSSG to AuNPs was completed because the red shifting of the peak maxima in the UV-Vis spectra proven the high aggregation state as a result of successful sensing moiety conjugation.

3.3 Conclusion

From the result obtained, it was concluded that AuNPs synthesized using the citrate reduction method yielded AuNPs with larger particle sizes at lower reaction temperature and higher reactant concentrations. Calculation of particle size and DLS analysis shown the synthesized AuNPs were measured at 4.89 – 34.40 nm and 18 – 80 nm of particle size and average hydrodynamic size, respectively. FTIR spectroscopy analysis also shown positive confirmation on citrate capping for conjugation of GSSG on AuNPs. Furthermore, functionalization of AuNPs with GSSG was proven successful by the red shifting of the peak maxima in the UV-Vis spectra.