**Bioynthesis of silver nanoparticles using *Graptophyllum pictum* leaves extracts**

Mohamed Syazwan Osman¹², Muhammad Ismail¹, Khairunnisa Khairuddin¹, Najwa Azis¹, Nur Syifa Salim¹, Nur Aqilah Zaini¹, and Norfezah Md Noor³

¹Faculty of Chemical Engineering, Universiti Teknologi MARA, 13500 Permatang Pauh, Penang, Malaysia.
²Molecular System Design & Optimization Research Group (MSORG), Faculty of Chemical Engineering, Universiti Teknologi MARA, 13500 Permatang Pauh, Penang, Malaysia.
³Faculty of Hotel Management & Tourism, Universiti Teknologi MARA, 13500 Permatang Pauh, Penang, Malaysia.

E-mail: syazwan.osman@uitm.edu.my

**Abstract.** Silver nanoparticles (AgNPs) have found significant application in industry especially in medical and catalysis field. Considerable attention has been made to synthesis AgNPs via various routes. However, in recent years, focus have been shifted to synthesis AgNPs using more environmentally friendly route. Attempts to synthesis AgNPs by using plant extracts as potential complex reducing agent have found significant interest to date. Hence, this work will explore the feasibility to synthesis AgNPs by using locally available *Graptophyllum pictum* leaves (*puding hitam*). The extracts were evaluated on the their total phenolic content to shed potential insights on the mechanism prior AgNPs synthesis. The feasibility of the AgNPs were evaluated by using UV-Vis and Transmission electron microscopy (TEM). In conclusion, *Graptophyllum pictum* leaves (*puding hitam*) could be a viable alternative for the environmentally friendly synthesis of AgNPs.

1. Introduction
The mainstream preparation of functional nanomaterials employs physical, chemical and mechanical techniques. However, the biotechnological approach for the synthesis of metallic NPs is becoming more important and it will hopefully overcome the abovementioned disadvantages of non-biotic methods. There is considerable recent demand for change in the way biosynthesized NPs are applied. The current literature not only suggests new protocols for biosynthesis of NPs which use various types of biomass but it also recommends immediate testing of developed nanostructures in numerous applications – most frequently in catalysis or as antimicrobial agents [1-3]

The biosynthesis of NPs is gaining greater attention; especially because the synthesis protocols are user-friendly and there is no special equipment, financially demanding devices or harmful chemicals required. The main advantages of NPs biosynthesis are; only a metal precursor is needed in most cases; reduction or chemical transformation and eventual NPs embedding occurs in one step; is mediated only by the (bio)chemicals contained in the biomass. Therefore, using plant extracts as a reducing agent in the synthesis of nanoparticles has received increasing interest. Being readily scalable and biocompatible, along with the availability of simple protocols, makes this approach a favorite procedure for the synthesis of nanoparticles. Moreover, compared with the plant in vivo synthesis of nanoparticles, the bio-reduction of metal ions by plant extracts provides a more flexible control over
the size and shape of the nanoparticles [4-5] Generally, biochemical reduction is initiated by addition of the metal salts solution to the plant extracts, and is followed by storing the mixture at room temperature with or without agitation until the reaction is complete (within minutes) depending on the nature of the plant extract. Color changes in the reaction mixture indicate the formation of the nanoparticles. For example, AgNPs solutions appear dark (yellow-brown) in color because of the surface plasmon resonance phenomenon. Plus, the formation of the nanoparticles can be confirmed by UV–visible spectrophotometer.

_Graptophyllum pictum_ is a traditional medicine plants from Papua, Indonesia [7] containing alkaloids [7]. Its secondary metabolite contents act as an alternative base source and capping agent in MNPs synthesis as hazardous chemicals substitute. In previous study, _Graptophyllum pictum_ (GP) leaves extract was reported contains alkaloids and flavonoids [7]. Alkaloids as a weak base are needed in MNPs preparation, and flavonoids have hydroxyl group to prevent MNPs agglomeration [7]. Both of functional materials are contained in GP as base source and capping agents.

2. Methodology

2.1. Materials

Silver nitrate (AgNO₃) was purchased from Fisher Scientific and used as received without further treatment. _Graptophyllum pictum_ (GP) leaves were purchased from a local supplier. The leaves were washed thoroughly with tap water to remove dirt and washed again with distilled water before being dried in oven at 40 °C for 48 hours. The dried MCV leaves were grinded into powder by using vertical pulverising machine (Hong Chun model RT-34) and stored at room temperature for further use. All the aqueous solutions were prepared by MilliQ water (18.2 Ωcm⁻¹) [8].

2.2. Preparation & characterization of MCV leaves extract and biosynthesis of silver nanoparticles

Dried GP leaves (0.5 g) was boiled in 20 mL deionized water at 100 °C for 30 min and then cooled to room temperature (30 °C). The final obtained extract was filtered through filter paper and used as reducing/stabilizing agents for the following experiments. In a scintillating vials, 20 mL of the GP leaves extract was reacted with 10 mM of AgNO₃ solution at room temperature under stirring conditions of 250 rpm. The colour change of the reaction was observed and the time taken for the changes was noted. The solution colour changes immediately from dark brownish to purple red colour indicating the formation of silver nanoparticles (Ag-NP). The Ag-NPs solution obtained was kept safely at 4 °C. Concentration of the AgNO₃ as precursor were varied from 10-50 mM to understand the influence of it from colloidal stability perspective. The Ag-NPs were then characterized using TEM for its morphology and UV-Vis to monitor the AgNP formation kinetics. Dynamic light scattering (DLS) method by using Malvern ZS were employed to study its colloidal stability [8].

3. Results and Discussion

3.1. Ag-NPs visual observation and structural morphology

Gross examination of the potential Ag-NPs formation were performed. After 6 hours of reaction, the solution color changes from dark brownish to purple red indicating potential reaction occur (Figure 1a). This observation were in agreement with findings from Shakeel and co-workers[4]. The sample from 50 mM AgNO₃ precursor concentration were then subjected to structural investigation with TEM (Figure 1b). It was observed the formation of AgNPs with satisfactorily spherical shape with approximate diameter around ~68 nm (based on N=30 AgNPs). This finding were consistently with other finding from Tippayawat and co-worker using aloe vera as reducing agent [1].
3.2. UV-Vis spectrophotometry
Parallel changes were observed when AgNO₃ concentration were subjected to different concentration range from 10 mM- 50 mM respectively. The experiment were conducted at room temperature. The interaction at room temperature (25 °C) has been reported to generate stable AgNPs [2]. It was observed that there is slight peaks consistently at around 450 nm correspond to AgNPs surface excitation band (Figure 2). Upon incremental of the AgNO₃ concentration, the intensity increase correspondingly. UV–Vis absorption spectrum of the reaction solution approved the formation of silver nanoparticles from silver ions with the obtained peak at 450 nm [6]. The nature and characteristics of nanoparticles can be detected from SPR peak. Spherical and uniform nanoparticles in the solution have a single SPR band in the absorption spectra. In other words the number of peaks develops as anisotropy increases [5]. These results consistent with the finding from TEM morphology above. So, it is concluded, the biosynthesized Ag NPs are satisfactorily spherical in shape.

Figure 1. (a) left vial- GP leaves extract before reaction; right vial – AgNPs at the end of reaction (~6hrs) (b) TEM micrograph of AuNPs derived from GP leaves extract.

Figure 2. Spectrophotometric observation of AgNPs samples with different initial concentration of AgNO₃.
3.3. DLS measurement

The aim of dynamic light scattering measurements was carried out to determine the size distribution and polydispersity of produced Ag-NPs with different pH. This was clearly elucidated by the intensity obtained during the DLS measurements. From the Table 1 shown below, there are significant differences when different concentration of AgNO₃ been employed. Hydrodynamic size to indicate colloidal size of Ag-NPs whereas zeta potential suggested the stability of the colloidal Ag-NPs respectively. It was suggested that higher concentration of AgNO₃ as precursor will result higher stability of the nanoparticles. For an instance, 50 mM AgNO₃ results in hydrodynamic diameter size around ~120 nm and zeta potential reading of -40 MV. The indicator whether the colloids are stable or not is based on zeta potential value above +/- 30 mV [8]. The Ag-NPs samples were then be subjected to same measurement after 7 days to gauge their long term stability and the same trends were observed. Even though the hydrodynamic size significantly increased across all samples potentially due to particles agglomeration, is still within threshold of colloidal stability for sample 50 mM. Further optimization are required for other process factors such as initial pH, reaction temperature and time in order to understand the behavior of the synthesis better [1].

Table 1. DLS measurement of hydrodynamic size and zeta potential of AgNPs derived from GP leaves extracts

| Sample ID | Hydrodynamic size (nm) | Zeta Potential (mV) |
|-----------|------------------------|---------------------|
|           | 24 hours | 7 days  | 24 hours | 7 days |
| 10 mM     | 311.5    | 678.5  | -18.5    | -2.5    |
| 20 mM     | 256.4    | 564.6  | -21.6    | -11.7   |
| 30 mM     | 180.8    | 496.2  | -27.8    | -17.6   |
| 40 mM     | 145.7    | 386.5  | -35.1    | -25.6   |
| 50 mM     | 119.5    | 245.8  | -40.6    | -33.5   |

4. Conclusions

From the research, we can conclude several conclusions which are;

i. Ag-NPs derived from GP leaves extracts were synthesized successfully by facile biological method.
ii. The morphology and surface plasmon resonance behavior of the as-made Ag-NPs were characterized.
iii. Colloidal stability of the as made Ag-NPs for different precursor initial concentration were investigated.

Acknowledgements

This work was financially and technically supported by Faculty of Chemical Engineering, Universiti Teknologi MARA cawangan Pulau Pinang

References

[1] Kratosovaa G et al 2019 Biotechnol. Advan. 37 154
[2] Ajitha B. et al 2016 J. Mol. Liq. 219 474
[3] Nadhirah M. et al 2014 Adv. Mater. Res. 832 350
[4] Ahmed S. et al 2016 J. Radiation Res. App. Sci. 9 1
[5] Fahimirada S et al 2019 Ecotoxicol. Environ. Safety 168 260
[6] Pirtarighata S. et al 2019 Mater. Sci. Eng. C 98 250
[7] Sari I.P. et al 2017 *Mater. Sci. Eng.* **191** 1
[8] Osman M.S. et al 2018 *Material Today Proc.* **5** 22050