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BIOSYNTHESIS OF SILVER NANOPARTICLES USING *Citrus grandis* PEEL EXTRACT

(Biosintesis Nanopartikel Perak dengan Menggunakan Ekstrak Kulit *Citrus grandis*)

Nur Syazana Jalani[1], Wong Michell[1], Wong Ee Lin[1], Sharifah Zati Hanani[1]*, Uda Hashim[2], Rozaini Abdullah[1]

[1]*Department of Chemical Engineering, Faculty of Engineering Technology, Universiti Malaysia Perlis, Kampus UniCITI Alam, 02100 Sungai Chuchuh, Padang Besar, Perlis, Malaysia*
[2]*Institute Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia*

**Corresponding author: sharifahzati@unimap.edu.my*

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Abstract

In this study, *Citrus grandis* or pomelo peel extract was used to synthesize silver nanoparticles (AgNPs). The effect of reaction time, amount of plant extract and concentration of silver nitrate which could influence the biosynthesis process were also studied. The synthesized AgNPs were then characterized by UV-Visible spectroscopy, Fourier transform infra-red spectroscopy (FTIR) and field emission scanning microscopy (FESEM) equipped with energy dispersive X-ray (EDX). The resulting UV-Vis spectra of synthesized AgNPs from pomelo peel extract showed standard surface plasmon resonance band in range of 401-433 nm which indicated the presence of AgNPs. FTIR spectra confirmed the existence of possible functional groups which might be responsible for the reduction of nanoparticles. Most of the synthesized AgNPs were observed in spherical shape which ranged between 20-30 nm under the Field Emission Scanning Electron microscope. The EDX analysis showed the strong signal at 3 keV from the silver atoms in the synthesized nanoparticles which confirmed that the synthesis of AgNPs was successful.

Keywords: silver nanoparticles, *Citrus grandis* peel extract, biosynthesis, surface plasmon resonance

Abstrak

Dalam kajian ini, ekstrak kulit *Citrus grandis* atau limau bali telah digunakan untuk menghasilkan nanopartikel perak. Pengaruh masa tindak balas, jumlah ekstrak kulit limau bali dan kepekatan perak nitrat ke atas biosintesis nanopartikel perak turut dikaji. Pencirian nanopartikel perak yang terbentuk kemudiannya dijalankan menggunakan spektroskopi sinar UV, spektroskopi infra merah transformasi Fourier (FTIR) dan mikroskopi imbasan pancaran medan (FESEM) dilengkapi dengan sinar-X tenaga serakan (EDX). Spektra UV-Vis nanopartikel perak dari ektrak kulit limau bali menunjukkan puncak resonansi plasmon sekitar 401-433 nm yang membuktikan pembentukan nanopartikel perak. Spektra FTIR membuktikan kewujudan beberapa kumpulan berfungsi yang mungkin terlibat dalam proses penurunan nanopartikel. Kebanyakan nanopartikel perak dilihat berbentuk sfera dan bersaiz di antara 20-30 nm melalui mikroskopi imbasan pancaran medan. Analisis EDX pula menunjukkan isyarat yang kuat daripada atom perak dalam nanopartikel yang telah disintesis dan ini membuktikan sintesis nanopartikel perak telah berjaya.

Kata kunci: nanopartikel perak, ekstrak kulit *Citrus grandis*, biosintesis, puncak resonansi plasmon

Introduction

Silver nanoparticles (AgNPs) have applications in various fields due to their unique optical, physical, electrical and medicinal properties. Silver nanoparticles can be incorporated into biosensor materials [1], antimicrobial applications [2], cosmetic products [3] and electronic components [4].

Commonly, AgNPs can be synthesized by physical, chemical and biological methods. Although physical and chemical methods may produce well-defined AgNPs, but these conventional methods have several disadvantages. These methods usually involve expensive equipment, high energy consumption and operation conditions such as temperature and pressure. Besides, utilizing hazardous chemicals as reducing agent in synthesizing AgNPs such as sodium borohydride, $NaBH_4$ gives an adverse effect to health due to the absorption of harmful chemicals on its surface. This concern has enhanced the researchers to develop safe consumption nanoparticles and environmentally friendly processes through other biological approaches.

Recently, biosynthesis of nanoparticles has been an emerging research area which offers an alternative to conventional chemical and physical methods. The biosynthesis of AgNPs employing either microorganisms or plant extract offers an environmentally friendly process by using less toxic chemicals. However, plants are preferred because it does not involve any process of maintaining cell culture. Moreover, use of plant extracts also reduces the cost of microorganism isolation and their culture media. The synthesizing AgNPs from plant extract such as *Azadirachta indica* [5], *Prosopis farcta* [6], *Peumus boldus* [7] and *Carica Papaya* [8] have been reported extensively. Different plant showed different morphology of synthesized nanoparticles. The spherical shaped AgNPs having size in range from 4.7-11.5 nm were achieved using *Azadirachta indica* extract as reducing and capping agent [5]. Meanwhile, spherical AgNPs with size 10.8 nm was successfully synthesized from *Prosopis farcta* extract [6]. The reaction conditions such as amount of plant extract, concentration of silver nitrate and reaction time is important to achieve maximum number of synthesized nanoparticles. These parameters also affected the shape and size of the synthesized nanoparticles [7, 8].

Even though AgNPs have been synthesized from various plants, the synthesis of AgNPs from *Citrus grandis* or pomelo peel waste has been not reported. In this study, pomelo peel extract was used as natural reducing agent for the synthesis of AgNPs. This pomelo peel extract has an ability to reduce and stabilize the metallic ions. Besides, the influence of reaction time, amount of plant extract and concentration of silver nitrate on the synthesis of AgNPs were also studied.

Materials and Methods

Materials

The fresh *Citrus grandis* fruit was purchased from nearest stall. Silver nitrate and all chemicals (analytical grade) were purchased from Bendosen Laboratory Chemicals, Norway. All the chemicals were used as received without further purification process.

Preparation of plant extract

The pomelo peels were dried in the oven at 60 °C for 16 hours. Then, the pomelo peels were blended into fine powder and sieved [9]. The preparation of plant extract was prepared following the modified procedure from [10]. An amount 1g of pomelo was dissolved in 50 ml of distilled water and then heated at 70 °C for 30 minutes in a water bath heater. After 30 minutes, the extract was removed from the water bath heater and filtered with a filter paper.

Biosynthesis of silver nanoparticles

The AgNPs were synthesized following the procedure by Nakhjiri et al. [11] with slight modification. In a typical experiment, 10 ml of pomelo peel extract was added to 30 ml of 1 mM silver nitrate solution and then kept in a water bath maintained at 70 °C for 2 hours. The formation of AgNPs were confirmed due to the colour changes of the solution. A series of experiments were conducted to investigate the effect of condition parameters including reaction time, amount of plant extract and concentration of silver nitrate. The effect of reaction time was evaluated by incubating the reaction mixtures for 1, 2, 3, 4 and 5 hours in a water bath. The amount of plant extract was varied to 2.5, 5.0, 7.5, 10.0 and 12.5 ml while the concentration of silver nitrate is constant at 1 mM. The effect of silver salt was determined by varying the concentration of silver nitrate (0.5, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 mM).

Characterization of synthesized silver nanoparticles

The UV-Vis spectrometer (Shimadzu, Model UV-1800 spectrophotometer) was used to measure the absorption spectra of the synthesized AgNPs against distilled water as blank. The absorbance of solution was recorded after diluting a small aliquot (0.5 ml) of sample with 5 ml deionized water.

FTIR analysis was carried out after the removal of the free biomass residue or compound that was not absorbed by repeated centrifugation and redispersion in water. Thereafter, the purified suspension was dried in hot air oven at 60 °C for 10-15 minutes. Finally, the dried nanoparticles were analyzed by FTIR spectrophotometer (Perkin Elmer) and the spectra was scanned in the range of 4000 cm^{-1} to 650 cm^{-1}. The sample was dispersed uniformly in a matrix of dry KBr and then compressed to form a clear disc which then examined directly.

The morphology of the synthesized AgNPs was determined using FESEM-EDX (Model FEI Nova NanoSEM 450) at the NOR Laboratory in University Science Malaysia by coating the nanoparticles with a fine layer of platinum and placed in the FESEM-EDX to view its surface image.

Results and Discussion

Visual observation and UV-Vis spectroscopy

The early prediction of the AgNPs formation by reduction of silver nitrate and pomelo peel extract can be observed by colour change of the mixture. The change of colour from colourless to yellowish-brown solution as shown in Figure 1 indicates the presence of AgNPs in the reaction mixture. The increase in colour intensity of reaction mixture could be due to increasing number of nanoparticles formed because of reduction of silver ions present in the aqueous solution. It is known that AgNPs appear yellowish-brown colour due to the excitation of the surface plasmon vibrations [12]. The surface plasmon resonance (SPR) phenomenon occurs when noble metal nanoparticles emit strong electromagnetic fields on the particle surface thus enhancing its radiative properties [13]. This leads to a strong absorption band at about 400-500 nm in UV-Vis spectra of AgNPs [14].

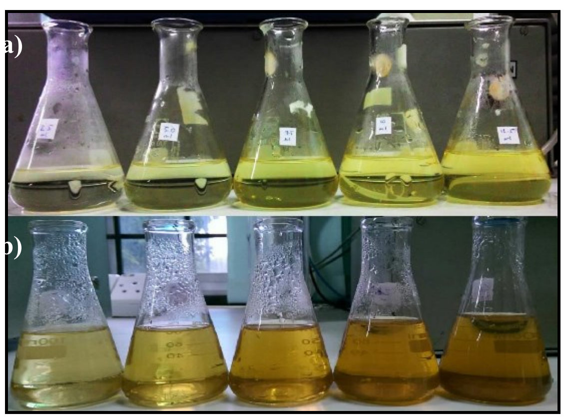

Figure 1. The colour of mixture reaction (a) before and (b) after the reaction

Effect of pomelo peel extract amount

Figure 2 shows the UV-Vis spectra of the synthesized AgNPs at different amount of pomelo peel extract from 2.5 ml to 12.5 ml. The UV-Vis spectra of synthesized AgNPs at pomelo peel extract from 5.0 ml to 12.5 ml showed the SPR peak obtained ranged from 401.6 nm to 407.6 nm as a result of silver plasmon resonance which confirmed the formation of AgNPs. The increase of pomelo peel extract results in increase of SPR intensity and the SPR band shifted to longer wavelength. The increase in intensity could be due to increasing number of nanoparticles formed because of reduction of silver ions present in the aqueous solution. Meanwhile the SPR band shifted to longer wavelength may be due to the amount of reducing agents found in the reaction mixture. As the amount of pomelo peel extract increases, more reducing agents are available. From Figure 2, the 12.5 ml of pomelo peels extract gave the highest SPR intensity where the reducing agent bounds itself to the surface of Ag^{+} and reduces it to Ag^{0} [15].

Meanwhile, no peak was observed at 2.5ml of pomelo peel extract. This is due to insufficient of reducing agent to complete the formation of AgNPs.

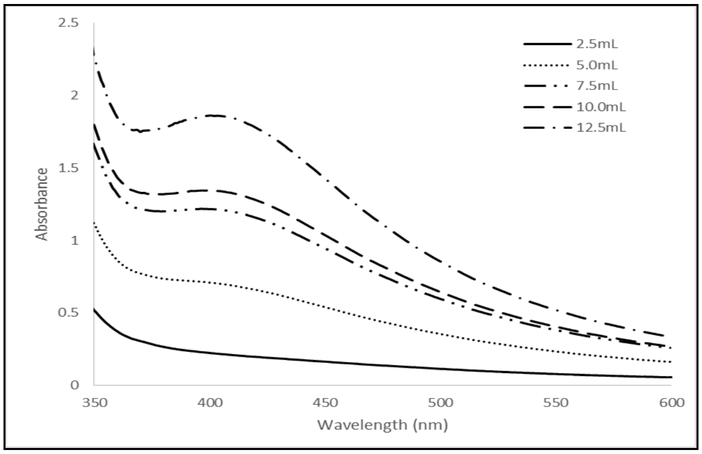

Figure 2. UV-Vis spectra of AgNPs synthesized at for various pomelo peel extract amount (Constant parameter: 1 mM $AgNO_3$, 70 ºC, 2 hours)

Effect of silver nitrate concentration

The UV-Vis spectra in Figure 3 shows the AgNPs were synthesized at different silver nitrate concentration. The SPR wavelengths vary from the range of 406 nm to 422 nm as the concentration silver nitrate increased from 1 mM to 5 mM. It was observed that as the concentration of silver nitrate increases, the SPR band shifts to the longer wavelength. This may suggest high concentrations of silver nitrate (3 mM to 5 mM) may cause the formation of large nanoparticles [16]. The highest SPR intensity can be observed at 2mM of silver nitrate. However, as the concentration further increase to 5 mM, the SPR intensity decreases. This phenomenon may occur due to the limited availability of functional groups in the pomelo peels extract to act as reducing agents [17]. Although the availability of silver ions for aggregation is high, the limited amount of reducing agent prevents the formation of AgNPs.

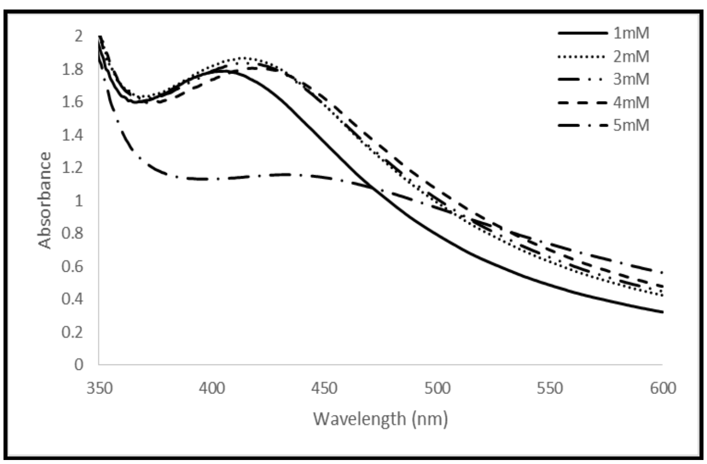

Figure 3. UV-Vis spectra of AgNPs at different concentration of silver nitrate (Constant parameter: 10 ml pomelo peel extract, 70 ºC, 2 hours)

Effect of reaction time

The UV-Vis spectra of synthesized AgNPs as shown in Figure 4 was synthesized at different reaction time. The SPR wavelength ranging from 401 nm to 415 nm as the reaction time increased from 1 hour to 5 hours. The SPR peak appeared at about 401 nm at the first hour and slowly shifts to the longer wavelength with increasing reaction time. The intensity of the SPR peak increased as the reaction time increased, which indicated the increased formation of the synthesized AgNPs. The increase in SPR intensity might be due to the transformation of OH groups to carbonyl groups because of silver ions reduction [18].

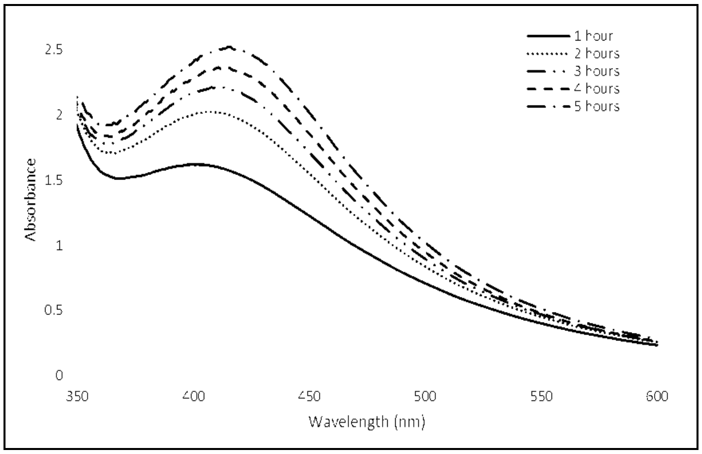

Figure 4. UV-Vis spectra of AgNPs at different reaction time (Constant parameter: 1 mM $AgNO_3$, 10 ml pomelo peel extract, 70 ºC)

FTIR analysis

The FTIR analysis was carried out to identify the major functional groups in pomelo peel and their possible involvement in AgNPs synthesis. Table 1 shows the shifting of peaks of peel extract which may suggest the involvement of the functional group in the formation of AgNPs. First, the shifting of the peak could be observed from 3357.90 cm^{-1} to 3380.21 cm^{-1} which is corresponds to H-bonded alcohols and phenols. Another shift is observed in peak from 1607.93 cm^{-1} to 1641.77 cm^{-1} which correspond to C=C of alkenes and C=N amine stretching. The shifting of these peaks indicates these functional groups might involve in the synthesis of AgNPs. Meanwhile, the observed peak at 1051.29 cm^{-1} denote –C-OC linkages and –C-O bonds which mainly attributed from flavonoids and terpenoids in pomelo peel extract [19]. The disappearance of these peaks in FTIR spectra of AgNPs suggest the involvement of these bioactive compounds in reducing silver ions to AgNPs.

Table 1. FTIR peak assignments of pomelo peel and synthesized AgNPs

Assignment	**Pomelo Peel**	**AgNPs**
H-bonded alcohols and phenols	3357.90 cm^{-1}	3380.21 cm^{-1}
C=C of alkenes and C=N amine stretching	1607.93 cm^{-1}	1641.77 cm^{-1}
C-N stretching and C-O stretching of amino and carboxyl groups	1051.29 cm^{-1}	-

Condition of synthesized AgNPs: 2 mM $AgNO_3$, 12.5 ml pomelo peel extract, 5 hours

FESEM-EDX analysis

The surface morphology of nanoparticles and their chemical composition was investigated using FESEM-EDX. Figure 5a and 5b show that most of the synthesized AgNPs are found to be in spherical shape and some are irregular which the size range between 20-30 nm. The aggregation of the synthesized AgNPs may be caused by cell components present on the nanoparticles surface that acts as a capping agent [20]. The EDX profile in Figure 5c shows a spectral signal in silver region (Ag) approximately at 3 keV which corresponded to the absorption of metallic silver due to SPR of AgNPs [21]. The others signal from Cl, C, O, N and S atoms are also recorded which might be attributed to pomelo peel extract used.

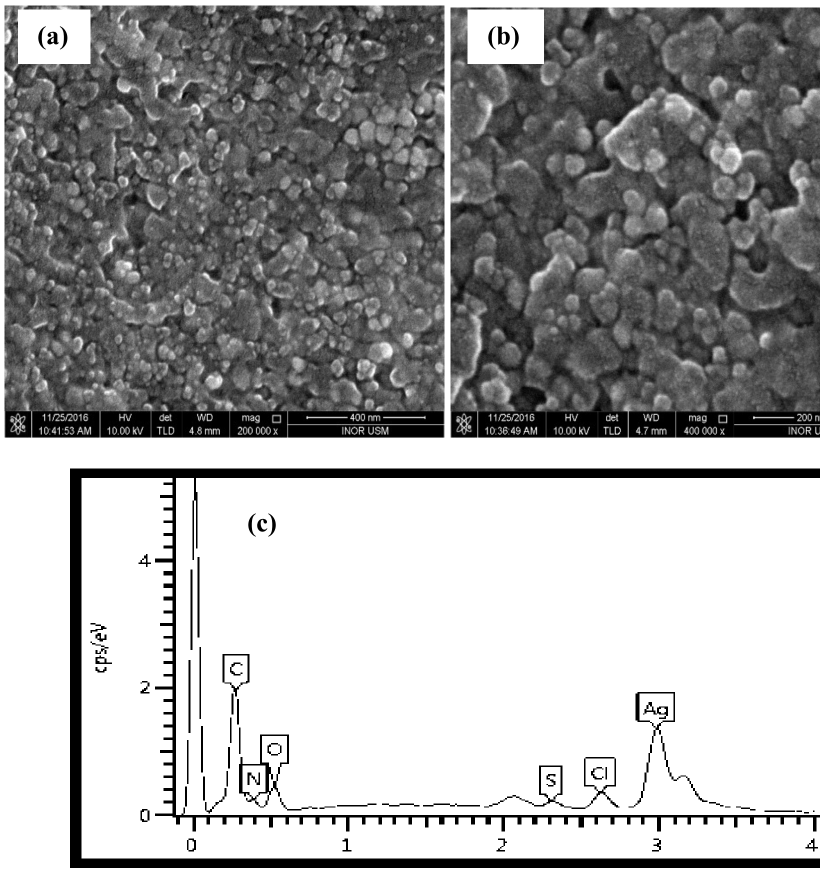

Figure 5. FESEM image of synthesized AgNPs at various resolutions (a) 200,000x, (b) 400,000x and (c) EDX profile showing the chemical composition (Condition of synthesized AgNPs: 2 mM $AgNO_3$, 12.5 ml pomelo peel extract, 5 hours)

Conclusion

The spherical and irregular AgNPs with size ranging from 20-30 nm were successfully synthesized using pomelo peel extract as reducing agent. The experiment done showed that parameters studied do affect the biosynthesis of AgNPs as the time and amount of peel extract was directly proportional to the number of AgNPs formed. In contrast, the number of AgNPs formed was inversely proportional to the concentration of silver nitrate.

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