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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM MARINE POLYCHAETE *DIOPATRA CLAPAREDII* GRUBE, 1878

Nur Shaheera Hussain[a], Noor Aniza Harun[a], Mohd Naim Fadhli Mohd Radzi[a], Izwandy Idris[b,c], Wan Iryani Wan Ismail[a*]

[a]School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[b]School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[c]South China Sea Repository and Reference Centre, Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

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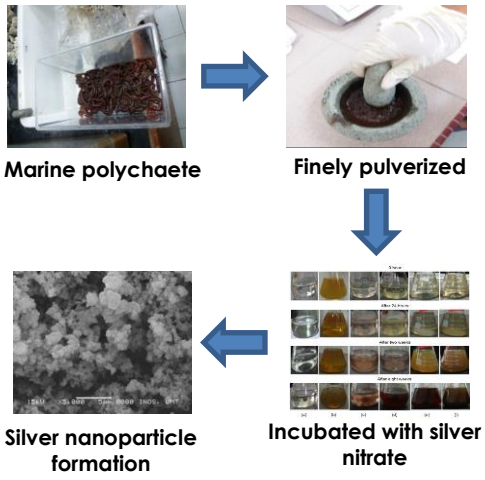
*Corresponding author
waniryani@umt.edu.my

Graphical abstract



Abstract

Silver nanoparticles (AgNPs) offer a broad range of high commercial value applications. However, current techniques to synthesize AgNPs using chemical and physical approaches are toxic to the environment and not cost-effective in the long-term. Therefore, utilization of green biological synthesis technique to produce AgNPs is one of the solutions. In this study, marine polychaete *Diopatra claparedii* was used to investigate its potential in synthesizing AgNPs. Specimens were collected from tidal flats adjacent to the mangrove forest in the west coast of Peninsular Malaysia and divided into two groups: freshly prepared (acclimatized in aquaria) and frozen (kept in the freezer) samples. Samples from both groups were cut, finely pulverized, filtrated, and mixed with silver nitrate ($AgNO_3$) at room temperature. Colour changes were recorded after 24 hours, 2 weeks, and 8 weeks of incubation. Formation of AgNPs was quantified using UV-Vis spectroscopy analysis, where surface Plasmon resonance (SPR) peaks were observed in the range of 400–440 nm, confirming the formation of the AgNPs. Subsequently, the synthesized nanoparticles were validated using scanning electron microscope. AgNPs showed weak antibacterial activity because of aggregation. Further studies are required to confirm the findings.

Keywords: Silver nanoparticles (AgNPs), green technology, marine organisms, polychaetes, Malaysian shore

Abstrak

Nanopartikel perak menawarkan pelbagai aplikasi dengan nilai komersial yang tinggi. Walau bagaimanapun, teknik semasa untuk menghasilkan nanopartikel perak menggunakan pendekatan kimia dan fizik adalah toksik kepada alam sekitar dan tidak kos efektif dalam jangka masa panjang. Oleh itu, penggunaan teknologi hijau untuk menghasilkan nanopartikel perak adalah salah satu penyelesaiannya. Dalam kajian ini, polychaete marin *Diopatra claparedii* digunakan untuk mengkaji potensinya dalam menghasilkan nanopartikel perak. Spesimen dikumpulkan dari pantai bersebelahan dengan hutan bakau di pantai barat Semenanjung Malaysia dan dibahagikan kepada dua kumpulan: sampel yang baru (diaklimatisasi dalam akuarium) dan beku (disimpan dalam peti sejuk). Sampel dari kedua-

dua kumpulan dipotong, dihancurkan, ditapis, dan dicampur dengan perak nitrat ($AgNO_3$) pada suhu bilik. Perubahan warna dicatatkan selepas 24 jam, 2 minggu, dan 8 minggu inkubasi. Pembentukan nanopartikel perak ditentukan melalui analisis spektroskopi ultra lembayung-cahaya-nampak, di mana puncak resonan Plasmon permukaan diperhatikan dalam julat 400-440 nm, untuk mengesahkan pembentukan nanopartikel perak. Selepas itu, nanopartikel yang disintesis telah disahkan menggunakan mikroskop pengimbas elektron. Nanopartikel perak menunjukkan aktiviti antibakteria yang lemah kerana penggumpalan. Kajian lanjut diperlukan untuk mengesahkan penemuan.

Kata kunci: Nanopartikel perak, teknologi hijau, hidupan laut, polikit, persisiran pantai Malaysia

1.0 INTRODUCTION

In the recent decades, the synthesis of metal nanoparticles has attracted major attention, particularly in electronics and photonics, catalysis, information storage, chemical sensing and imaging, environmental remediation, drug delivery, and biological-labeling applications [1-3]. Metal nanoparticles are made of a cluster of gold, silver, or copper with dimensions ranging 1–100 nm [4]. Among these metal nanoparticles, silver nanoparticles (AgNPs) are the most widely applied in products that directly come in contact with the human body, such as household items like detergents, soaps, shampoo, cosmetic products, toothpaste and also extensively utilized in the pharmaceutical and medical area [5-6].

Major methods to produce AgNPs are via chemical and physical approaches. However, these methods need certain conditions; for instance, physical syntheses of nanoparticles are through complicated evaporation and condensation processes, and involve high operation cost [7]. Meanwhile, chemical approaches use toxic chemicals, such as borohydride, citrate, ascorbate, and elemental hydrogen, and also release hazardous chemical by-products to the environment. Therefore, there is a need to develop an eco-friendly method to synthesize nanoparticles using green synthesis approach, for example, by using biological methods. One such method is using marine organisms, for instance polychaetes [8].

This study reports the synthesis and characterizations of AgNPs by a green approach. The AgNPs were prepared using silver nitrate as silver precursor and local polychaetes, *Diopatra claparedii* Grube, 1878 [9], as reducing agent. This species is abundantly available on the west coast of peninsular Malaysia and is currently utilized as baitworm [10]. The utilization of local polychaetes, *D. claparedii* Grube, 1878 in the synthesis of AgNPs may provide a new insight towards the development of environmentally friendly method to produce AgNPs.

2.0 METHODOLOGY

2.1 Sampling and Sample Maintenance

Sediment and specimens were collected from the lower tidal flat zone of local mangrove during low tide. The pH and salinity of the seawater were measured using pH meter and refractometer, respectively. The specimens were removed together with their tubes from the sediment using a shovel. The collected specimens were then stored in the ice chest and kept moist using wet towels while being transported to the laboratory. In addition, the sediment was collected and kept in the plastic bags. In the laboratory, the sediment was air dried before being transferred into aquaria. Live *D. claparedii* were placed and maintained in the aquaria filled with artificial seawater at 30 ppt and 27°C. Meanwhile, the dead specimens were kept at -20°C until further analysis.

2.2 Sample Preparation

Freshly prepared and frozen *D. claparedii* were cut and finely pulverized using mortar and pestle. Next, the pulverized polychaetes were weighed and divided into five different mass 1, 3, 5, 10, and 15 g. The crude extracts were then prepared by adding deionized water into each specimen to make up to 100 mL. Subsequently, the crude extract was filtrated using Whatman No.1 filter paper to obtain clean crude extract/pure extract [8].

2.3 Synthesis of Silver Nanoparticles

A total of 10 mL of the clean crude extract from each mass group was mixed with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution in a 100 mL Erlenmeyer flask. The sample was replicated into three samples for each group. In this experiment, a flask containing 10 mL of deionized water and 90 mL of 1 mM $AgNO_3$ solution was taken as a negative control while citrate-stabilized AgNPs, prepared using the method described by [20], was taken as a positive control. All samples were covered with aluminium foil, agitated at room temperature for 24 hours, and incubated for

2 weeks and eight weeks before colour observation [8]. The colour change was observed gradually for each day after 24 hours of agitation.

2.4 UV-Vis Spectroscopy

UV-visible absorption spectra of the AgNPs samples from both groups (fresh and frozen) were obtained using UV-Visible spectrophotometer (UV 1800, Shimadzu) at the broad range of wavelength of 190–800 nm.

2.5 Scanning Electron Microscope (SEM)

The selected samples of AgNPs were mounted on SEM holder, which was coated using poly-L-lysine. The morphology of AgNPs was observed using SEM (JEOL, JSM6360LA) at 10–15 kV accelerating voltage.

2.6 Assessment of Antibacterial Activity

The antibacterial activity of synthesized AgNPs from the polychaete extracts was assayed against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) strains respectively using Kirby-Bauer disk diffusion method. These bacteria are known to be resistant to various antibiotics [23]. In brief, Mueller-Hinton Agar plates were uniformly inoculated with 1.5 × 10^8 CFU/mL (0.5 McFarland Standard) of bacterial suspension, using sterile swabs in triplicates. Next, the filter paper disks were saturated with 25 µL of synthesized AgNPs from both fresh and frozen polychaete extracts using sterile forceps and incubated overnight at 37°C. In this experiment, penicillin and citrate-stabilized AgNPs were taken as positive control and dH2O and 1 mM of AgNO3 solution without polychaete extracts were taken as negative control. After 24 hours, the samples were observed and the diameter of inhibition zone was measured using a ruler [8].

3.0 RESULTS AND DISCUSSION

3.1 Colour Observation

Both freshly prepared and frozen samples at the weight of 1, 3, 5, 10, and 15 g showed colour changes after incubation with 1 mM of $AgNO_3$ solution. The colour gradually changed from light yellow after 24 hours of agitation to dark brown after 2 weeks of incubation (Figure 1). This colour observation is parallel to the dark brown colour reported by many researchers [11-13]. The colour changes in aqueous solution are attributed to the localized surface Plasmon resonance phenomenon and it occurred because of the reduction of Ag^+ into AgNPs during exposure to polychaete extracts. Moreover, the colour for positive control of citrate-stabilized AgNPs changed from light yellow to dark yellow after 2 weeks and 8 weeks of incubation, which indicates the formation of AgNPs. Meanwhile, the negative control solution did not exhibit any colour change.

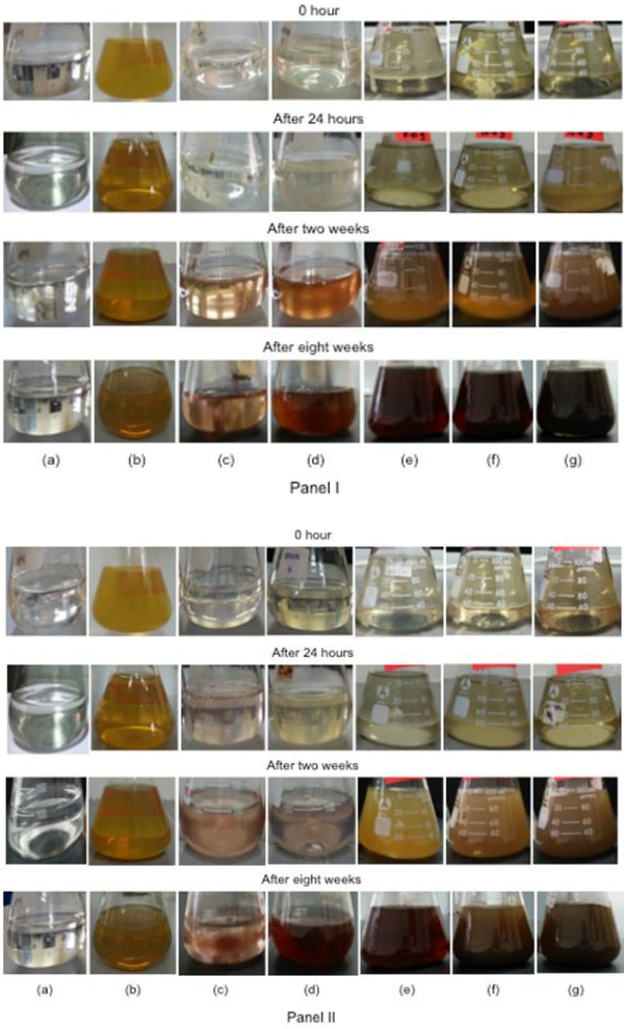


Figure 1 Colour changes from reducing activity of Malaysian marine worms (polychaete, *D. claparedii*) indicate the existence of silver nanoparticles. (Panel I:) freshly prepared samples, (Panel II:) frozen samples. (a) Negative control ($AgNO_3$ solution without polychaete extract) (b) Positive control ($AgNO_3$ solution with commercial silver) (c) AgNPs-1 g ($AgNO_3$ solution with 1 g of polychaete extract) (d) AgNPs-3 g ($AgNO_3$ solution with 3 g of polychaete extract) (e) AgNPs-5 g ($AgNO_3$ solution with 5 g of polychaete extract) (f) AgNPs-10g ($AgNO_3$ solution with 10 g of polychaete extract) (g) AgNPs-15 g ($AgNO_3$ solution with 15 g of polychaete extract)

3.2 UV-Vis Spectroscopy

UV-visible spectroscopy was performed to compare the Plasmon resonance bands of AgNPs synthesized using both fresh and frozen polychaete samples. UV-visible absorption spectra are formed because of the surface plasmon resonance (SPR) effect [14] and the evidence for the presence of AgNPs was the shift of the UV-Vis absorption peaks. The absorbance peaks of the AgNPs produced from fresh (411 nm) and

frozen polychaetes (414–442 nm) are red-shifted relative to positive control of citrate-stabilized AgNPs (394 nm) (Figure 2a–d). The differences in absorbance peak compared to positive control are believed to be caused by several factors including shape, size, and morphology of the synthesized AgNPs [14-15].

Both fresh and frozen polychaete samples of 5, 10 and 15 g displayed significant SPR bands of formation AgNPs. However, no evidence of the formation of AgNPs was observed from samples in lower weights (1 and 3 g) of fresh and frozen polychaetes (Figure 2a–d). This finding suggests that the optimum mass of polychaetes to be utilized as reducing agent to produce AgNPs is between 5 – 15 g. Interestingly, 3 g of frozen polychaetes demonstrated contradictory results, where the formation of SPR peak of AgNPs was observed. The observation may be attributed to the possible enzymes or other bioactive compounds that were released after the cessation of active processes in a certain cell, hence, facilitated for the AgNP formation [2].

Furthermore, AgNPs produced from fresh polychaete extract showed narrower peaks, suggesting that uniform and homogenous size of AgNPs were produced and observed through the UV-Vis absorbance bands (Figure 2a and c). In contrast, for the AgNPs prepared from frozen polychaete crude extracts, broad SPR spectra were observed, possibly the consequences of the self-aggregation of AgNPs during the synthesis process (Figure 2b and d).

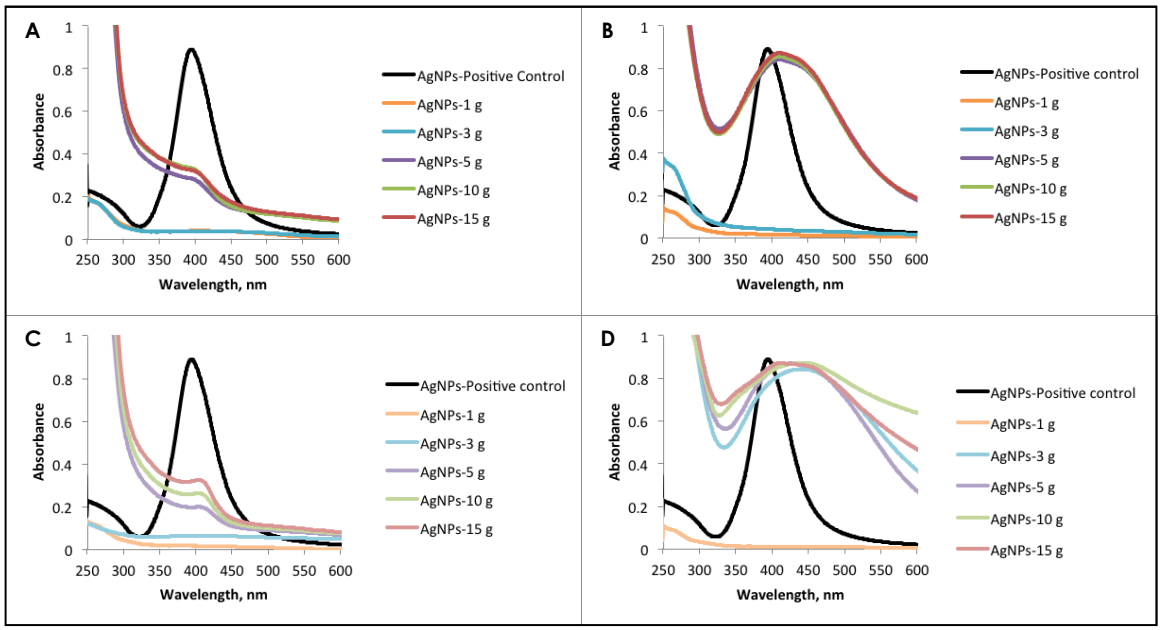


Figure 2 UV-Vis spectroscopy analysis of synthesized AgNPs at five different concentrations from freshly prepared polychaetes after incubated at room temperature for two weeks (A) and eight weeks (B), and frozen polychaetes after two weeks (C) and eight weeks (D)

3.3 SEM Analysis

The synthesized of AgNPs were characterized by SEM to provide further insight into their shape and morphology. It was assumed that the form of AgNPs produced from polychaete extract is a spherical-like shape, as reported in the previous studies [16-17]. In contrast, no single particle in spherical-like shape was obtained from the synthesis of AgNPs using both fresh and frozen polychaetes extracts (Figure 3). The SEM photographs obtained from AgNPs prepared using both fresh and frozen polychaete extracts show the existence of aggregations of spherical-like particles. This aggregation phenomenon was expected to be observed under SEM on account of nature of sample preparation for SEM during the drying process of the samples [18-19].

3.4 Assessment of Antibacterial Activity

Assessment of the antibacterial activity of the synthesized AgNPs from the polychaete extract showed a weak antibacterial effect against both strains as there was no clear zone formed (Table 1). Hence, it is believed that the synthesized AgNPs cannot inhibit the bacterial growth. These negative results might be because of clumping and bigger size of AgNPs synthesized from the polychaete extract. A previous study showed that the antibacterial effect was influenced by many factors such as the size,

shape, surface chemistry, and dose of AgNPs used [23]. However, the most important factor in determining the antibacterial potential of AgNPs is the size of AgNPs. This is because the bigger size of AgNPs has smaller surface area than the smaller size of AgNPs.

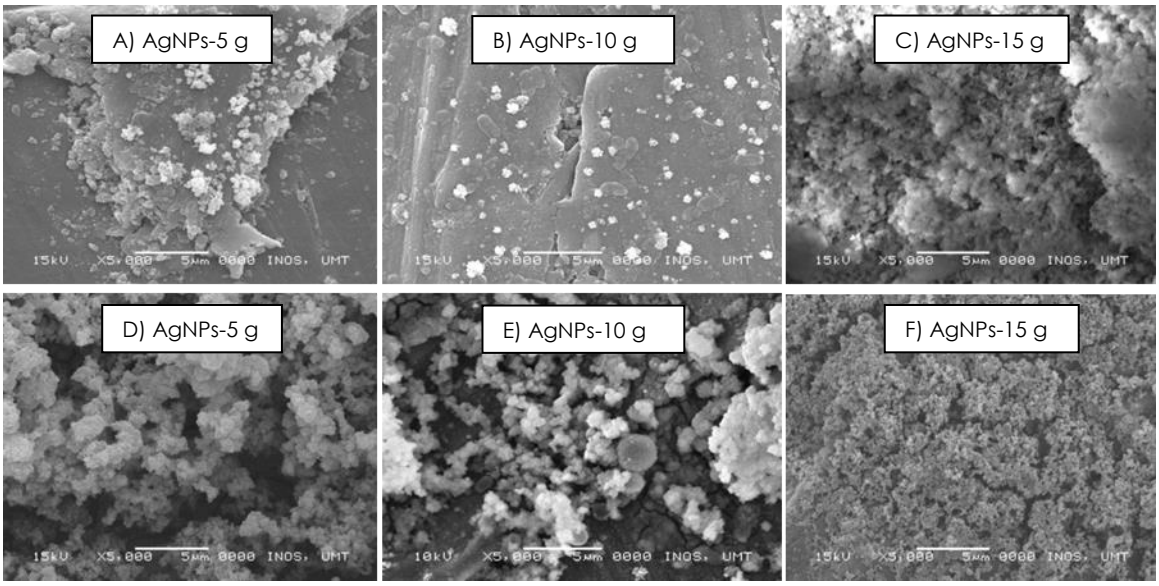


Figure 3 Magnified images of AgNPs of freshly prepared samples (A-C) and frozen samples (D-F) viewed under SEM. Scale: 5000x magnification

Table 1 The value of inhibition zone (mm) of the synthesised AgNPs from fresh and frozen samples at different concentrations against *Staphylococcus aureus* and *Escherichia coli*

Zone of Inhibition (mm)										
Microorganisms	AgNPs concentrations (g/ml)						Controls			
	Fresh			Frozen			Positive controls		Negative controls	
	1	5	15	1	5	15	Penicillin	Citrate stabilised AgNPs	dH_2O	1 mM $AgNO_3$ solution
S. aureus	0	0	0	0	0	0	20	0	0	0
E. coli	0	0	0	0	0	0	10	0	0	0

Moreover, the previous study observed that smaller size of AgNPs produced greater antimicrobial effect because of the larger surface area [24]. The bigger size of the AgNPs makes them difficult to penetrate inside the bacteria [25-26], thereby preventing them from inhibiting the bacteria. Another reason may be because of the weak electrostatic attraction between the nanoparticles and the bacterial cells [27]. Bigger size (clumping particles) of the AgNPs has a smaller surface area, which reduces the number of charge around the membrane of the synthesized AgNPs. Subsequently, the electrostatic attraction between the positive charge of the aggregation of AgNPs and the negative charge of the bacterial cells is weaker than non-aggregation AgNPs. A study by [28] suggested that a strong electrostatic attraction between nanoparticles and bacterial cells is the most suitable to be the bactericidal agent. Therefore, more studies need to be conducted to confirm these findings.

4.0 CONCLUSION

This study reveals the potential application of local polychaete (*D. claparedii*) in synthesizing AgNPs. From the results, fresh polychaete crude extract at the mass of 5, 10, and 15 g showed the evidence of the formation of AgNPs based on the UV-vis spectra. The spherical-like shape of synthesized AgNPs was recorded using SEM; however, aggregation of AgNPs was observed. The nanoparticles showed weak antibacterial effect because they were clumped and aggregated. In conclusion, the synthesized method used need improvement in synthesizing the AgNPs effectively. Further study on this species, the most appropriate synthesizing method and the mechanisms involved in the reactions are currently in progress.

Acknowledgement

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