

CHAPTER III

EXPERIMENTAL

This chapter describes the instruments and equipment, the chemicals, the chemical preparation, sample preparation and detection method used in this work.

3.1 Modification of AgNPLs with GSH and Cys

3.1.1 Instruments and equipment

The instruments and equipment for the modification of AgNPLs using GSH and Cys are listed in Table 3.1.

Table 3.1 List of instruments and equipment for the modification of AgNPLs using GSH and Cys.

Instruments/equipments	Suppliers
Milli-Q Water System, $R \geq 18.2 \text{ M}\Omega\text{cm}^{-1}$	Merck Millipore, Germany
Stirrer	Metrohm, Switzerland
Magnetic stirring bars, $8 \times 3 \text{ mm}$	SGS ICS, Switzerland
Stop watch	Mini Timer, Japan
Analytical balance, Mettler Toledo AB204-S	Mettler, Switzerland
Autopipette and tips	Eppendorf, Germany

3.1.2 Chemicals

The chemicals for the modification of AgNPLs using GSH and Cys are listed in Table 3.2

Table 3.2 List of chemicals used for the modification of AgNPLs using GSH and Cys.

Chemicals	Suppliers
L-Glutathione	Sigma Aldrich, USA
L-Cysteine	Sigma Aldrich, USA
Silver nanoplates (AgNPLs)	Sensor Research Unit at the Department of Chemistry, Faculty of Science, Chulalongkorn University

3.1.3 Chemical preparation

3.1.3.1 50 mM glutathione solution (25 mL)

50 mM glutathione solution was prepared using 0.0768 g of glutathione dissolved in 25 mL of Milli-Q water. After that, the solution was diluted with Milli-Q water to final concentration of glutathione at 50 mM.

3.1.3.2 50 mM cysteine solution (25 mL)

50 mM cysteine solution was prepared using 0.0303 g of cysteine dissolved in 25 mL of Milli-Q water. After that, the solution was diluted with Milli-Q water to final concentration of cysteine at 50 mM.

3.1.4 Methodology

The modified-AgNPLs was prepared by mixing 2.70 mL of 1.85 mM AgNPLs, 1000 μ L of 50 mM GSH, and 5000 μ L of 50 mM Cys into 41.30 mL of Milli-Q water. Next, the mixture solution was left to stir for about 2 h to ensure a complete self-assembly of the GSH and Cys onto the surface of AgNPLs.

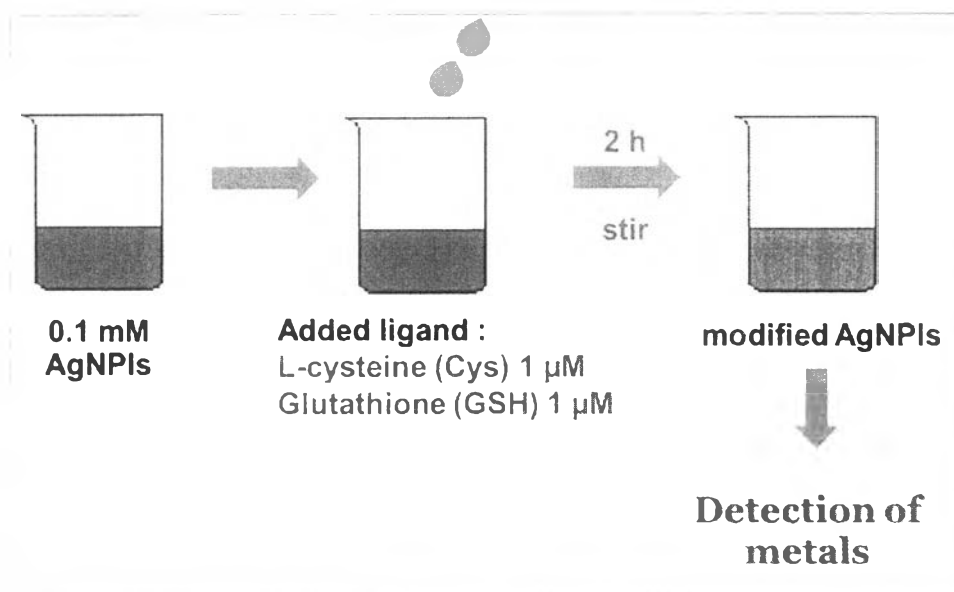


Figure 3.1 Schematic diagram for the modification of AgNPs with GSH and Cys.

3.2 Characterization of GSH-Cys-AgNPs

3.2.1 Instruments and equipments

The instruments and equipments for the characterization of GSH-Cys-AgNPs are listed in Table 3.3.

Table 3.3 List of instruments and equipments for the characterization of GSH-Cys-AgNPs.

Instruments/equipments	Suppliers
Milli-Q Water System, $R \geq 18.2 \text{ M}\Omega\text{cm}^{-1}$	Merck Millipore, Germany
UV-visible spectrometer, HP HEWLETT PACKARD 8453	Agilent Technologies, UK
Autopipette and tips	Eppendorf, Germany
Fourier transform infrared spectroscopy, Nicolet 6700	Nicolet, USA
Centrifuge tube	SPL Life Sciences, Korea

Instruments/equipments	Suppliers
Hettich centrifuge, Mikro 120	Hettich, UK
Freeze dryer, Freezeone 77520 Benchtop	Labconco, USA
Stop watch	Mini Timer, Japan
Cuvette quartz cell, 1 cm	

3.2.2 Methodology

The incorporation of GSH and Cys onto the AgNPLs surface was investigated using fourier transform infrared spectroscopy (FTIR) and UV-vis spectrophotometer for the identification of functional groups and absorption measurement, respectively. GSH-Cys-AgNPLs was initially suspended and then freeze dried. The FTIR spectra was recorded using KBr discs (Nicolet 6700) with 32 scans at resolution 4 cm^{-1} . A frequency of $400\text{-}4000\text{ cm}^{-1}$ was scanned using triglycine sulphate (TGS) detector. For the UV-vis spectra, the GSH-Cys-AgNPLs suspended in water was recorded using a UV-vis spectrophotometer (HEWLETT PACKARD 8453).

3.3 GSH-Cys-AgNPLs based colorimetric detection of nickel

3.3.1 Instruments and equipment

The instruments and equipments for the GSH-Cys-AgNPLs based colorimetric detection of nickel are listed in Table 3.4.

Table 3.4 List of instruments and equipments for the GSH-Cys-AgNPLs based colorimetric detection of nickel.

Instruments/equipments	Suppliers
Milli-Q Water System, $R \geq 18.2 \text{ M}\Omega\text{cm}^{-1}$	Merck Millipore, Germany
UV-visible spectrometer, HP HEWLETT PACKARD 8453	Agilent Technologies, UK
Autopipette and tips	Eppendorf, Germany
pH meter, S220 SevenCompact™ pH/Ion	Mettler Toledo, Switzerland
Stop watch	Mini Timer, Japan
Cuvette quartz cell, 1 cm	

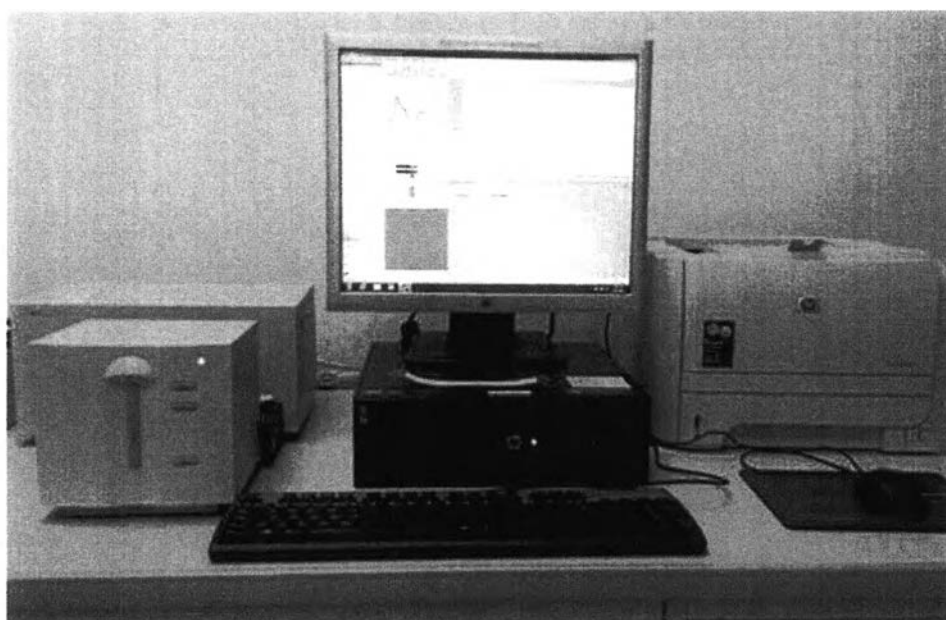


Figure 3.2 UV-visible spectrometer HP HEWLETT PACKARD 8453 (Agilent Technologies, UK).

3.3.2 Chemicals

The chemicals for the GSH-Cys-AgNPLs based colorimetric detection of nickel are listed in Table 3.5. All reagents are analytical grade. The $18 \text{ M}\Omega \text{ cm}^{-1}$ resistance water, obtained from a Millipore Milli-Q purification system, was used to prepare solution in this experiment.

Table 3.5 List of Chemicals for the GSH-Cys-AgNPLs based colorimetric detection of nickel.

Chemicals	Suppliers
Nickel standard solution 1000 mg/L	Merck, Germany
Mercury standard solution 1000 mg/L	Spectrosal, UK
Arsenic standard solution 1000 mg/L	Spectrosal, UK
Cobalt standard solution 1000 mg/L	Spectrosal, UK
Zinc standard solution 1000 mg/L	Merck, Germany
Gold standard solution 1000 mg/L	Merck, Germany
Platinum standard solution 1000 mg/L	Merck, Germany
Rhodium standard solution 1000 mg/L	Merck, Germany
Silver standard solution 1000 mg/L	Merck, Germany
Iron sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	Merck, Germany
Copper sulphate (CuSO_4)	Analar, France
Cadmium sulphate (CdSO_4)	Baker Analyzed, USA
Lead nitrate ($\text{Pb}(\text{NO}_3)_2$)	Spectrosal, UK
Sodium chloride (NaCl)	Merck, Germany
Potassium chloride (KCl)	Univar, USA
Calcium chloride (CaCl_2)	Laboratory chemicals, USA
Magnesium sulphate (MgSO_4)	Sigma-Aldrich, USA
Disodium hydrogen phosphate (Na_2HPO_4)	Merck, Germany
Potassium dihydrogen phosphate (KH_2PO_4)	Sigma-Aldrich, USA
Sodium hydroxide (NaOH)	Merck, Germany
Ortho-phosphoric acid (H_3PO_4) 85%	Merck, Germany
Nitric acid (HNO_3) 65%	Merck, Germany
GSH-Cys-AgNPLs solution	

3.3.3 Chemical preparation

3.3.3.1 0.05 M phosphate buffer pH 8.0 (500 mL)

0.05 M phosphate buffer was prepared by mixing 0.05 M 18.50 mL of KH_2PO_4 and 0.05 M 481.50 mL of Na_2HPO_4 . After that, the mixture solution was adjusted to pH 8.0 using H_3PO_4 and NaOH .

3.3.3.2 0.1 M nitric acid solution (1000 mL)

0.1 M nitric acid solution was prepared by dilution of 65% nitric acid with Milli-Q water to the final concentration of 0.1 M.

3.3.3.3 Nickel stock solution (25 mL)

A stock solution of 70 mg/L nickel was prepared by dilution of 1000 mg/L nickel standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.4 Mercury stock solution (25 mL)

A stock solution of 70 mg/L mercury solution was prepared by dilution of 1000 mg/L mercury standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.5 Arsenic stock solution (25 mL)

A stock solution of 70 mg/L arsenic solution was prepared by dilution of 1000 mg/L arsenic standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.6 Cobalt stock solution (25 mL)

A stock solution of 70 mg/L cobalt solution was prepared by dilution of 1000 mg/L cobalt standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.7 Zinc stock solution (25 mL)

A stock solution of 70 mg/L zinc solution was prepared by dilution of 1000 mg/L zinc standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.8 Gold stock solution (25 mL)

A stock solution of 70 mg/L manganese solution was prepared by dilution of 1000 mg/L manganese standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.9 Platinum stock solution (25 mL)

A stock solution of 70 mg/L platinum solution was prepared by dilution of 1000 mg/L platinum standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.10 Rhodium stock solution (25 mL)

A stock solution of 70 mg/L rhodium solution was prepared by dilution of 1000 mg/L rhodium standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.



3.3.3.11 Silver stock solution (25 mL)

A stock solution of 70 mg/L silver solution was prepared by dilution of 1000 mg/L silver standard solution with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.12 Iron (III) stock solution (25 mL)

A stock solution of 70 mg/L iron (III) solution was prepared using 0.12 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.13 Iron (II) stock solution (25 mL)

A stock solution of 70 mg/L iron (II) solution was prepared using 0.12 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.14 Copper stock solution (25 mL)

A stock solution of 70 mg/L copper solution was prepared using 0.06 g of CuSO_4 dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.15 Cadmium stock solution (25 mL)

A stock solution of 70 mg/L cadmium solution was prepared using 0.17 g of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.16 Lead stock solution (25 mL)

A stock solution of 70 mg/L lead solution was prepared using 0.04 g of $\text{Pb}(\text{NO}_3)_2$ dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.17 Sodium stock solution (25 mL)

A stock solution of 70 mg/L sodium solution was prepared using 0.06 g of NaCl dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.18 Potassium stock solution (25 mL)

A stock solution of 70 mg/L potassium solution was prepared using 0.05 g of KCl dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.19 Calcium stock solution (25 mL)

A stock solution of 70 mg/L calcium solution was prepared using 0.07 g of CaCl_2 dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.

3.3.3.20 Magnesium stock solution (25 mL)

A stock solution of 70 mg/L magnesium solution was prepared using 0.12 g of MgSO_4 dissolved in 25 mL of 0.1 M HNO_3 . After that, the solution was diluted with 0.05 M phosphate buffer pH 8.0 to the final concentration of 70 mg/L.



3.3.4 Methodology

The colorimetric detection of nickel solution was performed at room temperature by mixing 1500 μL of 70 ppb nickel solution and 1500 μL of the GSH-Cys-AgNPLs in conventional 1 cm cuvette quartz cell. For the optimization of Ni(II) detection using UV-vis spectroscopy, 3 significant parameters including modifier ratio, effect of pH, and incubation times were examined. The ratio of modifying agent (GSH and Cys) for AgNPLs was varied within the range of 0.5-5 μM . The effect of pH on the aggregation of GSH-Cys-AgNPLs and Ni(II) was studied over the pH range from 2.5 to 11.0. Next, the aggregation of AgNPLs induced by Ni(II) was investigated at various incubation times (0-10 min) by using absorption measurement at 618 nm. The optimal conditions consisting of GSH and Cys concentration ratio of 1:5, pH 8.0, and incubation time at 6 min were selected and used for further experiments (see results and discussion in the chapter IV).

To investigate the selectivity of the colorimetric detection toward Ni(II) using GSH-Cys-AgNPLs, sixteen different metal ions (Hg(II), As(III), Co(II), Zn(II), Au(III), Pt(II), Rh(II), Ag(I), Fe(III), Fe(II), Cu(II), Cd(II), Pb(II), Na(I), K(I), Ca(II) and Mg(II)) were introduced as common ions and analyzed under the optimal conditions.

3.4 Characterization of GSH-Cys-AgNPLs aggregated with nickel

3.4.1 Instruments and equipments

The instruments and equipments for the characterization of GSH-Cys-AgNPLs aggregated with nickel are listed in Table 3.6.

Table 3.6 List of instruments and equipments for the characterization of GSH-Cys-AgNPLs aggregated with nickel.

Instruments/equipments	Suppliers
Milli-Q Water System, $R \geq 18.2 \text{ M}\Omega\text{cm}^{-1}$	Merck Millipore, Germany
UV-visible spectrometer, HP HEWLETT PACKARD 8453	Agilent Technologies, UK
Transmission electron microscope, H-7650	Hitachi Model, Japan
Autopipette and tips	Eppendorf, Germany
Stop watch	Mini Timer, Japan
Cuvette quartz cell, 1 cm	

3.4.2 Methodology

The aggregation of GSH-Cys-AgNPLs induced by Ni(II) was studied using transmission electron microscope (TEM) and UV-vis spectroscopy for morphology and absorbance, respectively. The reaction between GSH-Cys-AgNPLs and Ni(II) were monitored using a UV-vis spectrophotometer (HP HEWLETT PACKARD 8453) (see the results and discussion in the chapter IV). For the TEM characterization of GSH-Cys-AgNPLs aggregation, GSH-Cys-AgNPLs and Ni(II) were mixed. And after 6 min, a drop of colloidal suspension was placed on a carbon-coated copper grid. This colloidal suspension was allowed to dry at room temperature and inspected by TEM operated at 200 kV (Hitachi Model H-7650).

3.5 Analytical performance

3.5.1 Linearity

The absorption of GSH-Cys-AgNPLs mixed with Ni(II) standard solution in the concentration range of 10-150 ppb was measured using UV-vis spectrophotometer (HP HEWLETT PACKARD 8453) under the optimal conditions. The average values of triplicate measurements were used to plot calibration curve between 10 to 150 ppb from which the linear range can be obtained.

3.5.2 Limit of detection (LOD)

The limit of detection (LOD) was determined by statistical method from the calibration curve in the range of 10-150 ppb and calculated from $3SD_{bl}/S$. SD_{bl} is the standard deviation of blank measurement ($n=11$), and S is the sensitivity of the method obtained as the slope of the linearity.

3.5.3 Limit of quantitation (LOQ)

The limit of quantitation (LOQ) was determined by statistical method from the calibration curve in the range of 10-150 ppb and calculated from $10SD_{bl}/S$. SD_{bl} is the standard deviation of blank measurement ($n=11$), and S is the sensitivity of the method obtained as the slope of the linearity.

3.5.4 Repeatability

The repeatability was studied by eleven replicated measurements of the analyte solution. The repeatability was assessed in terms of the relative standard deviation (% RSD), using the following formula:

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

3.6 The determination of nickel in real sample

3.6.1 Instruments and equipments

The instruments and equipments for the determination of nickel in real sample are listed in Table 3.7.

Table 3.7 List of instruments and equipments for the determination of nickel in real sample.

Instruments/equipments	Suppliers
Milli-Q Water System, $R \geq 18.2 \text{ M}\Omega\text{cm}^{-1}$	Merck Millipore, Germany
UV-visible spectrometer, HP HEWLETT PACKARD 8453	Agilent Technologies, UK
iCAP 6000series inductively coupled plasma optical emission spectrometer	Thermo Fisher Scientific, USA
pH meter, S220 SevenCompact™ pH/Ion	Mettler Toledo, Switzerland
Hot plate, C-MAG HS 10	IKA®, Thailand
Ultrasonic steri-cleaner	Sturdy, Taiwan
Autopipette and tips	Eppendorf, Germany
Stop watch	Mini Timer, Japan
Cuvette quartz cell, 1 cm	

3.6.2 Chemicals

The Chemicals for the determination of nickel in real sample are listed in Table 3.8. All reagents are analytical grade. The $18 \text{ M}\Omega \text{ cm}^{-1}$ resistance water, obtained

from a Millipore Milli-Q purification system, was used to prepare solution in this experiment.

Table 3.8 List of Chemicals for the determination of nickel in real sample.

Chemicals	Suppliers
Nitric acid (HNO ₃) 65%	Merck, Germany
Gold plating solution	Gem and Jewelry Institute of Thailand (Public Organization)
GSH-Cys-AgNPLs	

3.6.3 Sample preparation and validation of proposed method with ICP-OES

The jewelry plating sample solution was prepared by mixing with HNO₃, and the solution was heated at 200 °C to evaporate the solution. After that, the sample was made up with Milli-Q water and heated at 100 °C until dryness (repeated three times). Then, 0.05 M phosphate buffer pH 8 was added into the digested sample. Next, the pH samples were adjusted to pH 8.0 by the addition of 3 M sodium hydroxide. The sample was then sonicated for 30 min and clear solution obtained was ready for analysis. Under the optimal conditions, the proposed method was applied for the determination of nickel in these jewelry plating solutions. The results of this proposed method was validated by ICP-OES method.