GREEN SYNTHESIS OF GOLD NANOPARTICLES USING PALM OIL LEAF EXTRACT

by

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14893

Dissertation submitted in partial fulfilment of
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CERTIFICATION OF APPROVAL

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Approved by

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(AP. Dr. Sekhar Bhattacharjee)

UNIVERSITI TEKNOLOGI PETRONAS

January 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my on except as specified in the references and acknowledgement, and that the original work contained herein have not been undertaken or done by unspecified sources or persons

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(FATIN NASUHA BINTI MOHD TAJUDIN)

ABSTRACT

The present study reports the green synthesis of gold nanoparticles (AuNPs) in which palm oil leaf extract was used as reducing agent to reduce gold ions in chloroauric acid into AuNPs. Palm oil leaf is chosen because it possesses anti-oxidant properties, under-utilised and considered as a major waste in palm oil industry. The synthesized AuNPs were characterized using UV-Vis spectroscopy, Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). The effect of varying concentration of chloroauric acid, volume ratio, temperature and reaction time towards the morphology and size of synthesized AuNPs were analyzed. TEM imaging done of the sample confirms the presence of polydispersed AuNPs with mean diameter of 29.49 nm. In addition, the synthesized AuNPs composed of hexagonal and orthorhombic crystalline structures. Bio-molecules in palm oil leaf extract such as protein, flavonoids or phenols that contain abundant of C=O, N-H or O-H groups might be accountable for the reductions of gold ions into AuNPs because decrease in intensity of gold solution was observed at 3349.79 cm^{-1}.

ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

1.1 Background

Nanotechnology is expected to be the foundation of many main technological innovations of the 21st century. According to Dubey, Lahtinen and Sillanpaa (2010), particle less than 100 nm in size are generally known as nanoparticles. Recently, the demand for nanoparticles has been greatly increased due to its wide applications such as biomedicine, catalysis, bio-sensing, electronic and magnetic devices (Gan et al., 2012).

Synthesis of metal nanoparticles such as gold nanoparticles (AuNPs) have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties (Bar et al., 2009; Tuutijarvi et al., 2009; Rassaei et al., 2008). There are several physical and chemical methods have been developed to synthesize well-defined metal nanoparticles. However, the energy intensive chemical and physical methods of synthesizing AuNPs are not favoured when nanoparticles are to be used in medical application because it involves toxic and aggressive chemicals as reducing and capping agents (Milaneze et al., 2013). As a result, there is a possibility of producing residual toxic chemicals and by-products which might adsorbed on the surface of nanoparticles (Gan et al., 2012).

Therefore, biosynthesis of AuNPs has become a major focus of researchers in order to overcome those problems. As an alternative, microbes, fungi and plant extracts can be used to replace the role of hazardous reducing and capping agents. Among all, plants seem to be more feasible in producing large amount of nanoparticles and it can potentially serve as long-lasting and environmentally

benign reservoirs as compared to microorganisms. Another advantage of using plant extract in synthesizing AuNPs is it contains phytochemicals, minerals, vitamins and proteins which are capable to serve as reducing and stabilizing agents (Chakraborty et al., 2013).

Though green synthesis of AuNPs using plants extracts has been carried out by many scientist and researchers, very limited attempt was done to synthesize AuNPs using biomass. Most of developed green synthesis approaches are using plant species which are difficult to be found in Malaysisa such as Morinda citrifolia L, Terminalia arjuna and Rosa rugosa. So far there is no report on the use of palm oil (elais guineensis) leaf extract as a reducing agent in synthesizing AuNPs.

Therefore, this study will explore the possibility of synthesizing AuNPs using palm oil leaf extract as an alternative to the available methods. In addition, the palm oil leaf are chosen over the other parts of the palm oil tree because it possesses anti-oxidant properties (Yin et al., 2013), under-utilised and considered as a major waste in the oil palm industry.

1.2 Problem Statement

Some of gold nanoparticles (AuNPs) produced through physical and chemical methods are not safe to be used in biomedical industry since the synthesis process involves hazardous chemicals as reducing and stabilizing agents. Therefore, scientists and researchers are currently working on developing green approaches of synthesizing AuNPs. However, most of the established green approaches used plant species which are difficult to find or have other significant roles. Hence, there is a need for developing an approach of synthesizing AuNPs using plant species or biomass which are widely available specifically in Malaysia.

1.3 Objective of Study

Objectives of this study are as follows:

- To synthesize gold nanoparticles (AuNPs) using palm oil leaf extract.

- To study morphological characteristics of the synthesized gold nanoparticles.

1.4 Scope of Study

Gold nanoparticles (AuNPs) in this study are synthesized by reducing chloroauric acid ($HAuCl_4$) with palm oil leaf extract. The optimum conditions for the formation of AuNPs will be determined by manipulating reaction parameters such as concentration of acid, volume of leaf extract, temperature and reaction time. Characterization on the synthesized AuNPs will be done using UV-Visible Spectrophotometer, Fourier Transform Infrared (FTIR) and Transmission Electron Microscopy (TEM).

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of Gold Nanoparticles

Gold nanoparticles (AuNPs) own a unique and tuneable Surface Plasmon Resonance (Philip, 2009). Being size-dependent, the Surface Plasmon Resonance (SPR) of AuNPs is very sensitive to any changes in refractive index of the medium surrounding the particles. Every species adsorbed on the surface of nanoparticles will exhibit a colour change which will eventually shift the position of SPR peak proportional to the magnitude of refractive index change near the nanoparticles surface (Pedersen et al., 2005). Colloidal solution of AuNPs is reported to have a distinctive red colour with absorbance spectra between 510 nm to 550 nm (Abdelhalim et al., 2012).

Other than that, AuNPs also have a unique optical and electrical property (Sujitha et al., 2012). AuNPs are well known as excellent scatterers and absorbers of visible light due to their strongly enhanced Surface Plasmon Resonance at optical frequencies. Besides that, the optical properties of the AuNPs can be tuned simply by manipulating the size and shape of the AuNPs (Jain et al., 2006). Unlike metal in a bulk form, AuNPs also exhibit excellent catalytic activity due to their high surface area to volume ratio and their interface-dominated properties.

2.2 Application of Gold Nanoparticles

Gold nanoparticles (AuNPs) are versatile materials with a broad range of applications in a variety of fields which include medicine, catalysis and electronics (Amin et al., 2013). One of significant applications of AuNPs is in the

detection and treatment of cancer cells (Soppimath and Betageri, 2008). Unlike current methods of cancer diagnosis and treatment which are harmful and costly, AuNPs provide an inexpensive and safer route for targeting only cancerous cells without harming the other healthy cells (Pellequer and Lamprecht, 2009).

In photodynamic therapy or also known as hyperthermia therapy, AuNPs will be injected first to the infected body and accumulate at the tumor site. AuNPs will release heat when they are excited by laser light at wavelengths ranging from 700 to 800 nm. As a result, the localized heat produced by the AuNPs will eradicate the targeted tumors (Pattani et al., 2012).

Other than that, AuNPs also serve as contrast agents in cellular and biological imaging. Cellular imaging is crucial in diagnosis of cancer and several other disorders in which it utilizes microscopy techniques and optical contrast agents to provide anatomic details of cells and tissue architecture (Jain et al., 2006).

Currently in electronic industries, AuNPs are mainly being used to connect resistors, conductors, and other elements of an electronic chip (Chandra et al., 2013). Magnetic and optical properties own by the AuNPs are also useful for data storage. Therefore according to Rowles, there are extensive researches being done to incorporate AuNPs into CDs, DVDs and flash memory devices, which would improve their storage capacity.

In addition, AuNPs are widely being utilized in the design and development of biological sensors (Chakraborty et al., 2013). For example, AuNPs are used in colorimetric sensor in order to determine the suitability of foods for consumption (Chandra et al., 2013).

Apart from that, AuNPs also has a major application in catalysis industries due to their large surface area. AuNPs serve as catalysts in several chemical reactions in which the surface of AuNPs will be used for oxidation or reduction of certain chemicals (Chandra et al., 2013).

2.3 Plant Extraction

Green synthesis of gold nanoparticles (AuNPs) using plant extract is considered as cost-effective approach as it normally utilized biomass such as leaves, roots and fruit peels just to name a few. The plant was normally dried using various methods such as sun-dried, oven-dried or freeze-dried in order to ensure complete removal of water. This is because Naithani, Nair and Kakkar (2006) claimed that antioxidant content of the plant which might act as reducing and stabilizing agents reduced at low rate when it was totally dried.

According to Nurul et al., (2014) grinding of dried plant into powder was normally done in order to increase the surface area hence increasing the extraction rate. Besides that, type of solvent used also play a vital role in determining the success of extraction process. Compounds with similar polarity will be extracted once the solvent diffused into plant tissues. Hence, the amount of reducing agent extracted is greatly dependant on type of solvents used. Reis, Rai and Abu Ghannam (2012) claim that water extraction was favoured over organic solvents due to its simplicity as well as to avoid contamination.

On the other hand, concentration of antioxidant can be increased by increasing the temperature during the extraction process. However, the antioxidant was reported to degrade at temperature higher than 90 °C (Yu et al., 2012). Therefore, proper extraction temperature should be selected in order to obtain optimum yield in reducing agents.

2.4 Synthesis of Gold Nanoparticles

Most common synthesis method of AuNPs is through reduction of metal salts using citrates, as introduced by Turkevich (Pedersen et al., 2005). In the synthesis process via chemical, physical or photochemical methods, Au^{3+} in chloroauric acid ($HAuCl_4$) will be reduced to Au^0 with the aid of a reducing agent (Dubey et al., 2010).

Chemical methods normally involve the use of hazardous solvents and harmful reducing agents such as sodium borohydride (Van Hyning and Zukoski, 1998) and hydrazine (Guzman et al., 2009). In addition, most of AuNPs produced

through these methods are unstable and therefore require a stabilizing agent in the process of synthesis. As a result, some toxic chemical species might be absorbed on the surface of AuNPs and may cause adverse effect if it is being used in medical applications (Gan et al., 2012).

Therefore, biosynthesis of AuNPs using microorganisms or plants could be advantageous since biomass is capable of secreting biomolecules which can act as both reducing and capping agents during the reaction and hence, producing AuNPs which are more biocompatible (Lukman et al., 2011). However, microbial synthesis is not preferred as compared to plant because it is time consuming, tedious, and the size distribution, shape and crystallinity of synthesized AuNPs are hard to control (Narayanan and Sakthivel, 2010).

On the other hand, plant is favoured for biosynthesis of AuNPs because they contain natural reducing agent such as citric acid, ascorbic acid, flavonoids, reductases and dehydrogenases and extracellular electron shuttlers that might play significant role in synthesizing AuNPs (Pandey et al., 2012). It is also reported that, nanoparticles produced by plants are more stable and the rate of synthesis is much faster compared to the one using microorganisms (Philip et al., 2012). Due to that, green synthesis of AuNPs using various type of plant extract has received tremendous attention among scientist and chemist in the recent years.

TABLE 1 shows some of previous research done on the synthesis of AuNPs using various plant extracts and the size of resulting AuNPs:

Table 2.1: Plant species used in synthesizing AuNPs

No	Plant Species	Size of AuNPs (nm)	Reference
1	*Cinnamomum zeylanicum*	25	Smitha et al. (2009)
2	*Mangifera indica*	20	Philip (2010)
3	*Rosa rugosa*	11	Dubey et al. (2010)
4	*Momordica charantia*	10 – 100	Pandey et al. (2012)
5	*Plantago ovato*	8 – 30	Amin et al., (2013)
6	*Citrus limon, Citrus reticulate, Citrus sinensis*	15 – 60	Sujitha and Kannan (2012)
7	*Moringa oleifera*	20 – 60	Chakraborty et al. (2013)
8	*Mimosa pudica*	40	Iram et al., (2014)
9	*Pheonix dactylifera L.*	32 – 45	Zayed and Eisa (2014)
10	*Kiwi*	boiling water: 10-20 room temperature: 5-50	Ying et al., 2014

The size, shape and stability of AuNPs produced are greatly dependents on reaction parameters during the synthesis process. According to Iram et al (2014) in their study using *Mimosa Pudica*, the smallest nanoparticles size is obtained by optimizing the reaction conditions such as amount of extract, pH, reaction time and temperature. The above statement is proved by Ying et al (2014) in their study of synthesizing AuNPs using kiwi fruit as a reducing agent wherein an analysis done using TEM proved that the diameter of AuNPs synthesized in boiling water is in the range of 10–20 nm, while the one synthesized at room temperature are observed to be in the range of 5–50 nm. This finding proves that the reaction temperature have a significant role in controlling the size distribution of synthesized AuNPs as well.

According to Pedersen et al., (2005), aggregations and precipitations tend to occur as the particle size increases thus making the AuNPs unstable. However, the stability of synthesized AuNPs can be controlled by adjusting certain reaction parameters such as the temperature and pH. As for AuNPs produced using kiwi extract, they were initially unstable but turn out to be stable for more than 2 months after adjusting the pH value to 8.

2.5 Palm Oil Tree (*Elaeis Guineensis*)

Figure 2.1: Palm Oil Tree

Palm oil tree or scientifically known as Elaeis Guineensis is classified as tropical tree which normally grown and harvested for industrial production of vegetable oil. According to Verheye (n.d), oil is normally found concentrated in the fruit pulp as well as in the fruit kernel. Oil content in the fruit pulp is around 50 % to 60 % while 48 % to 52 % in the fruit kernel.

Currently, Malaysia is ranked as the world's largest producer of palm oil whereby most of the palm oil produced is being exported to more than 170 countries worldwide (Yean et al., 2012).

Palm oil is a perennial crop which has a lifespan of 30 years. The tree produces highest crop yields at the age of 9 to18 years and the yield will gradually decrease thereafter. Therefore, re-planting is recommended once the yield of the old palm oil tree has drops significantly (Shean, 2014). According to Abdullah and Sulaiman (2013), one major drawback of re-planting process is the huge amount of waste generated. It has been reported that, around 8.36 million tonnes dried biomass which composed of 7.02 million tonnes of trunk and 1.34 million tonnes of leaves will be generated at one time of re-planting process.

In the recent years, there have been several researches done on the palm oil leaf as an effort to transform the waste into valuable products. It is reported that juice squeezed from oil palm leaves is capable to enhance wound healing process (Sasidharan et al., 2012). Other than that, the palm oil leaf is also useful for treatment of cancer, rheumatism, headaches as well as an aphrodisiac liniment and diuretic (Irvin, 1985). Therefore, interests in investigating the constituent of the palm oil leaf have grown over the past few years since it possesses a lot of medical values (Yin et al., 2013).

In addition, the palm oil leaf is reported to contain levelled amount of bioactive compounds which are more diverse and higher in concentration compared to oil palm fruit (Ibraheem et al., 2012). Phytochemical screening done on the palm oil leaf extract confirms the presence of phenolic, flavonoids, tannins, coumarins, alkaloids, saponins, terpenoids and steroids as phytochemical constituents in palm oil leaf which could be responsible for the biological activities. Besides, the high content of phenolic, tannin and flavonoid compounds in the palm oil leaves extract may contribute to its antioxidant activity (Yin et al., 2013).

According to Arunachalam et al., (2013), the water soluble phytochemical compounds present in the leaf extracts such as flavonoids, and saponins are accountable for reducing gold ions to AuNPs by capping around the nanoparticles. However, the reduction rate of HAuCl will varies and the colour intensity differs due to reaction of the phytochemical compunds with the AuNPs.

CHAPTER 3

METHODOLOGY

3.1 Research Methodology

An extensive research was done on several journals and research papers in order to obtain a feasible and executable project plan. Figure 3.1 shows the project plan developed in order to achieve the objectives of the project.

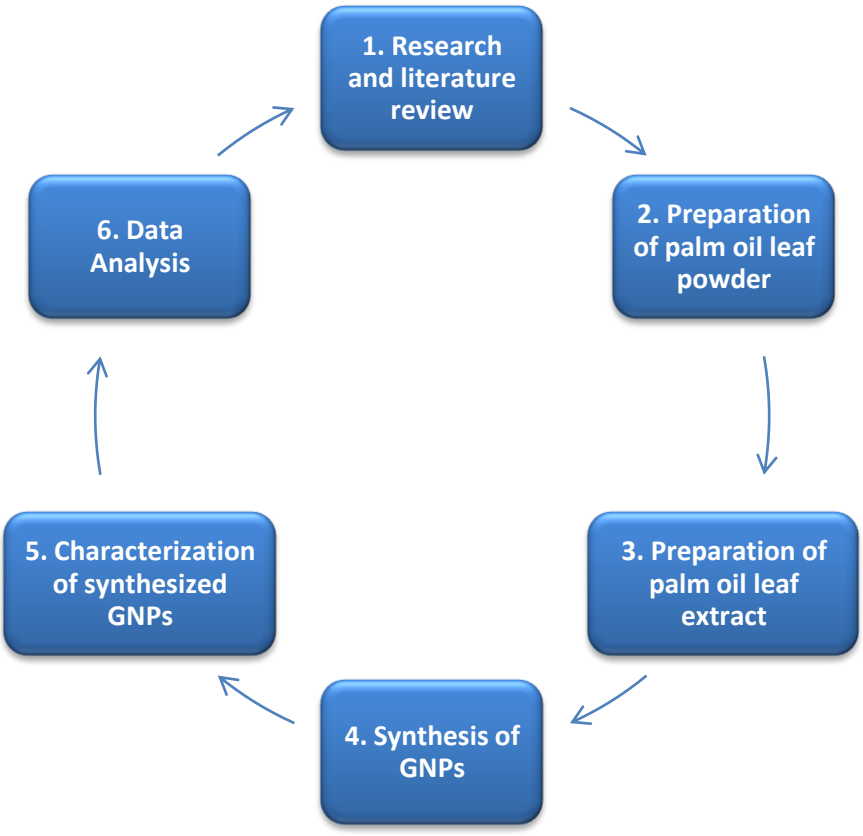

Figure 3.1: Project Plan

3.2 Experimental Methodology

3.2.1 Materials

Several materials were used in order to complete this study. Chloroauric acid (HAuCl4.3H2O) was purchased from Sigma-Aldrich, palm oil leaves were obtained from Felcra Berhad Nasaruddin Oil Palm Mill located in Bota, Perak and triple distilled water was obtained from Chemical Engineering Laboratory of Universiti Teknologi PETRONAS.

3.2.2 Apparatus and Equipment

Laboratory apparatus needed for the experiment are measuring cylinder, conical flask, Erlenmeyer flask, beaker, hot plate, magnetic stirrer, thermometer, filter funnel and filter paper. For storage purpose, small vials were used to keep the colloidal solution of AuNPs. IKA Grinder with a sieve of 0.25 mm was also used to grind the dried palm oil leaves into powder.

Characterization of AuNPs was done using several equipment namely Ultraviolet (UV) Visible spectrophotometer, Fourier Transform Infrared (FTIR) and Transmission Electron Microscopy (TEM).

3.2.3 Procedure for Preparation of Palm Oil Leaf Powder

1. Palm oil leaves were cut into small pieces and sun-dried for 2 weeks.
2. The leaves were rinsed several times with distilled water in order to remove dust particles.
3. The leaves were oven-dried at 50 °C for 8 hours to removes excess water.
4. The dried leaves were then grinded into powder using IKA grinder with 0.25 mm sieve.
5. The leaf powder was stored in an airtight container for further use.

3.2.4 Procedure for Preparation of Aqueous Palm Oil Leaf Extract

1. A beaker containing 180 ml of distilled water was heated up to 90 °C.

2. 18 g of palm oil leaf powder was added to the beaker containing hot distilled water and the mixture was stirred using a magnetic stirrer at 900 rpm for 10 minutes.
3. The solution was filtered by gravity filtration using a Whatman No. 1 filter paper. The supernatant liquids was collected and stored in a freezer at 4^{o}C in order to retain the activity of enzymes until further use.

3.2.5 Procedure for Synthesis of Gold Nanoparticles

1 wt % (2.943 mM) Stock solution of chloroauric acid was prepared by adding 500 mg of gold (III) chloride hydrate to 50 ml of distilled water in a 50 ml volumetric flask. The stock solution was further diluted to several concentrations such as 0.22 mM, 0.2943 mM, 0.4415 mM and 1.0000 mM which will be used to study the effect of chloroauric acid concentration on the synthesis of AuNPs. The working solution used for the experiments were prepared using equation $M_1V_1 = M_2V_2$ where M_1 is molar concentration of the stock solution, M_2 is molar concentration of the working solution, V_1 is volume of stock solution and V_2 is volume of working solution.

Procedure to synthesize AuNPs using palm oil leaf extract was developed as below:

1. 2 ml of palm oil leaf extract was added in a drop-wise manner to an Erlenmeyer flask containing 20 ml of distilled water at room temperature.
2. 2 ml of 1.0000 mM chloroauric acid was added into the mixture and the solution was stirred using a magnetic stirrer at 360 rpm for 45 minutes.
3. Time required for the solution to change colour into wine-red colour was recorded.
4. The gold colloidal was stored in a vial and kept in cold water at 4 ^{o}C in order to freeze the reaction.
5. The maximum absorbance peak of the gold colloidal was then measured using UV-Vis spectrophotometer.

Several parameters such as chloroauric acid concentrations, reaction temperature, reaction time as well as volume ratio between palm oil leaf extract and chloroauric acid were varied in order to determine the optimum conditions for synthesizing AuNPs using palm oil leaf extract.

3.2.6 Procedure for Characterization of Gold Nanoparticles

i) Ultraviolet (UV) Visible spectrophotometer

Analysis from this equipment will confirm the presence of AuNPs in the colloidal solution. The output of this analysis will be in a graph of absorbance vs. wavelength. Presence of AuNPs will result in a peak in the absorption band at wavelength between 510 to 550 nm which correspond to Surface Plasmon Resonance (SPR) of AuNPs (Chakraborty et al., 2013).

ii) Fourier Transform Infrared (FTIR)

FTIR analysis was performed to identify the possible bio-molecules inside palm oil leaf extract which responsible for the reduction of the Au^{3+} ions in chloroauric acid into AuNPs.

iii) Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy was used to determine the size and morphology of AuNPs. TEM analysis provides the size distribution of AuNPs through a micro image produced under different magnification. Besides that, growth pattern and distribution of shapes of the nanoparticles can also be determined using this technique.

A drop of colloidal gold solution was placed on carbon coated TEM copper grid. The film was allowed to dry for 30 minutes. The size distribution of particles was measured from enlarged images of TEM by taking different count for each sample.

3.2 Project Key Milestones

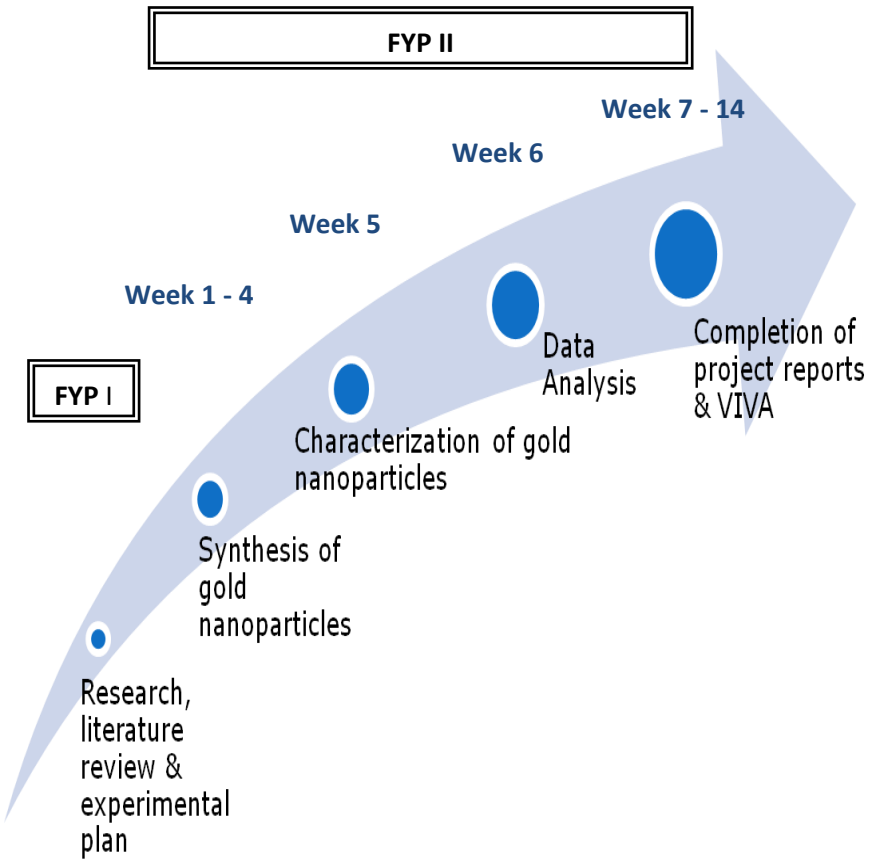

Figure 3.2: Project Key Milestones (FYP I & FYP II)

3.4 Gantt Chart

Table 3.1: FYP II Gantt Chart

Project Related Activities	Week													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Preparation of leaf extract														
Extract analysis using FTIR														
Synthesis of AuNPs														
Study of optimum conditions for synthesizing AuNPs														
Characterization of synthesized AuNPs														
Data Analysis														
Submission of Draft Progress Report														
Submission of Progress Report														
Pre-SEDEX														
Submission of Draft Final Report														
Submission of Dissertation (soft bound)														
Submission of Technical Paper														
Viva														
Submission of Project Dissertation (Hard Bound)														

CHAPTER 4

RESULT & DISCUSSION

4.1 Palm Oil Leaf Extract

Palm oil leaf extract should be prepared correctly as it will determine the ability of the extract to synthesize gold nanoparticles (AuNPs). In completing this research, a numbers of extracts were prepared and it was found that the drying process of the leaves is the most crucial part. Extract prepared using palm oil leaves which were oven-dried at 60 °C for 5 hours and extract prepared using palm oil leaves which were sun-dried for 1 week did not result in absorption peak in the rage of 510 nm to 550 nm. This indicates that AuNPs were unable to be synthesized using those extracts. According to Chen, Yang and Liu (2011), phenolic content of plant has a direct relationship with drying temperature. Thus, the drying process of the palm oil leaves should not be performed at a very high temperature as it might change the structure of bio-molecules or even degrade the bio-molecules present in the palm oil leaves. Besides, Han and May (2010) reported that dried palm oil leaves results in extracts with higher anti-oxidative power than the extract prepared using wet leaves. Therefore, the duration of drying the leaves should be long enough to ensure complete removal of water and moisture.

After extensive research and several trials of preparing the extract using various drying methods of palm oil leaves, it was found that the extract prepared using leaves which were sun-dried for two weeks and then oven-dried at 50 °C for 8 hours is the best. This is because the extract was able to reduce gold ions in chloroauric acid into AuNPs and results in an absorption peak near 540 nm.

The extract was prepared by adding 18 g of palm oil leaf powder to 180 ml of distilled water. Since the solution was stirred for only 10 minutes, the extract was

prepared at 90 °C in order to increase the extraction rate of compound with similar polarity as the water molecules. During the mixing, it can be seen that the powder was initially immiscible with the distilled water. Therefore, the stirring speed was increased up to 900 rpm to ensure all the powder dissolved in the hot distilled water.

Amount of extract collected after being gravitationally filtered overnight at room temperature was 100 ml. The volume of extract was lesser than the initial volume of the solvent because some of the liquid were vaporized and escaped to the surrounding when it was heated at 90 °C. However, the volume of extract collected was sufficient to be used for the entire experiments. The extract was stored in a freezer in order to retain the activity of enzyme until further used.

Figure 4.1: Aqueous Palm Oil Leaf Extract

It was found that the fresh palm oil leaf extract was dark brown in colour as shown in Figure 4.1. However, the extract turned cloudy after about 1 months eventhough it was kept in the freezer. An experiment conducted using the cloudy extract shows that the extract still has the ability to reduce gold ions in chloroauric acid into AuNPs and formed a maximum absorbtion peak in the AuNPs' range.

4.2 General Observations

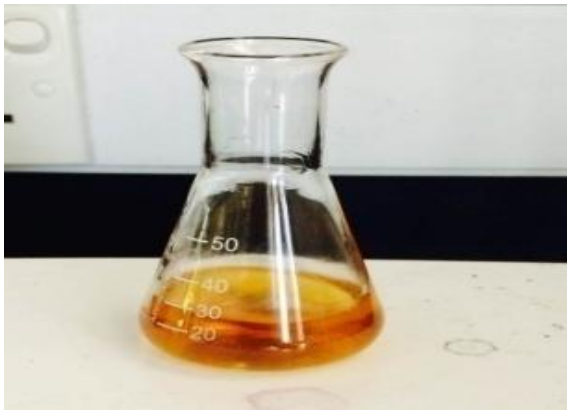

Figure 4.2: Colour of solution after addition of acid into aq. palm oil leaf extract

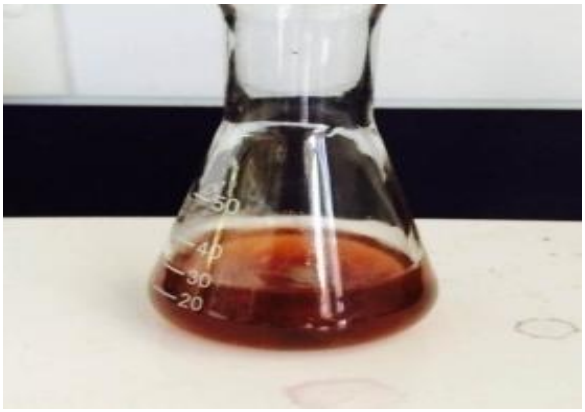

Figure 4.3: Colour of solution after 45 minutes of reaction at room temperature

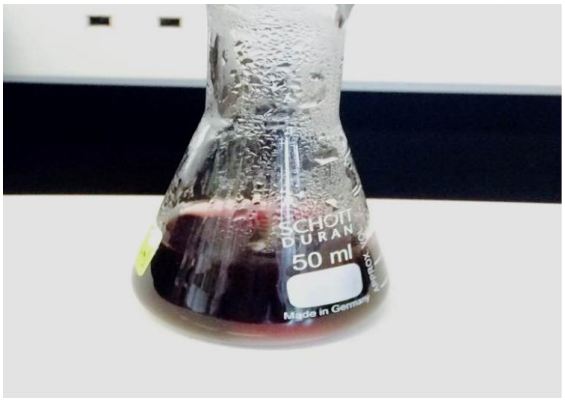

Figure 4.4: Colour of solution after 5 minutes of reaction at 50 °C

According to Gan et al., (2012), colour changes of the solution might be an indication of gold bio-reduction and formation of AuNPs. His results indicate that AuNPs synthesized using palm oil mill effluent (POME) showed a colour changes

from pale yellow to purplish pink when the initial pH is between 2.0 and 3.0, wine red when the initial pH is between 4.0 and 5.0 and followed by violet and grayish blue when the initial pH was further increased. UV-Vis analysis done on the samples formed an absorption peak around 530 to 560 nm which confirms the presence of AuNPs. Hence, visual observation on the colour changes of the solution can act as a preliminary assessment on the presence of AuNPs in this research.

Figure 4.2 shows the colour of solution right after addition of chloroauric acid into aqueous palm oil leaf extract. After some time, the colour of the solution started to change. Verma et al., (2012) reported that the colour of gold solution varies according to the size of particles which strongly dependant on several parameters such as acid concentration, volume of extract and temperature. Figure 4.3 shows the colour of solution after 45 minutes of reaction at room temperature and Figure 4.4 shows the colour of solution after 5 minutes of reaction at 50 °C. It can be seen that wine-red colour was formed when the reaction took place at room temperature while violet colour was formed when the reaction was carried out at 50 °C. The darkening of solution prepared at 50 °C indicates that at higher temperature, the process of aggregation of smaller AuNPs to form larger particles was favored over nucleation to form new AuNPs (Gan et al., 2012).

Table 4.1: Observations on colour changes of the solution

Acid Conc. (mM)	**Vol. of Acid (ml)**	**Vol. of Extract (ml)**	**Temp. (°C)**	**Reaction Time (min)**	**Duration to Change Colour (min)**	**Colour of Final Solution**
0.2200	2	2	25	45	17	Pale brown
0.2943	2	2	25	45	15	Wine-red
0.4415	2	2	25	45	20	Wine-red
1.0000	2	2	25	45	38	Wine-red
1.0000	2	1	25	45	> 45	No colour change
1.0000	2	3	25	45	23	Dark wine-red
1.0000	2	4	25	45	18	Dark wine-red
1.0000	2	2	50	5	1 ½	Violet
1.0000	2	2	70	5	1	Dark violet
1.0000	2	2	90	5	½	Dark violet

Based on Table 4.1, it can be seen that solution produced using 0.22 mM of chloroauric acid took only 17 minutes to change colour. However, the colour of final solution was pale brown which is not the colour of AuNPs solution. As the concentration of chloroauric acid increased to 0.2943 mM, the solution took 15 minutes to change into wine-red colour. This shows that 0.2943 mM was the minimum concentration of chloroauric acid required for the solution to form wine-red colour which is an indicator for the presence of AuNPs in the solution. Time required for the solution to turn into wine-red colour increased with respect to concentration of chloroauric acid used. This is because more gold ions were present in the acid with higher concentration thus longer time was required to reduce all the gold ions into AuNPs.

Next, volume of extract was varied while maintaining the volume of chloroauric acid at 2 ml in order to study the effect of volume ratio on the synthesis of AuNPs. It was observed that, solution prepared using 1 ml of palm oil leaf extract does not change colour even after 45 minutes of reaction. This shows that 1 ml of extract was not sufficient to reduce gold ions present in 1 mM chloroauric acid. As the volume of extract was increased, time required for the solution to change into wine-red colour was very much reduced. This is because by using high volume of extract, more reducing agents were available hence, shorten the time required to reduce all gold ions present in the solution. However, the colour of the solution was darker when higher volume of extract was used which indicates that agglomeration occurred in the reaction.

Lastly, the effect of temperature was studied by varying the reaction temperature. Initially, the reaction of 2 ml palm oil leaf extract with 2 ml of 1 mM chloroauric acid at room temperature took about 38 minutes to turn into wine-red colour. As the reaction temperature increased, time required for the solution to change colour reduced significantly and the solution changed colour almost instantly when the reaction was performed at 90 °C. Thus, it can be conclude that temperature play a role in increasing the reaction rate. However, the colour of solution prepared at higher temperature was very much darker than the one prepared at room temperature which indicates that agglomeration of particles might took place inside the reaction mixture.

4.3 UV-Vis Analysis

Light excitation of AuNPs result in a strong absorption band which falls under the visible region. This phenomenon occurs when the frequency of the electromagnetic field is resonant with the coherent electron motion or better known as Surface Plasmon Resonance (SPR) absorption. Based on the features, it is determined that UV-Vis spectroscopy is the most suitable and widely used technique to confirm the presence of AuNPs (Zayed and Eisa, 2014).

According to Chakraborty et al., (2013), AuNPs will exhibit surface plasmon resonance in the range of 510 nm to 550 nm. The position of SPR band in UV-Visible spectra is influenced by various parameters such as particle size, shape, local refractive index and its interaction with medium (Aromal et al., 2012).

The results obtained from UV-Vis analysis for the entire experiments of synthesizing AuNPs using palm oil leaf extract are tabulated in Table 4.2.

Table 4.2: Experimental Results

Acid Conc. (mM)	Vol. of Acid (ml)	Vol. of Extract (ml)	Temperature (°C)	Reaction Time (min)	Max. Wavelength (nm)	Absorbance (A)
0.2200	2	2	25	45	No peak	N/A
0.2943	2	2	25	45	548.63	1.68
0.4415	2	2	25	45	543.70	2.60
1.0000	2	2	25	45	540.68	3.09
1.0000	2	1	25	45	560.02	2.34
1.0000	2	3	25	45	545.94	3.90
1.0000	2	4	25	45	553.00	4.04
1.0000	2	2	50	5	537.00	3.70
1.0000	2	2	70	5	537.67	4.07
1.0000	2	2	90	5	538.00	4.42
1.0000	2	2	25	10	No peak	N/A
1.0000	2	2	25	20	No peak	N/A
1.0000	2	2	25	30	No peak	N/A
1.0000	2	2	25	40	541.69	2.22
1.0000	2	2	25	50	542.00	3.00
1.0000	2	2	25	60	541.68	3.12

4.3.1 Effect of chloroauric acid concentration on the synthesis of AuNPs

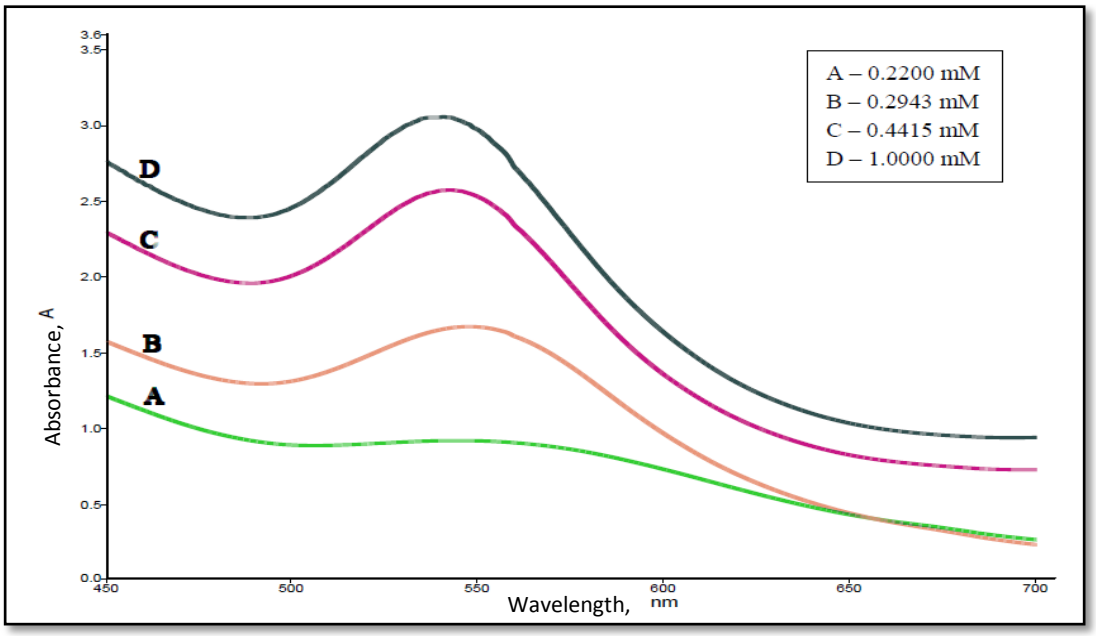

Figure 4.5: Absorption spectra as a function of wavelength at different acid concentrations

The effect of chloroauric acid concentration on the synthesis of AuNPs was studied by manipulating the acid concentration while fixing other parameters. The reaction was carried out by adding 2 ml of chloroauric acid into an Erlenmeyer flask containing 2 ml of palm oil leaf extract and 20 ml of distilled water. The solution was stirred using a magnetic stirrer at 360 rpm at room temperature for 45 minutes.

Based on Figure 4.5, it can be concluded that there was no formation of AuNPs when 0.22 mM chloroauric acid was used as it does not yield absorption peak in the range of AuNPs. This is supported by Gan et al., (2012) who reported that AuNPs was unable to be produced by using 0.10 mM and 0.25 mM gold chloride in his studies of synthesizing AuNPs using palm oil mill effluent (POME). In present study, an absorption peak was only available once 0.2943 mM of chloroauric acid was used and it results in absorption peak at 548.63 nm which is in the range of AuNPs. The broad peak observed might indicates the formation of larger and polydispersed AuNPs in the solution (Madhuri Sharon et al., 2012).

As the concentration of chloroauric acid was increased, the absorption peak shifted to the left with increasing absorbance. Increase in the absorbance value indicating that

the number of particles formed increases with increase in acid concentration. On the other hand, shifting of absorption peak to the shorter wavelength indicates that the size of AuNPs produced was reduced. This is contradicting the finding of most researchers who claimed that the size of nanoparticles increases with increasing acid concentration. This is because the amount of reducing agent available to reduce the gold ions inside the reaction mixture was relatively low when high concentration of chloroauric acid was used. Therefore, the small particles will aggregate to bigger ones.

Reason for the contradiction might be due to the reaction time. As discussed earlier, the solution with low concentration of acid took shorter time to change into wine-red colour which indicates that AuNPs were formed within that short time. However, the reaction was still allowed to continue since the reaction time for all the samples was fixed at 45 minutes. As the reaction proceeds, the nucleation of new AuNPs was not favored because of limited number of gold ions present in the solution. Therefore, the small particles inside the solution tend to combine with one another thus increasing the particle size. This might gives an insight that the palm oil leaf extract was able to act as reducing agent but not as capping or stabilizing agent since the particles tend to grow to form larger particles.

From visual observation, it can be concluded that the solution prepared using higher acid concentration took longer time to form AuNPs since the time required to observe colour changes on the solution was very much longer than those prepared using low concentration of acid. As the reaction time approached 45 minutes, the particles did not agglomerate as the reduction of gold ions into AuNPs was still the dominant reaction in the process. Thus, smaller particles were formed.

Among all acid concentrations studied, 1 mM chloroauric acid was found to be the best concentration for synthesizing AuNPs because it yields an absorption peak at 540.68 nm which is the closest to the desired peak (540 nm) and with the highest absorbance. Therefore, 1 mM chloroauric acid will be used for the entire experiments.

4.3.2 Effect of volume ratio on the synthesis of AuNPs

The effect of volume ratio on the synthesis of AuNPs was studied by manipulating the volume of extract while fixing other parameters. The reaction was carried out by adding 2 ml of 1 mM chloroauric acid into an Erlenmeyer flask containing palm oil leaf extract and 20 ml of distilled water. The solution was stirred using a magnetic stirrer at 360 rpm at room temperature for 45 minutes.

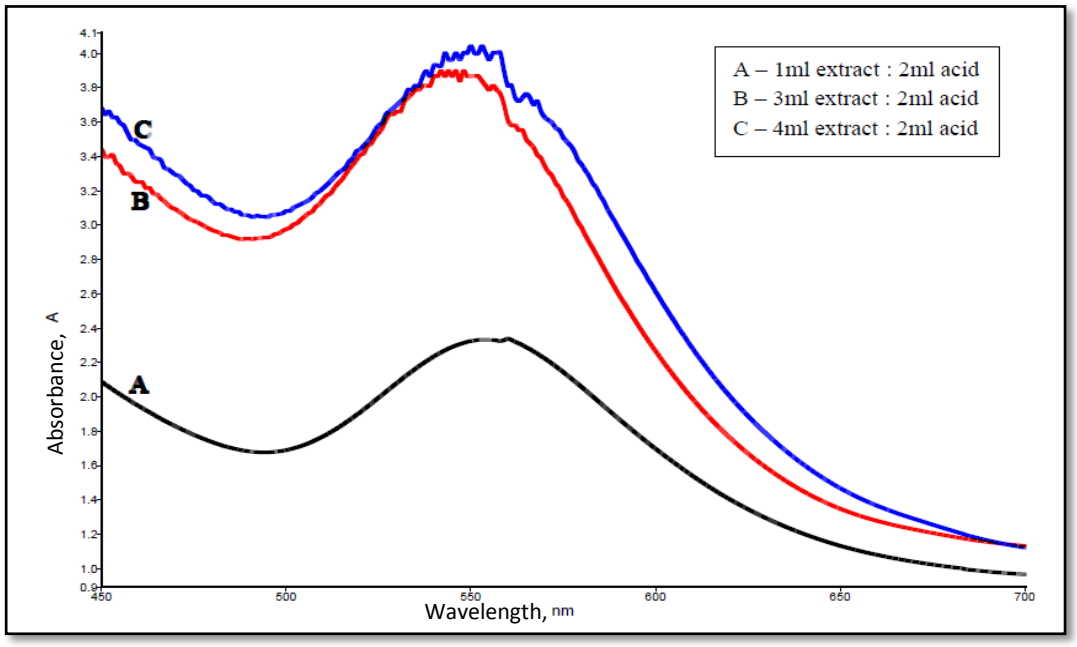

Figure 4.6: Absorption spectra as a function of wavelength at different volume ratio

Based on Figure 4.6, it was observed that 1 ml of extract yield an absorption peak at 560.02 nm which was not in the range of AuNPs. Hence, it can be conclude that 1 ml of extract was insufficient to reduce gold ions present in 1 mM chloroauric acid into AuNPs. This explain the reason why the solution does not change colour even after 45 minutes of reaction. As the volume of palm oil leaf extract increased to 2 ml, it took 38 minutes for the solution to turn into wine-red colour and absorption peak was observed to be at 540.68 nm with absorbance of 3.09 as shown in Figure 4.5 (line D). Hence, it can be concluded that AuNPs started to form when the volume of extract was the same as volume of acid.

As the volume of palm oil leaf extract surpassed the volume of acid, the absorbance value increased accordingly which reflects the increase in number of AuNPs produced. This is because with high volume of extract, more reducing agents were

available to reduce gold ions present in the solution into AuNPs. 3 ml of extract was found to be sufficient to reduce most of gold ions in 2 ml of 1 mM chloroauric acid since there was only a small jump in absorbance when 4 ml of extract was used. In most experimental works done by other researchers, the amount of acid used was in great excess thus the reducing agents tend to reduce all the gold ions into smaller particles. However in present study, only 2 ml of 1 mM chloroauric acid was used thus limiting the extract from reducing more gold ions into AuNPs.

In term of wavelength, it can be seen that the absorption peak shifted to longer wavelength as higher volume of extract was used. According to Wulf (2012), absorbance peak at longer wavelength indicates the formation of larger particles. However, Sujitha et al., (2013) reported that with greater volume of extract, the particles size reduced due to greater interaction between protective bio-molecules and surface of nanoparticles.

The reason for the contradiction might be due to inability of bio-molecules present in palm oil leaf extract to act as stabilizing agent. As discussed earlier, shorter time was required to observe colour changes on the solution when volume of extract was increased. Absence of stabilizing agents inside the solution will caused the small particles to agglomerate and increase in particles size as the reaction proceed. Therefore, the absorption peak shifted towards longer wavelength. The agglomeration was proved by the formation of wavy absorption band as shown in Figure 4.5 (line B and C). Therefore, 1:1 ratio (2ml extract : 2ml acid) seems to be the optimum condition to synthesize AuNPs using 1 mM chloroauric acid at room temperature as it does not result in agglomeration and the absorption peak was nearest to the desired peak.

4.3.3 Effect of temperature on the synthesis of AuNPs

In order to study the effect of temperature on the synthesis of AuNPs, the reaction temperature was varied while other parameters were kept constant. The synthesis process was carried out by using 2 ml of 1 mM chloroauric acid, 2ml of palm oil leaf extract in 20 ml of distilled water and the solution was stirred using a magnetic stirrer at 360 rpm for 5 minutes. The reaction was allowed to continue for only 5 minutes because reaction rate was significantly increased with increasing reaction

temperature. Therefore, shorter time was required to observe the formation of violet colour inside the solution which indicates the formation of AuNPs.

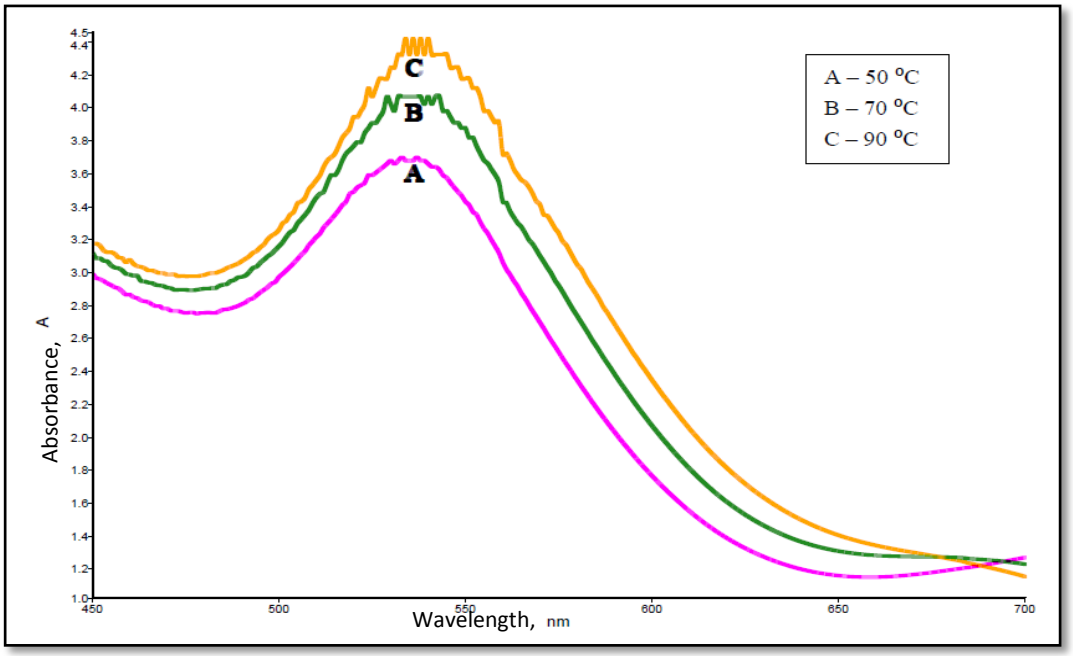

Figure 4.7: Absorption spectra as a function of wavelength at different temperature

Figure 4.7 shows that sharp peaks were observed when the reaction temperature was increased. This shows that monodispersed AuNPs were formed in the solution. As the reaction temperature increased, slight shift of the absorption peak towards longer wavelength was observed. This might indicate the formation of AuNPs with larger particles size which was supported by Gan et al., (2012) who claimed that at higher temperature, darkening of the solution reflects the process of aggregation of smaller AuNPs to form larger particles.

The agglomeration occurred might be due to high reaction temperature. Theoretically, particles will get excited at high temperature and collide with each other more frequently. This might destroyed the stabilizing agent present on surface of AuNPs thus allowed the particles to agglomerate with each other. This is supported by Madhuri Sharon et al., (2012) who claimed that agglomeration occurred due to high temperature which might destruct the stabilizing agent.

Therefore, it can be concluded that reaction conducted at room temperature using 2 ml of palm oil leaf extract and 2 ml of 1 mM chloroauric acid in 20 ml of distilled

water was the optimum conditions to synthesize AuNPs as it result in absorption peak at 540.68 nm, high absorbance and does not results in agglomeration.

4.3.4 Effect of reaction time on the synthesis of AuNPs

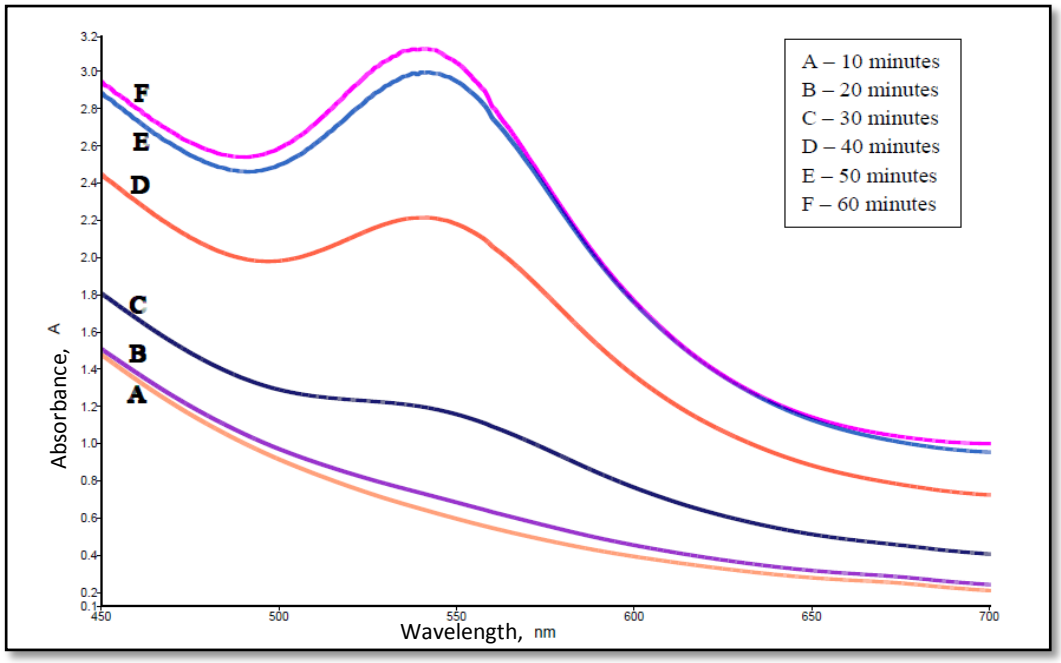

Figure 4.8: Absorption spectra as a function of wavelength at different time

Time-dependent UV-Vis spectra were recorded to give an insight on the processes of nucleation and growth of AuNPs reduced with the aid of palm oil leaf extract. According to Zayed and Eisa (2012), the formation of AuNPs started with reduction of gold ions, nucleation of metallic gold and followed by growth of individual nuclei.

In this experiment, the amount of extract, acid and distilled water used was doubled in order to ensure the volume of solution was sufficient for sampling at 10 minutes interval for 1 hour duration. Thus the solution was prepared using 4 ml of extract, 4 ml of 1 mM chloroauric acid in 40 ml of distilled water and stirred at 360 rpm for 1 hour at room temperature. At every 10 minutes interval, some of the solution was sucked using a dropper and placed in a vial for measurement of absorption peak using UV-Vis spectrophotometer.

Figure 4.8 shows the evolution of absorption spectra with respect to time. It can be seen that flat absorption band was produced by the samples taken after 10 minutes

and 20 minutes of reaction. Absorption peak at 541.69 nm was only available after 40 minutes of reaction with absorbance of 2.22. This shows that the reducing agent in palm oil leaf extract tooks about 40 minutes to reduce gold ions present in the solution into AuNPs. Hence, it can be concluded that the nucleation process was considerably slow.

Increase in absorbance with respect to time might be due to increase in number of AuNPs formed as a result of reduction of gold ions in the solution. However, very small increase in absorbance was observed for the sample taken at 60 minutes. Therefore, it can be concluded that after 50 minutes of reaction, almost all of gold ions present in the solution were reduced hence, further increase in reaction time does not result in significant increase of absorbance.

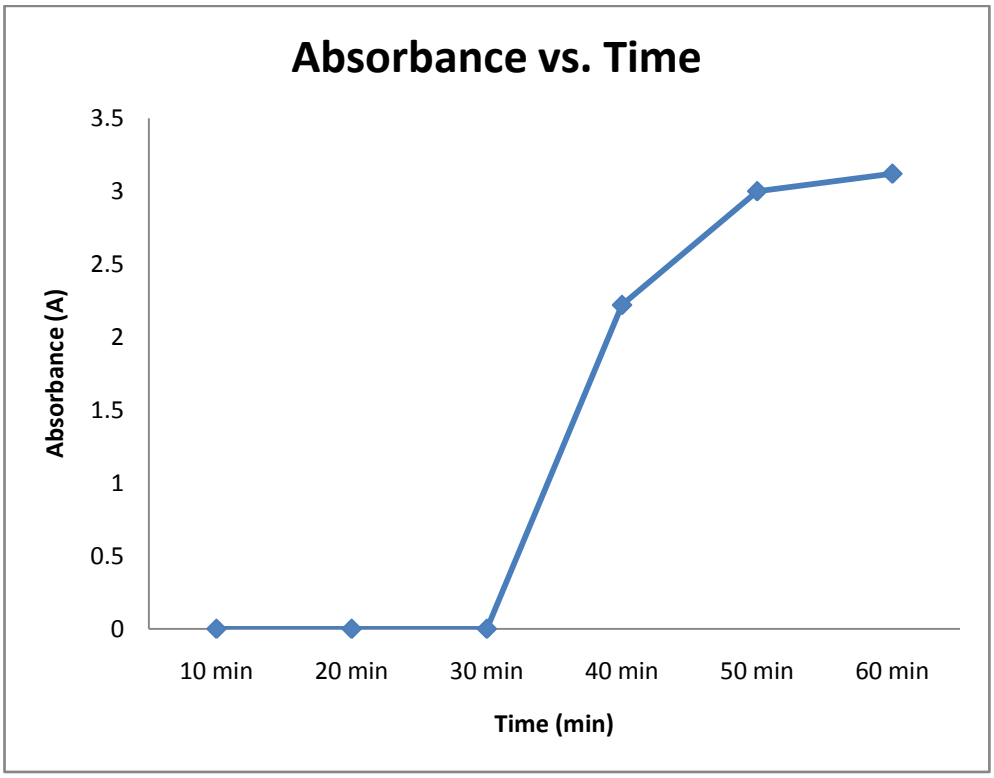

Figure 4.9: Evolution of absorbance over time

Based on Figure 4.9, it can be seen that the reduction and nucleation rate were at maximum in between 30 minutes to 40 minutes as substantial increase in absorbance value was observed. As the reaction proceed, the increase in absorbance value was not very significant and almost constant as it approached 60 minutes.

4.3 Stability of synthesized AuNPs

Eventhough palm oil leaf extract was only able to act as reducing agent but not as capping agent, the agglomeration can still be hindered by keeping the solution at low temperature. It was found that the gold solution which was kept in a cold water and stored in a freezer at 4 °C was still stable even after 12 days. This is based on UV-Vis analysis done on the sample which does not show any evolution of wavelength and absorbance from the day it was first prepared. Hence, it can be concluded that keeping the solution at low temperature (4 °C) will prevent the particles from undergo agglomeration process.

4.4 TEM Analysis

Transmission Electron Microscopy was used to determine the size, size distribution and shape of the AuNPs synthesized with the aid of palm oil leaf extract. Effects of two different concentration of chloroauric acid were studied in this analysis. The concentrations used were 0.2943 mM and 1 mM. Figure 4.10, 4.11, 4.12 and 4.13 shown images of TEM obtained for different concentration of chloroauric acid under different magnification. Remaining TEM images were included in APPENDICES.

4.4.1 TEM Images

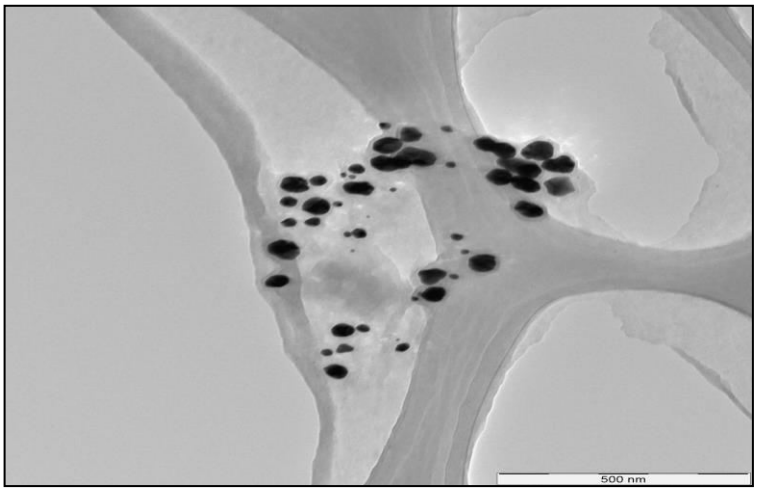

Figure 4.10: TEM image of AuNPs formed using 0.2943 mM of chloroauric acid (Magnification: 500 nm)

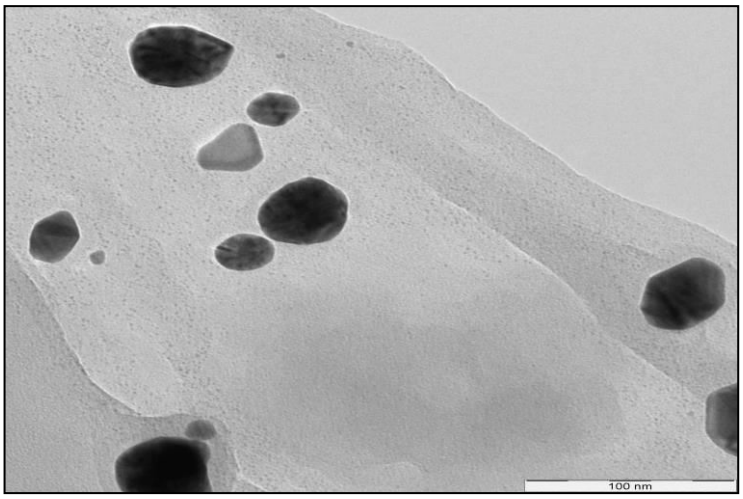

Figure 4.11: TEM image of AuNPs formed using 0.2943 mM of chloroauric acid (Magnification: 100 nm)

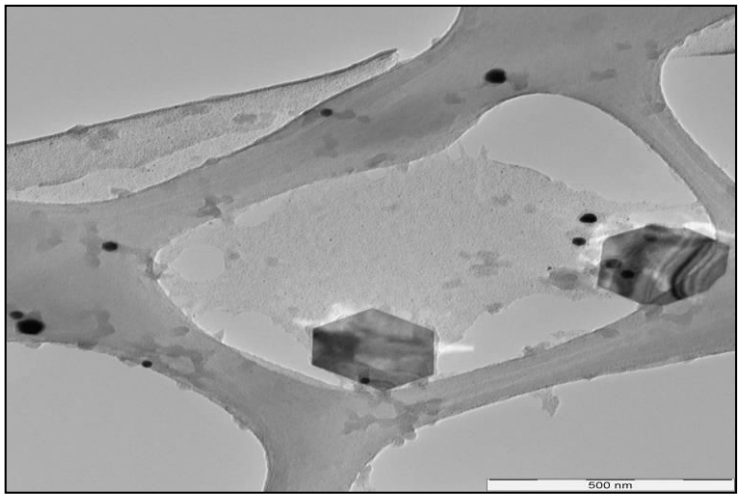

Figure 4.12: TEM image of AuNPs formed using 1.0000 mM of chloroauric acid (Magnification: 500 nm)

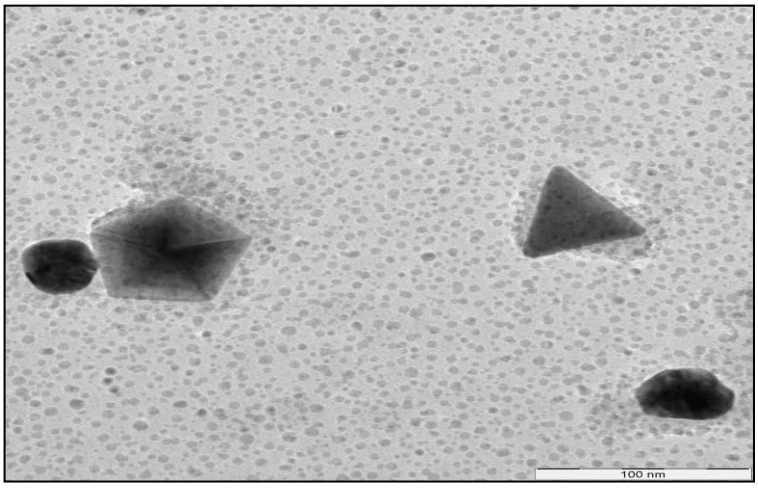

Figure 4.13: TEM image of AuNPs formed using 1.0000 mM of chloroauric acid (Magnification: 100 nm)

TEM images confirm the formation of AuNPs. It was observed that AuNPs formed in both solutions composed of various sizes and shapes. Truncated triangle plate, spherical and hexagonal shapes of AuNPs was observed in the gold solution produced using 0.2943 mM chloroauric acid. However, some of the particles were agglomerated hence producing AuNPs with undefined shapes. On the other hand, AuNPs formed using 1.000 mM chloroauric acid results in shape such as triangle, hexagonal, spherical and pentagon which were more distinct than those found in solution prepared using 0.2943 mM acid. The formation of AuNPs with various shapes might be due to the inconsistent motion of magnetic stirrer during the synthesis process.

4.5.2 Particle Size Distribution

Number of particles counted in one drop of gold solution prepared using 0.2943 mM and 1 mM chloroauric acid was 231 and 335 respectively. This shows that more AuNPs was formed when higher concentration was used. This is supported by the value of absorbance obtained from UV-Vis analysis done on the two samples. Solution prepared using 0.2943 mM chloroauric acid has an absorbance value of 1.68 while the one prepared using 1 mM chloroauric acid results in absorbance value of 3.09.

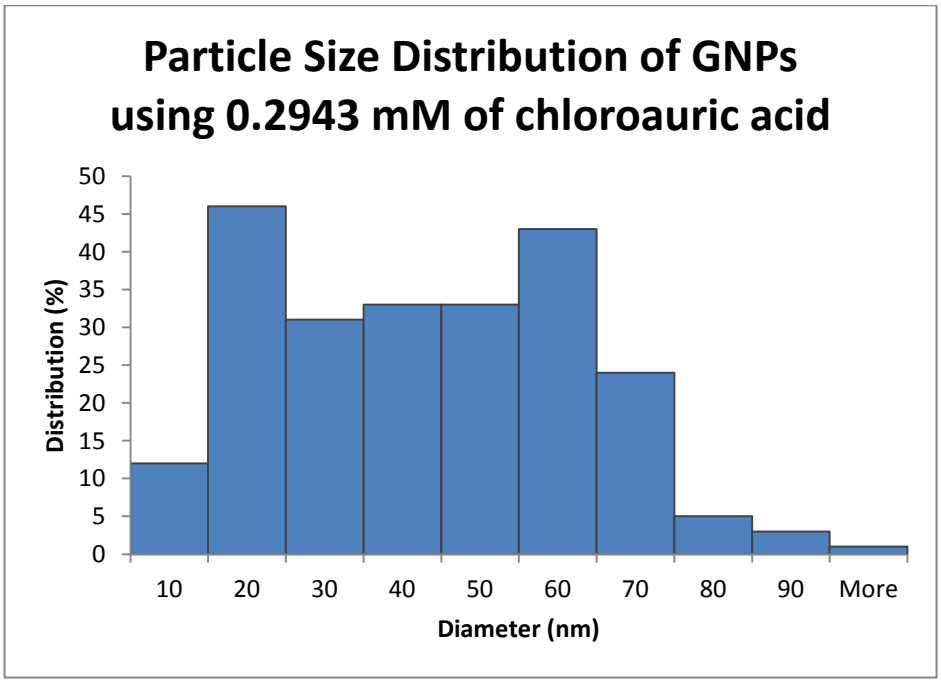

Figure 4.14: Size distribution of the AuNPs using 0.2943 mM chloroauric acid

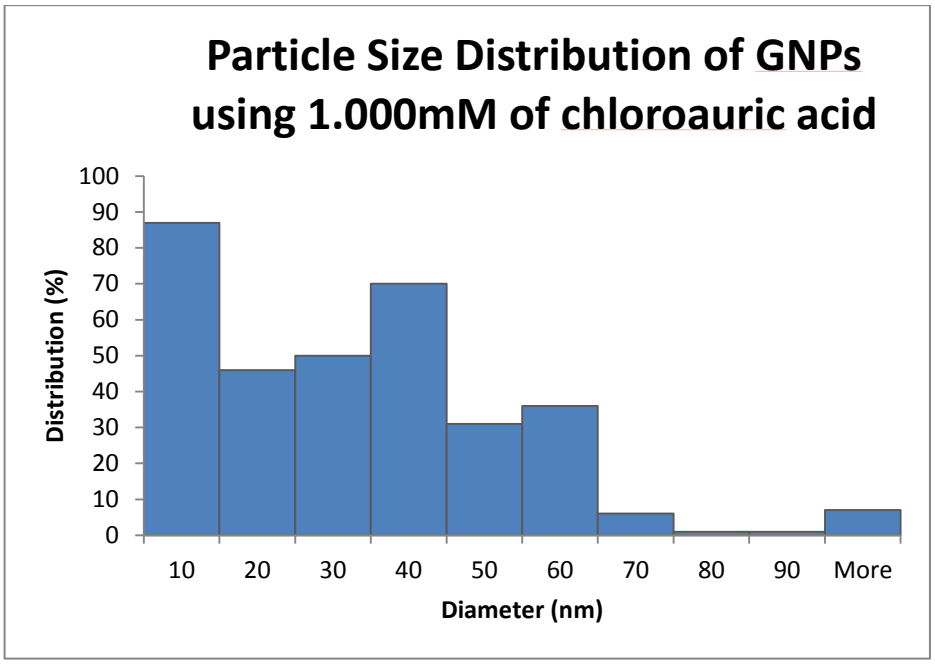

Figure 4.15: Size distribution of the AuNPs using 1.0000 mM chloroauric acid

Wide size distribution of AuNPs present in both solutions indicates that the AuNPs produced under those conditions were polydispersed. This finding agrees with the results obtained from UV-Vis analysis of the two samples which shown the formation of broad spectrums. Size of AuNPs synthesized using 0.2943 mM of chloroauric acid was found to be between 4.92 nm to 91.79 nm with average diameter of 38.22 nm.

On the other hand, AuNPs synthesized using 1 mM chloroauric acid results in particles size ranging from 2.25 nm to 296.18 nm. However, there was only 6 particles which diameter larger than 100 nm. The average diameter of AuNPs was 29.49 nm which was smaller than those synthesized using 0.2943 mM of chloroauric acid. This support the finding of UV-Vis analysis done on the two samples which shows that gold solution produced using 0.2943 mM and 1 mM results in absorption peak at 548.63 nm and 540.68 nm respectively. The co-existence of both small and large AuNPs might be due to AuNPs which were formed at early and later stage of the reaction. This shows that both nucleation to form new AuNPs and the aggregation to form larger particles occurred in a consecutive manner (Gan et al., 2012).

4.5.3 Diffraction Pattern

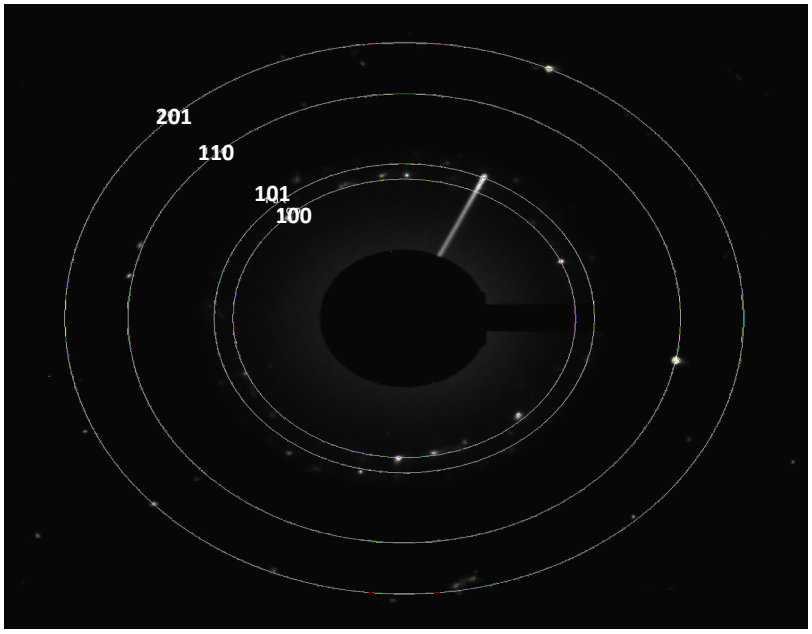

Figure 4.16: Diffraction pattern of AuNPs synthesized using 0.2943 mM acid

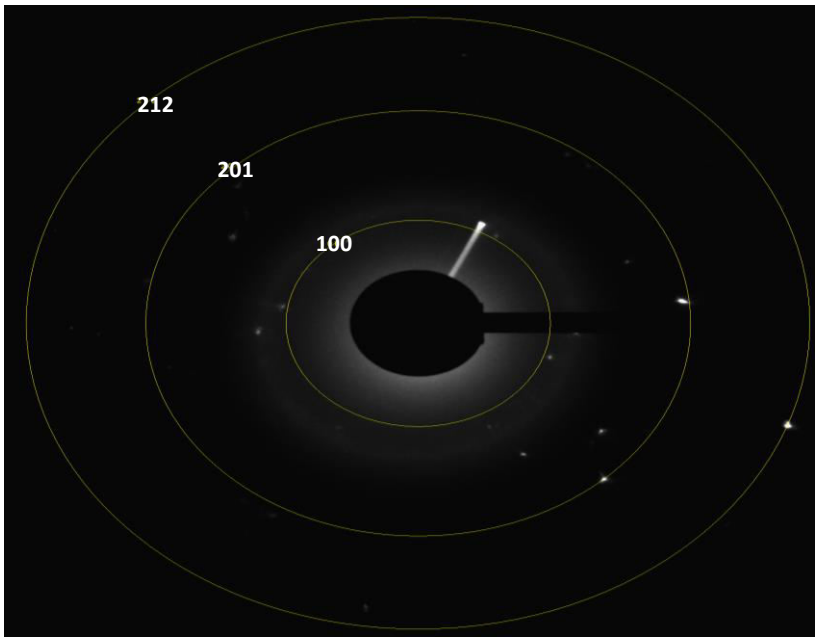

Figure 4.17: Diffraction pattern of AuNPs synthesized using 1.000 mM acid

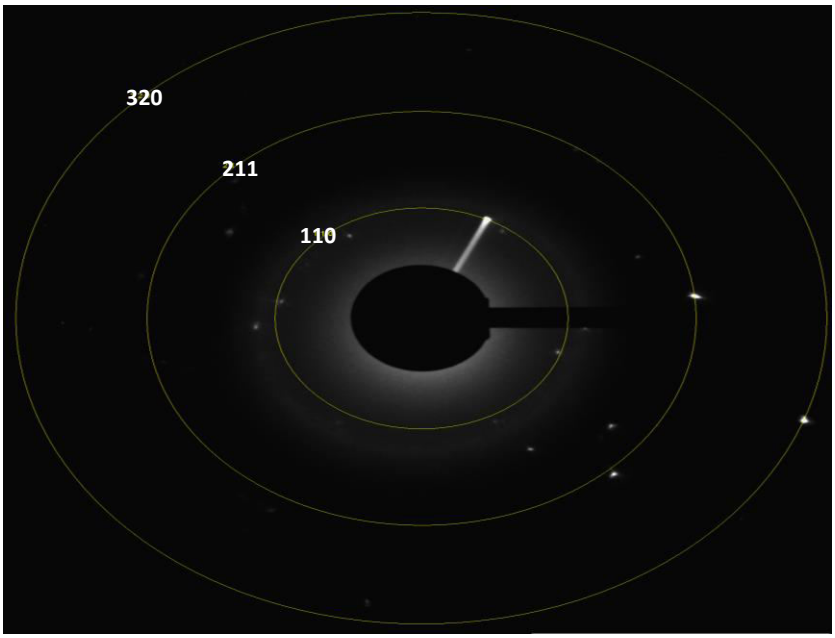

Figure 4.18: Diffraction pattern of AuNPs synthesized using 1.000 mM acid

Figure 4.16 shows the diffraction pattern of AuNPs synthesized using 0.2943 mM chloroauric acid. The clear lattice fringes in high resolution image and selected area electron diffraction (SAED) pattern with bright circular rings corresponding to (100), (101), (110) and (201) are the reflections of the hexagonal structure of AuNPs.

On the other hand, Figure 4.17 and Figure 4.18 show the diffraction patterns of AuNPs synthesized using 1 mM chloroauric acid. Rings in Figure 4.17 corresponding to the (100), (201) and (212) are the reflections of hexagonal structure while rings in Figure 4.18 corresponding to the (110), (211) and (320) are the reflections of orthorhombic structure. Thus, it can be conclude that AuNPs synthesized using 1 mM chloroauric acid composed of two crystalline structures namely hexagonal and orthorhombic.

4.6 FTIR Analysis

FTIR analysis was performed on palm oil leaf extract and gold solution which was prepared at the optimum conditions in order to identify the possible functional group which responsible in reducing gold ions present in the solution into AuNPs.

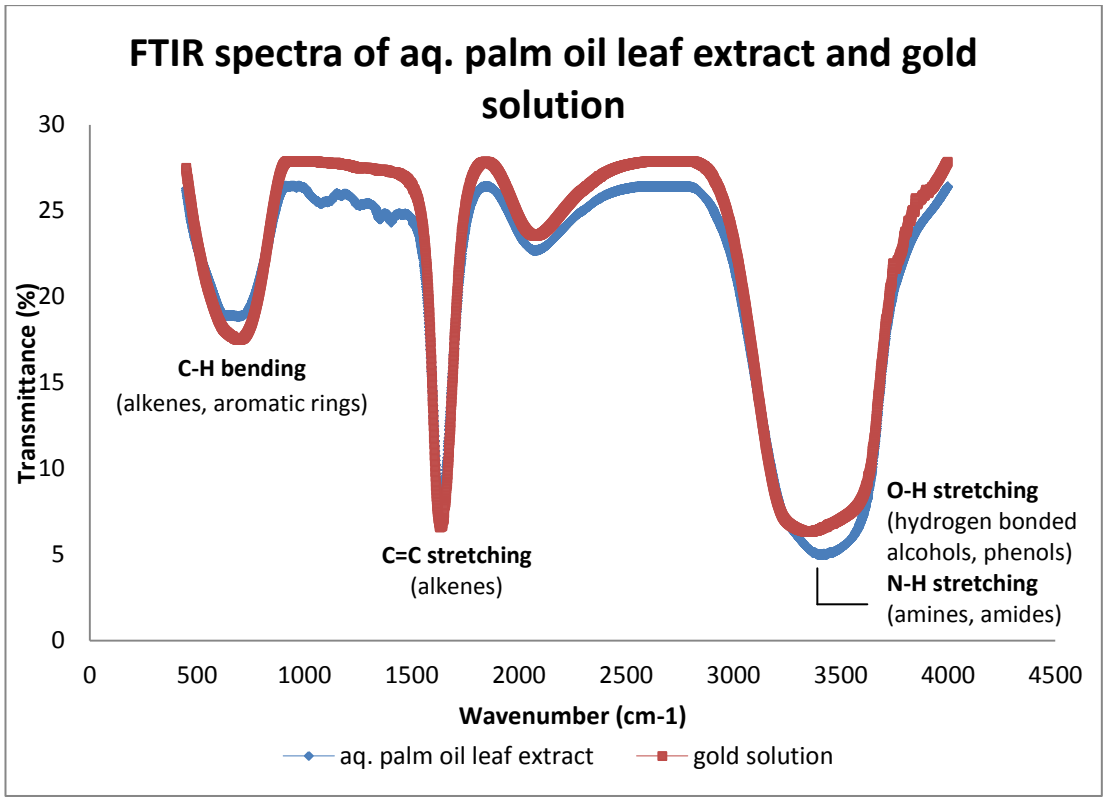

Figure 4.19: FTIR spectra of aqueous palm oil leaf extract and gold solution

FTIR spectra illustrated in Figure 4.19 shows that aqueous palm oil leaf extract formed a strong band at 3400.59 cm^{-1} correspond to O-H stretching of hydrogen bonded alcohols, phenol and N-H stretching of amines or amides. Whereas, the band at 1634.12 cm^{-1} correspond to C=C stretching of alkenes and the low band at 696.05 cm^{-1} correspond to C-H bending of alkenes and C-H bending of aromatic rings.

On the other hand, an FTIR spectrum of gold solution shows a slight shift of strong band towards 3349.79 cm^{-1} with a reduction in intensity. Hence, bio-molecules in palm oil leaf extract such as protein, flavonoids or phenols that contain abundant of C=O, N-H or O-H groups might be accountable for the reductions of gold ions into AuNPs.

The other bands were similar with the ones found in palm oil leaf extract. However, a slight increase in the concentration of alkenes or aromatic rings and the formation of bands at 2075.01 cm^{-1} and 2078.88 cm^{-1} are unexplainable.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Green synthesis of gold nanoparticles (AuNPs) using aqueous palm oil leaf extract has been demonstrated to be a cheaper, non-toxic, safer and environmentally-friendly method. It can be concluded that the size and morphology of synthesized AuNPs can be tuned by manipulating reaction parameters such as concentration of chloroauric acid, volume ratio between palm oil leaf extract and chloroauric acid, reaction temperature and reaction time. AuNPs with absorption peak of 540.68 nm was successfully produced by reacting 2 ml of palm oil leaf extract and 2 ml of 1 mM chloroauric acid in 20 ml distilled water at room temperature for 45 minutes. The solution turns into wine-red colour after 38 minutes and does not result in any agglomeration. TEM imaging done of the sample confirmed the presence of polydispersed AuNPs with mean diameter of 29.49 nm. On the other hand, TEM diffraction done on the sample revealed the formation of AuNPs with hexagonal and orthorhombic crystalline structures. Bio-molecules in palm oil leaf extract such as protein, flavonoids or phenols that contain abundant of C=O, N-H or O-H groups might be accountable for the reductions of gold ions into AuNPs because decrease in intensity of gold solution was observed at 3349.79 cm^{-1}.

For future findings, it is highly recommended to produce palm oil leaf extract using different polar and non-polar solvents to determine the effect of solvents in synthesizing AuNPs since different solvents have different resolving strength towards plant constituents (Yin et al., 2013). It is also recommended to use centrifuge for mixing purposes during AuNPs synthesis so that the solution will be well mixed as well as to avoid loss of volatile liquid to surrounding. Other than that, adding stabilizing agent to the reaction mixture can also be considered in order to avoid agglomeration of synthesized AuNPs.

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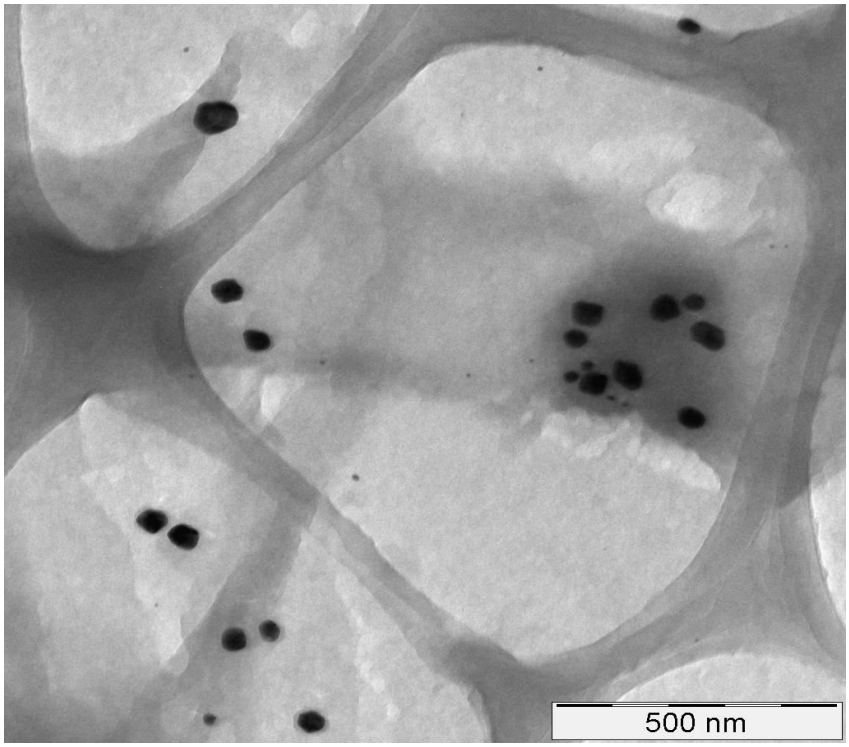
APPENDICES

Appendices 1: Literature Review Matrix

Reference	Suman et al., 2013	Zayed et al., 2013	Gopinath et al., 2013	Arunachalam et al., 2013	Geetha et al., 2013	Amin et al., 2012	Smitha et al., 2009
Plant species (Reducing agent)	Root of Morinda Citrifolia L	Phoenix Dactylifera L	Terminalia Arjuna	Memecylon umbellatum	Couroupita Guianensis	Arabinoxylan	Cinnamomum zeylanicum
PREPARATION OF EXTRACT							
Amount of powder	8 g	20 g	10 g	20 g	1 g	5 g	24 g
Volume of solvent	100 ml	100 ml	100 ml	100 ml	50 ml	200 ml	300 ml
Ratio	1 to 12.5	1 to 5	1 to 10	1 to 5	1 to 5	1 to 40	1 to 12.5
Type of solvent	distilled water	ethanol/water	double distilled water	sterile distilled water	double distilled water	Nanopure water	deionized water
Reaction Temperature	100 deg C	room temperature	100 deg C	100 deg C	room temperature	room temperature	100 deg C
Stirring Speed	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Duration	15 min	7 days	5 min	5 min	N/A	24 hrs	5 min
SYHTHESIS OF GNPs							
Volume of Extract	3 ml	Exp A: 0.1 ml Exp B: 0.2 ml Exp C: 0.4 ml	1 ml	Exp A: 5 ml Exp B: 10 ml Exp C: 15 ml	2 ml	8 ml	4 ml
Volume of Distilled Water	N/A	N/A	N/A	N/A	N/A	2 ml	N/A
Volume of Acid	34 ml	10 ml	100 ml	10 ml	8 ml	10 ml	30 ml
Concentration of Acid	1 mM	0.4 mM	1 mM	1 mM	1 mM	1 mM	0.2 mM
Type of Acid	aqueous chloroauric acid	aqueous chloroauric acid	aqueous chloroauric acid	aqueous chloroauric acid	aqueous chloroauric acid	aqueous chloroauric acid	aqueous chloroauric acid
Reaction Temperature	room temperature (incubated at dark)	incubated at room temperature	room temperature	incubated at room temperature	room temperature	80 deg C	room temperature
Stirred (yes/no)	No	No	No	Yes (150 rpm)	No	No	Yes (2 min)
Reaction Time	24 hr	Exp A: 40 min Exp B: 15 min Exp C: 3 min	15 min	2 min	5 min	40 min	1 hr
CHARACTERISTIC OF SYNTHESIZED GNPs							
Size	15 nm	Exp A: 45 nm Exp B: 36 nm Exp C: 32 nm	20 - 50 nm	15 - 25 nm	7 - 48 nm	10 - 25 nm	25 nm
Maximum Peak	540 nm	Exp A: 552 nm Exp B: 544 nm Exp C: 538 nm	530 nm	540 nm	534	535 nm	
Shape	face centered cubic	Exp A: irregular shape (triangles, tetragonals & nanorods) Exp B: Spherical shape Exp C: Spherical shape	spherical	Triangular, circular & hexagonal	Spherical, triangular, tetragonal, pentagonal	face centered cubic	prism & spherical

Appendices 2: TEM images for AuNPs synthesized using 0.2943 mM chloroauric acid



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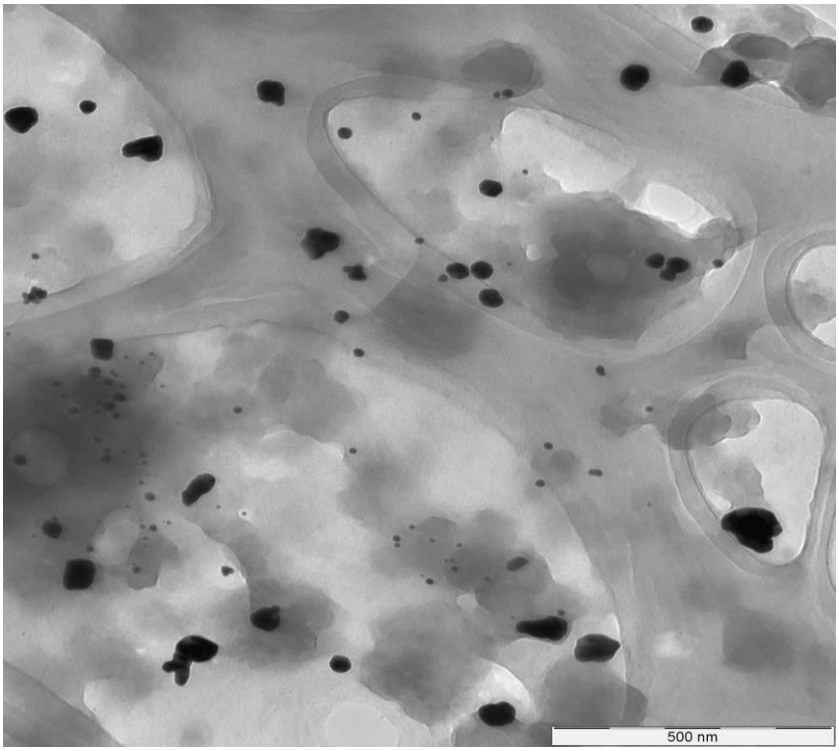

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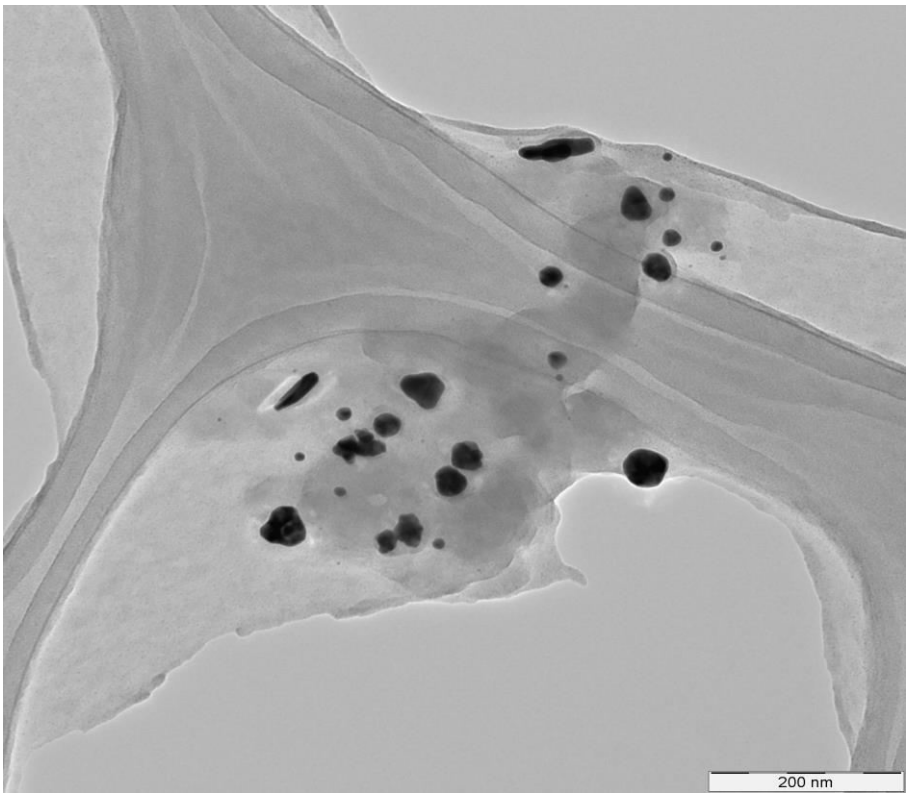

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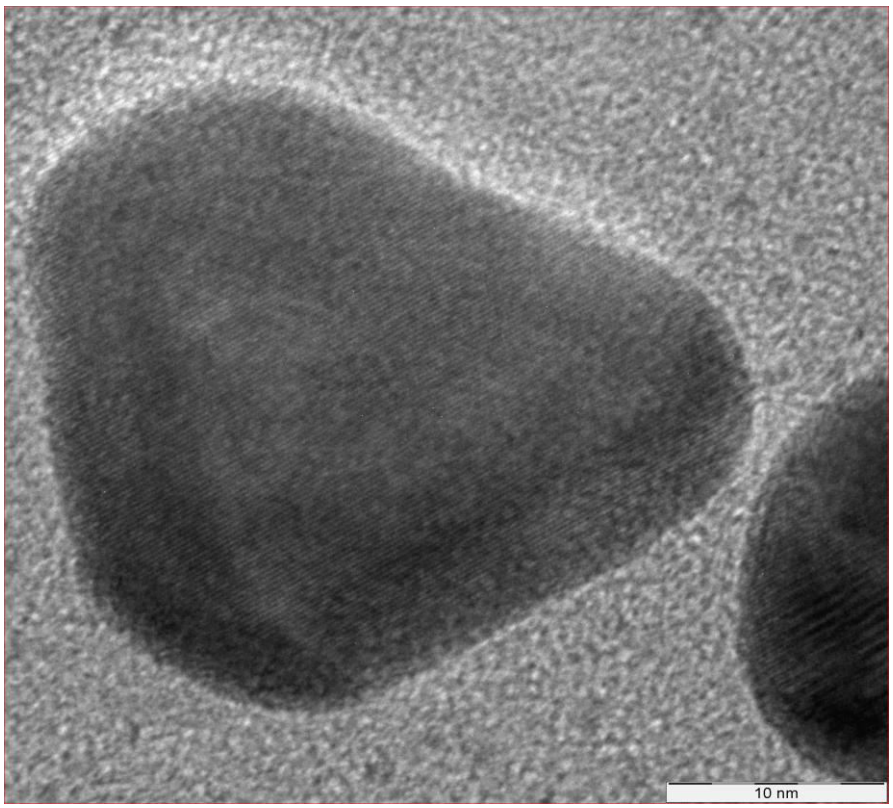

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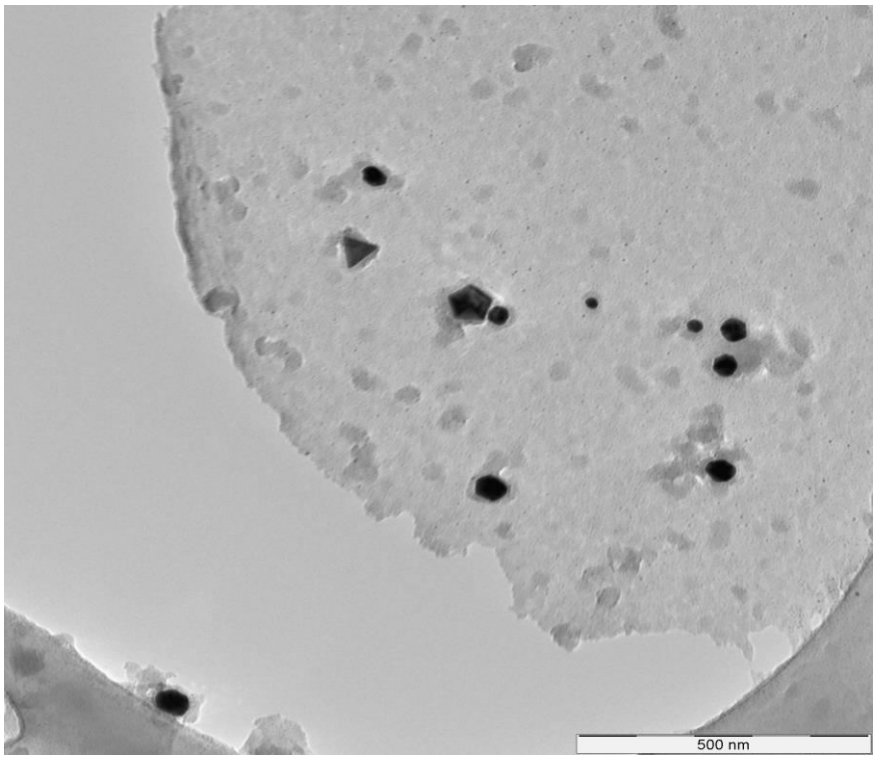
Appendices 3: TEM images for AuNPs synthesized using 1 mM chloroauric acid



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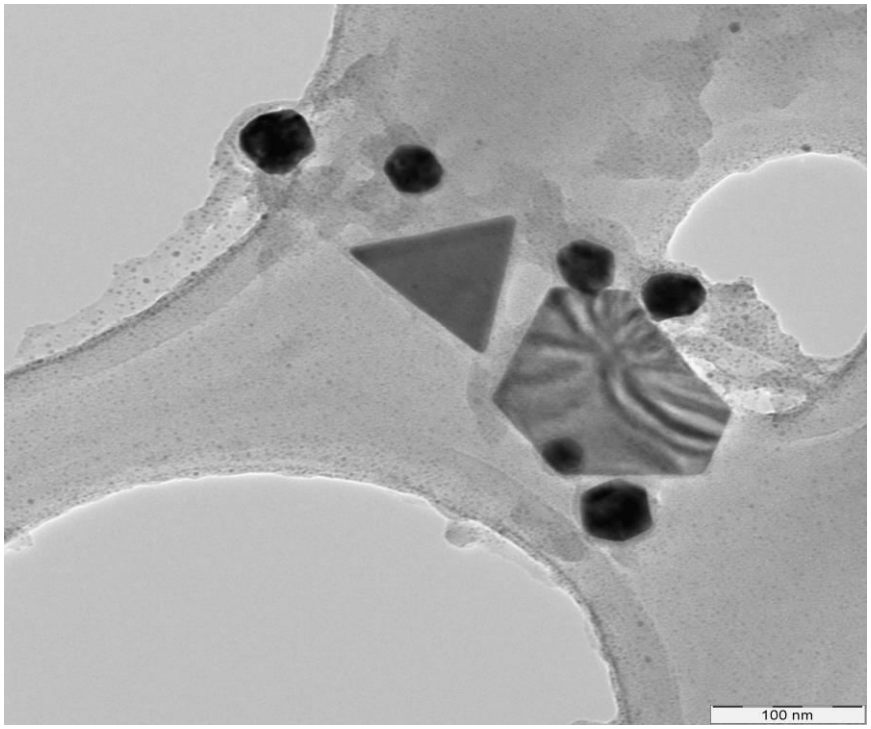

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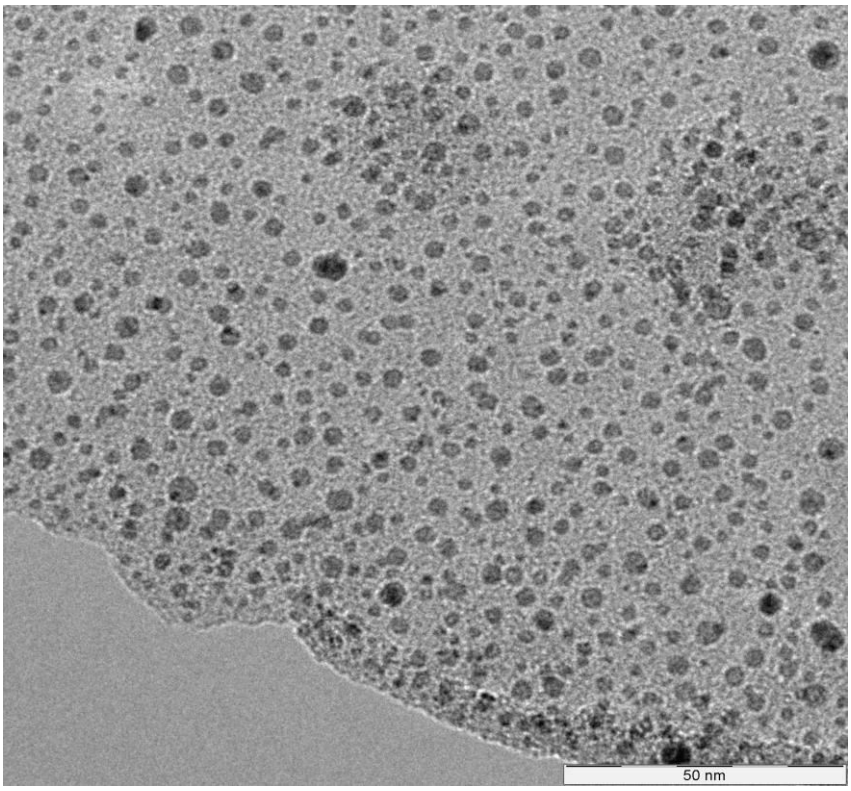

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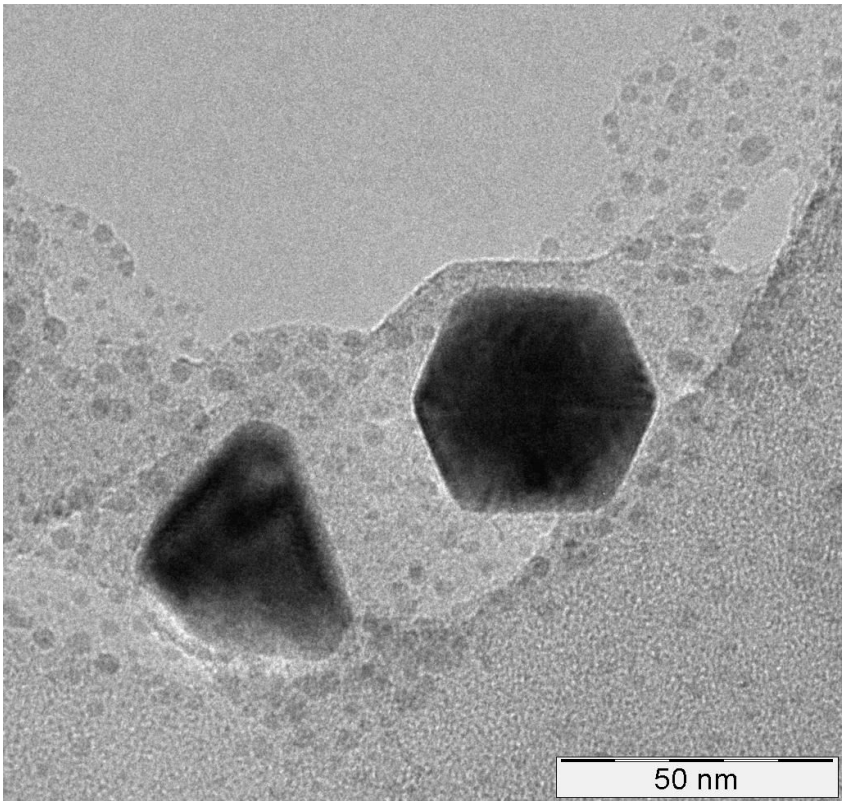

Magnification: 50 nm