

Green Synthesis of Ag Nanoparticles and Their Performance towards Antimicrobial Properties

(Sintesis Hijau Nanozarah Ag dan Prestasi ke Arah Sifat Antimikrob)

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ABSTRACT

*Green synthesis is a forthcoming trend in the nanotechnology field where classical methods of synthesis are now replaced by inexpensive and eco-friendly methods. In this study, a green method has been developed for the synthesis of silver nanoparticles (AgNPs) where AgNPs were synthesised using water-based facile hydrothermal method. Silver nitrate (AgNO_3) and polyvinylpyrrolidone (PVP) were used as precursor and reducing agents to produce AgNPs. The molar ratio effect of the precursor and stabiliser, its reaction temperature and reaction time were investigated. X-ray Powder Diffraction (XRD), Field-Emission Scanning Electron Microscope (FESEM) and UV-Vis Spectrometry were used to characterise the AgNPs. The as-synthesized AgNPs with different molar ratios of the precursor to stabiliser were tested for antibacterial activities using Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*). All the AgNPs samples exhibited antibacterial activities that were stronger against Gram-negative bacteria, as compared with Gram-positive bacteria. The diameter of the zone of inhibition (ZOI) increased with the increase of the AgNO_3 : PVP molar ratios. The results obtained proved that uniform AgNPs synthesized via green techniques have a high potential of influencing applications involving antimicrobial properties.*

Keywords: Antibacterial activities; green synthesis; hydrothermal; nanoparticles; silver

ABSTRAK

*Sintesis hijau adalah trend akan datang dalam bidang nanoteknologi yang menggantikan kaedah sintesis tradisi dengan kaedah yang mesra alam dan murah. Dalam kajian ini, kaedah hijau telah dibangunkan untuk mensintesis nanozarah perak (AgNPs) dengan AgNPs disintesis menggunakan air-berdasarkan kaedah reaksi mudah hidroterma. Perak nitrat (AgNO_3) dan polivinilpirolidon (PVP) telah digunakan sebagai pelopor dan ejen penurunan untuk menghasilkan AgNPs. Kesan nisbah molar pelopor dan penstabil, suhu tindak balas dan masa tindak balas telah dikaji. Ujian X-Ray Pembelauan Serbuk (XRD), Pancaran Medan Mikroskop Elektron Pengimbas (FESEM) dan Spektroskopi UV-Vis digunakan untuk mencirikan AgNPs. AgNPs disintesis dengan nisbah molar yang berbeza daripada pelopor dan penstabil telah diuji untuk aktiviti antibakteria menggunakan bakteria Gram-positif (*Bacillus subtilis*) dan bakteria Gram-negatif (*Escherichia coli*). Semua sampel AgNPs menunjukkan aktiviti anti-bakteria yang kuat terhadap bakteria Gram-negatif, berbanding dengan bakteria Gram-positif. Zon diameter perencatan (ZOI) meningkat dengan peningkatan nisbah molar AgNO_3 : PVP. Keputusan yang diperolehi membuktikan bahawa AgNPs berseragam yang disintesis melalui teknik hijau berpotensi tinggi untuk mempengaruhi aplikasi yang melibatkan sifat antimikrob.*

Kata kunci: Aktiviti anti-bakteria; hidroterma; nanozarah; perak; sintesis hijau

INTRODUCTION

Metal nanoparticles particularly silver nanoparticles (AgNPs) are very attractive because of their size and shape-dependent properties. In recent years, research on the synthesis of AgNPs employing green materials, commonly known as green synthesis, has been studied and exploited to a large extent. AgNPs material have a wide spectrum of potential applications due to their unique properties, which are in many ways different compared to their bulk form. It was stated that nanosilver had been utilised for roughly 120 years, more commonly known as ‘colloidal silver’ (Yang 2017). Any noble metals employed in optical detection are usually used to obtain the surface plasmon resonance (SPR) effect. In essence, silver shows the highest SPR band

intensity as compared to any other NPs like copper and gold (Nurani et al. 2015). Moreover, nano-sized silver has distinct chemical, biological and physical properties when compared with macro-sized silver. Their unique physico-chemical properties allow them to display a wide spectrum of bactericidal and fungicidal activities (Srikanth et al. 2016) that have made them enormously popular as antimicrobial agents, in healthcare-related products, cosmetics, coatings for medical devices and optical sensors (Zhang et al. 2016).

In order to fulfill the requirement of AgNPs as antibacterial agents in healthcare-related products and food industries, numerous techniques have been developed for synthesizing AgNPs with enhanced optical, electrical, thermal and biological properties. These include biological

synthesis (Banik & Dhekial 2015; Chauhan et al. 2013; Gandhi & Khan 2016), chemical synthesis (Gudikandula & Charya Maringanti 2016; Khodashenas & Ghorbani 2015; Landage 2014; Muzamil et al. 2014), physical synthesis (Pacioni et al. 2015; Tran et al. 2013) and polyol synthesis (Hsu & Wu 2011; Korte 2007; Yan et al. 2012). Evaporation-condensation and laser ablation are classified as foremost in vital physical approaches. In spite of the fact that the physical synthesis of AgNPs employing a tube furnace at an atmospheric pressure is able to deliver uniform and size-controlled AgNPs, it still has some drawbacks (Natsuki 2015; Niknejad et al. 2015). For example, tube furnace consumes an abundant amount of energy and space, which leads to a rise in the environmental temperature surrounding the source material, and requires a lot of time to achieve thermal stability (Abou El-Nour et al. 2010; Pacioni et al. 2015; Zewde et al. 2016). Polyol and other chemical reducing methods are common techniques in synthesizing metal nanoparticles. Reducing agents such as sodium borohydrate or hydrazine (Mehr et al. 2012; Noordeen et al. 2013; Zhong et al. 2016) in a silver salt solution are widely employed in the synthesis of AgNPs due to the fast reduction of Ag^+ to Ag^0 . However, most of the chemical techniques used for the synthesis of nanoparticles are too expensive and also involve the utilisation of harmful and unsafe chemicals. These techniques cover complex multi-procedures during synthesis, and cause various biological risks. Continuous dependence on chemical synthesis can also lead to serious economical and environmental problems. Economic issues are expected to arise from a consistent increase of toxic chemical reagents because of its continuous demand but restricted supply (Rashid et al. 2013; Zhou & Wang 2012). Excessive use of solvents or chemicals and conducting synthesis under high energy over the long run may lead to further damage to our ecosystem. Greener routes have been explored for over a decade to reduce the environmental impact of nanoparticle synthesis. The standards of green chemistry were presented by Anastas and Warner (Zhang et al. 2011) who created 12 principles that persuasively depicted a green chemistry approach.

The green chemistry approach is a technique used to discover alternative effective reaction conditions that use environmentally-friendly reaction media and renewable resources to complete the preferred chemical transformations with reduced by-products or waste, as well as eliminating the use of conventional organic solvents wherever possible. Three fundamental principles for the designation of nanoparticles in a green synthesis approach are the choice of the solvent medium (ideally water), non-toxic reducing agent and an eco-friendly stabiliser for the stabilisation of the nanoparticles (Anastas & Eghbali 2010; Genuino et al. 2012; Maratha 2011).

Chemical-reducing approaches using a solution as media have high possibilities of obtaining nanostructured metals with distinct morphologies and a high-end product. In general, refluxing and boiling are well-known for heat-induced methods in the synthesis of AgNPs. However,

hydrothermal techniques are rarely utilised for the synthesis of nanoparticles, although this process can deliver an eco-friendly outcome and is a more energy-efficient approach to synthesis well-crystallized and uniform metal nanoparticles (Zou et al. 2007). Thus, in this study, we synthesized AgNPs *via* a toxic free hydrothermal process by employing water as the medium. Polyvinylpyrrolidone (PVP) is then employed as the stabiliser and reducing agent. In order to obtain an optimal condition for the fabrication of AgNPs, the effects of various parameters such as the hydrothermal temperature, AgNO_3 :PVP molar ratio and reaction time of the hydrothermal process on the size of AgNPs were evaluated.

EXPERIMENTAL DETAILS

Chemicals All the chemicals and reagents used in this study were of analytical grade without further purification. Silver nitrate, AgNO_3 (Bendosen), polyvinylpyrrolidone, PVP, average molecular weight, $M_w = \sim 1\,300\,000$ (Sigma-Aldrich, Co. USA), bacteria cultures (*Escherichia coli* and *Bacillus subtilis*) were obtained from the Institute of Research for Molecular Medicine (INFORMM), Universiti Sains Malaysia (USM)'s laboratory stock culture, antibiotic (Chloramphenicol, Duchefa Biochemie, Netherlands), Mueller-Hinton (MH) agar culture medium (HiMedia, Mumbai, India).

Synthesis Silver nanoparticles (AgNPs) were synthesised using a facile hydrothermal method. The synthesis was carried out based on the previous study by Zhou and Wang (2012) with some modifications. In this process, all the materials were added together at room temperature and the reaction was carried out by heating in a closed hydrothermal system. 0.612 g of AgNO_3 and 0.4 g of PVP were dissolved, respectively, in a 20 mL of distilled water. The molar ratio between the repeating unit of PVP and AgNO_3 was $R=1$. Both solutions were simultaneously added dropwise into 50 mL of distilled water. Constant magnetic stirring was applied for 15 min. The entire mixture was then transferred into a Teflon vessel (capacity=100 mL), which was placed in a stainless steel hydrothermal reactor and heated in the oven at 160°C for 24 h. Finally, the prepared sample was washed and centrifuged for 10 min. This washing process was repeated four times to remove possible contamination (excess PVP) and then freeze-dried overnight.

The AgNPs were prepared using several molar ratios ($R=N$) between AgNO_3 and PVP, where N values are 0.5, 1, 3, 5, 7, and 10. The reaction time and temperature of synthesis was fixed at 24 h and 160°C , respectively. All the synthesised samples under different molar ratio were further tested for antimicrobial activities in order to determine its optimum molar ratio parameter before varying the temperature and reaction time parameters. Besides, the effect of temperature and reaction time during synthesis of AgNPs was evaluated by reducing the temperature from 160°C into 140°C and 120°C and reaction times were changed to 6, 12, 18, and 36 h.

Characterisations The crystallinity and phase purity of the synthesised AgNPs were analyzed by powder X-ray diffraction (XRD) (PW 3040/60 X'PERT PRO, PANalytical) using CuK_α (1.541 Å) radiation in the range $2\theta = 10\text{-}90^\circ$. UV-Vis spectra from 200 nm-800 nm for all samples prepared under different conditions were performed on the LAMBDA 25 UV/Vis Systems spectrometer. The morphology of the as-prepared AgNPs powder was characterised by Field Emission Scanning Electron Microscope (FESEM) (Leo Supra 50 VP Field Emission SEM). An acceleration electron voltage of 10 kV voltages was applied to obtain the FESEM images. Further analysis was carried out using high-resolution Transmission Electron Microscopy (HRTEM) (TECNAI G2 20 S-TWIN, FEI) at 200 kV.

Antimicrobial activities of AgNPs The antimicrobial test of AgNPs were quantitatively conducted by the bacterial inhibition ring method (agar plate disc diffusion assay) against Gram-negative bacterium *Escherichia coli* and Gram-positive bacterium *Bacillus subtilis*. Nutrient agar medium was prepared by Mueller-Hinton (MH) agar culture medium in 1 L distilled water at pH7.2. The agar medium and empty Petri plates were autoclaved for sterilization purpose. Then, the agar medium was then cast into the Petri plates and cooled down (Shateri Khalil-Abad & Yazdanshenas 2010). The bacteria were inoculated in Petri dishes with MH medium agar. Then, paper discs of 5 mm in diameter were planted on the inoculated test organism agar at equidistant spots. Diluted solution (30 $\mu\text{g/mL}$) of AgNPs synthesised using different molar ratio of precursor to stabiliser were applied (10 μL) on the inoculated disc and left for 10 min at room temperature for the compound diffusion (Muniandy et al. 2017). Chloramphenicol (30 $\mu\text{g/mL}$) was used as the control for

the antimicrobial test. The presence of clear zone around the discs, also known as zone of inhibition (ZOI) on the plates was recorded as an inhibition against the microbial species. The mean and standard deviation reported for all samples and each microbial strain were tested in triplicate.

RESULTS AND DISCUSSION

UV-VIS SPECTROSCOPY

Effect of Molar Ratio (AgNO_3 :PVP) on the Formation of AgNPs Figure 1 shows a single absorption band at 415-425 nm for all the spectra of the hydrothermally synthesized AgNPs using different molar ratio (AgNO_3 :PVP) under a constant reaction time (24 h) and at the temperature of 160°C . The observation of this absorption band is attributed to the characteristics of the surface plasmon resonance (SPR) of AgNPs. Generally, no significant peak was observed for the sample synthesised using molar ratio (1:0.5) which indicated the absence of AgNPs due to very low PVP ratio that led to a lack of nucleation sites for AgNPs. After increasing the AgNO_3 :PVP molar ratio to 1:1, the UV-vis absorption measurements showed a broad weak peak (~ 455 nm), signifying the formation of Ag colloids with nanometre-sized nuclei. When the molar ratio was further increased to 1:3, 1:5 and 1:7, the resonance peaks were blue-shifted to 432, 425 and 409 nm, respectively, and the extinction bands became narrower. A slight shift towards shorter wavelengths is attributed to the decrease of the nanoparticle size whereas, a uniform size would cause narrower extinction bands.

The intensity of λ_{max} increased with the increase in the molar ratio of AgNO_3 :PVP from 1:1 to 1:5, suggesting

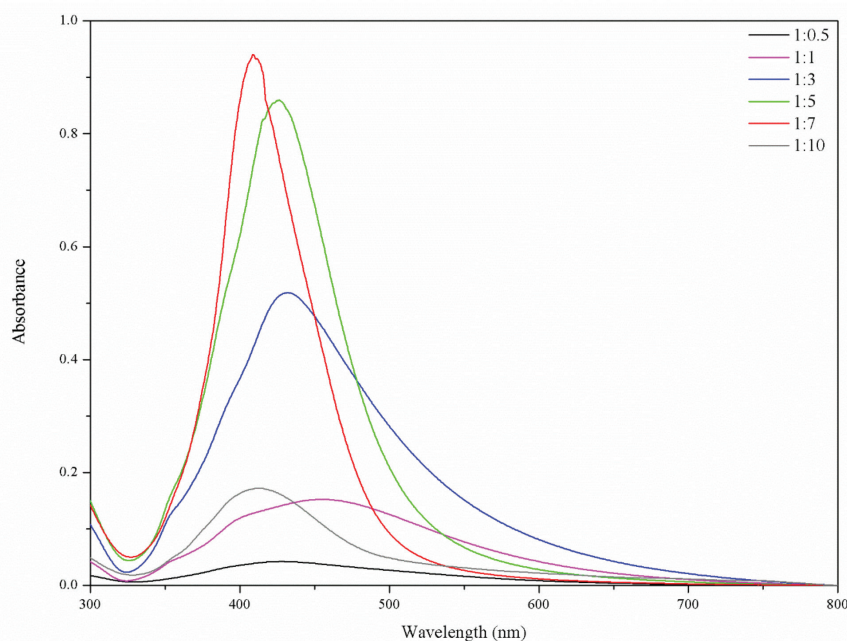


FIGURE 1. UV-Vis spectra of synthesised AgNPs at various molar ratio of AgNO_3 :PVP under a constant temperature (160°C) and at reaction time of 24 h

a continued reduction of Ag^+ ions. However, when the molar ratio increased to 1:7 the intensity of λ_{max} remained the same, indicating that the PVP amount is already saturated when it reached the ratio of 1:7. In this study, the absorption band of the as-synthesised sample was sharp and intensive, but its intensity and sharpness decreased after the molar ratio increased to 1:10. Moreover, the position of the adsorption peak shifted towards a longer wavelength (413 nm). The reduction in its intensity may be due to the decrease in the concentration of AgNPs. The growth of the AgNPs gradually approached saturation for ratio 1:10. The higher content of PVP may lead to saturation of PVP against AgNO_3 where it enhanced fast nucleation of AgNPs and led to lower yield of AgNPs. The red shift implied the increase in size and transition in the shape of silver nanoparticles. Thus, AgNO_3 :PVP (1:5) was selected as the optimum molar ratio even though 1:7 suggested the smaller particle size. This is because at the molar ratio of 1:5, the complete reduction of Ag^+ ions had occurred (Song et al. 2014).

Effect of Reaction Time on the Formation of AgNPs The obtained UV-Vis spectra for all samples synthesised using different reaction time (6, 12, 18, 24 and 36 h) under a constant AgNO_3 :PVP molar ratio (1:5) and temperature (160°C) is presented in Figure 2. For the sample with a reaction time of 6 h, the formation of small Ag nuclei was revealed as the appearance of light yellow was observed clearly. As time increased, the moderate fading of the yellow colour into sage green colour demonstrated the formation of homogeneous Ag nanoparticles. These changes can be evidenced clearly in the spectra for all

the samples. The SPR peak for AgNPs with the reaction time for 6 h located at 413 nm with low λ_{max} intensity may be caused by the formation of very small Ag nuclei. After 12 h, the SPR of AgNPs appeared at 426 nm. This resonance peak became broader and red-shifted to 440 nm at 18 h. Then, by further increasing time, this SPR slightly blue shifted to 425 nm at 24 h. When the reaction time further increased to 36 h, the solution turned into a dark greyish green and the resonance peak shifted towards 439 nm, revealing the formation of AgNPs with increasing size (Kan et al. 2008). From these experimental results, 24 h was selected as the best reaction time for complete nucleation and the formation of uniform small size AgNPs.

Effect of Temperature on the Formation of AgNPs Figure 3 shows the spectra for AgNPs obtained from varying different hydrothermal temperatures. All spectra have similar SPR peaks (425 nm), except the peak which belongs to the sample synthesised at 140°C . This sample exhibited an additional peak around 490 nm. According to Chou and Lai (2014), the new peak at this high wavelength may be attributed to different sizes or shape factors. However, when the temperature increased to 160°C , the spectrum gradually changed back to the single peak similar to those obtained using 120°C as shown in Figure 3. The obtained results proved that lowering the reaction temperature led to insufficient energy and incomplete growth of the AgNPs. Hence, in this study, 160°C is found to be the optimum temperature to carry out the synthesis of AgNPs under hydrothermal method.

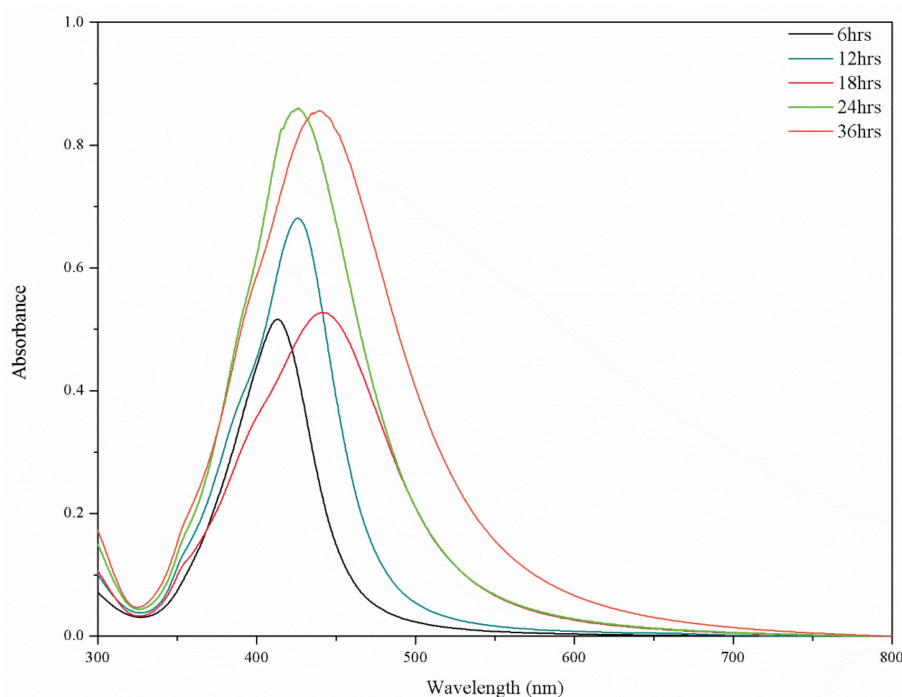


FIGURE 2. UV-Vis spectra of synthesised AgNPs at different reaction times at 160°C and molar ratio of AgNO_3 :PVP (1:5)

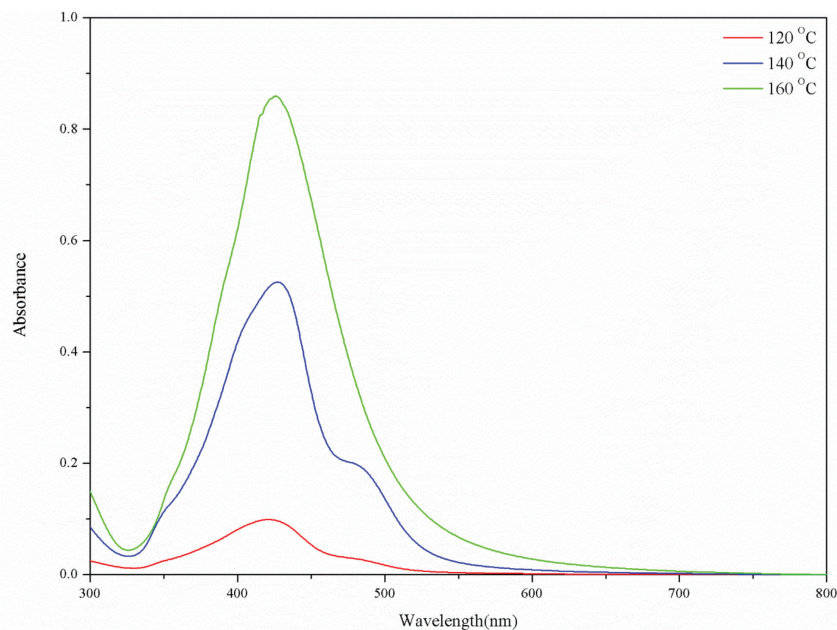


FIGURE 3. UV-Vis spectra of synthesised AgNPs at different temperatures under at 24 h and molar ratio of AgNO_3 :PVP (1:5)

X-RAY DIFFRACTION (XRD)

The X-Ray powder diffraction pattern for silver nanoparticles is shown in Figure 4. A comparison of our XRD pattern with the PDF 98-008-3898 defined that the silver particles formed were in the form of nanocrystals, as shown by the peaks at 2θ values of 38.3° , 44.0° , 64.3° , and 77.3° corresponding to (111), (200), (220) and (311) Bragg reflections, respectively. The obtained spectrum proved that the synthesised AgNPs belonged to the face-centered cubic structure. X-ray diffraction results showed that the presence of very sharp diffraction peaks indicates the high crystallinity of fine AgNPs. The mean crystallite sizes of the AgNPs obtained *via* Rietveld refinement was 19.3 nm. The unassigned peaks indicated by (*) in Figure

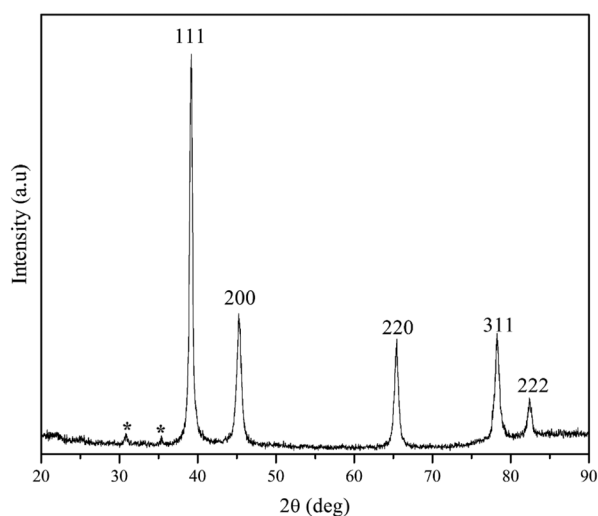


FIGURE 4. XRD pattern of the as-synthesised AgNPs using 1:5 AgNO_3 :PVP at 160°C for 24 h

4 belong to the amorphous and crystalline organic phases of stabilisers (Khademalrasool & Farbod 2015; Naima et al. 2015)

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

Effect of Molar Ratio (AgNO_3 :PVP) on the Formation of AgNPs The evolution and change in particle size can be clearly observed in Figure 5. The FESEM micrographs of AgNPs synthesized at different molar ratio with constant reaction time (24 h) and temperature (160°C) show homogeneous uniform nanospheres formation. The only difference observed in the micrographs (Figure 5) showed that as the molar ratio of AgNO_3 :PVP increased, the particle size of AgNPs decreased. The sample synthesised using 1:1 of AgNO_3 :PVP had the largest particle size distribution which is 65.08 ± 2.7 nm. Reduction in particle size of AgNPs to 50.11 ± 2.6 nm is noticeable as the molar ratio AgNO_3 :PVP increased to 1:7. This was further confirmed with the HRTEM image (insert of Figure 5(d)) which indicated that the size of synthesised AgNPs was in the nano range (40-70 nm).

This could be due to the formation of thicker coating over the surface of AgNPs at a higher concentration of PVP. This phenomenon led to a weaker selective adsorption of PVP which induced isotropic growth into the nanospheres. In addition, high concentration of PVP also offered a stronger stable capacity to prohibit the aggregation of nanoparticles as large amount of PVP covered all the silver seed surfaces (Song et al. 2014).

Effect of Reaction Time on the Morphology of AgNPs The effect of reaction time on the morphology of AgNPs was estimated by varying the reaction time to 6, 12, 18, and 24

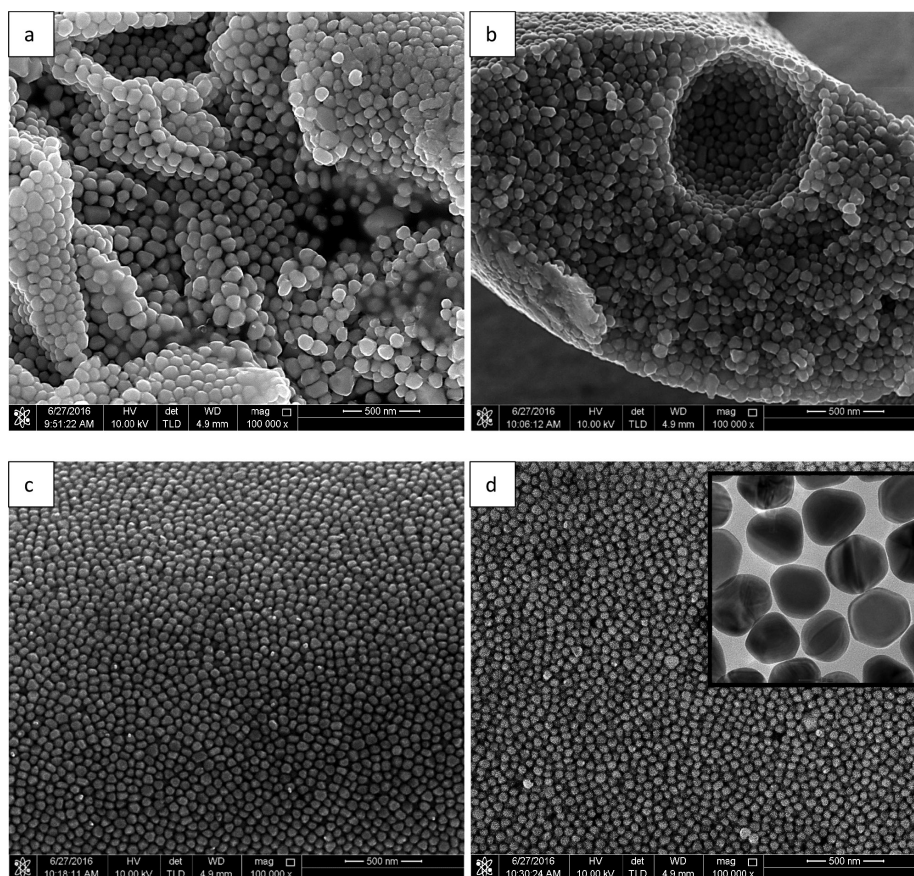


FIGURE 5. FESEM images showing AgNPs synthesized using different molar ratio AgNO_3 :PVP (a) 1:1, (b) 1:3, (c) 1:5 and (d) 1:7 at 160°C for 24 h

h under a constant temperature (160°C) and AgNO_3 :PVP molar ratio (1:5). The development of Ag nanoparticles can be observed in Figure 6. When the reaction stopped at 6 h, only large agglomerated clumps of silver material were observed. Highly agglomerated irregular small particles were observed after increasing the reaction time to 12 h. Smaller particles were formed at this phase which meant that they were not stable and if the process was stopped at this stage, the end-product would be less.

When the reaction time was prolonged to 18 h, bigger nanoparticles with less homogeneity were visible. Finally, once the reaction time was increased up to 24 h, homogeneous AgNPs without any agglomeration were formed. Thus, we propose that 24 h of synthesis is the optimum reaction time required to produce uniform AgNPs *via* hydrothermal process.

Effect of Temperature on the Morphology of AgNPs
Generally, chemical processes accelerate with an increase in temperature. Thus, alteration in temperature affects growth rate and nucleation in colloidal nanoparticles.

At a higher reaction temperature, more nuclei are formed which leads to the formation of nanoparticles with various sizes. We also observed this phenomenon in our synthesis where the AgNO_3 :PVP molar ratio (1:5) and reaction time (24 h) were kept constant as shown in Figure

7. Irregular clumps were obtained when the reaction was carried out at 140°C and small uniform nanoparticles were obtained at elevated temperatures (160°C). It could be that the enlarged rate of nucleation and growth of particles at higher temperatures resulted in even smaller particles (Shankar et al. 2011).

POSSIBLE FORMATION MECHANISM OF AgNPs

PVP is frequently used as a capping agent, which prompts the formation of AgNPs, but also stabilises the resultant colloidal suspension *via* steric repulsion. It has been suggested that the electron could be strongly partial to the oxygen in the polar carbonyl group ($\text{C}=\text{O}$), leaving a negative charge localised at the oxygen atom and a positive charge localised at the nitrogen atom as shown in Figure 8 (Gharibshahi et al. 2017). The interaction between the PVP as the capping agent and the ions (Ag^+) is strong by way of ionic bonds between the Ag ions and the amide group *via* oxygen in the PVP chain. The PVP stabilised the silver nitrate by means of the amide group steric and electrostatic stabilisation (Oku 2016).

Thus, chemical reduction may occur between the PVP and metal ions through a process in which metal ions could receive electrons from $\text{C}=\text{O}$ and form atomic metal as seen in Figure 9. After this reduction occurs, the bonding between the oxygen in the carbonyl group and the silver

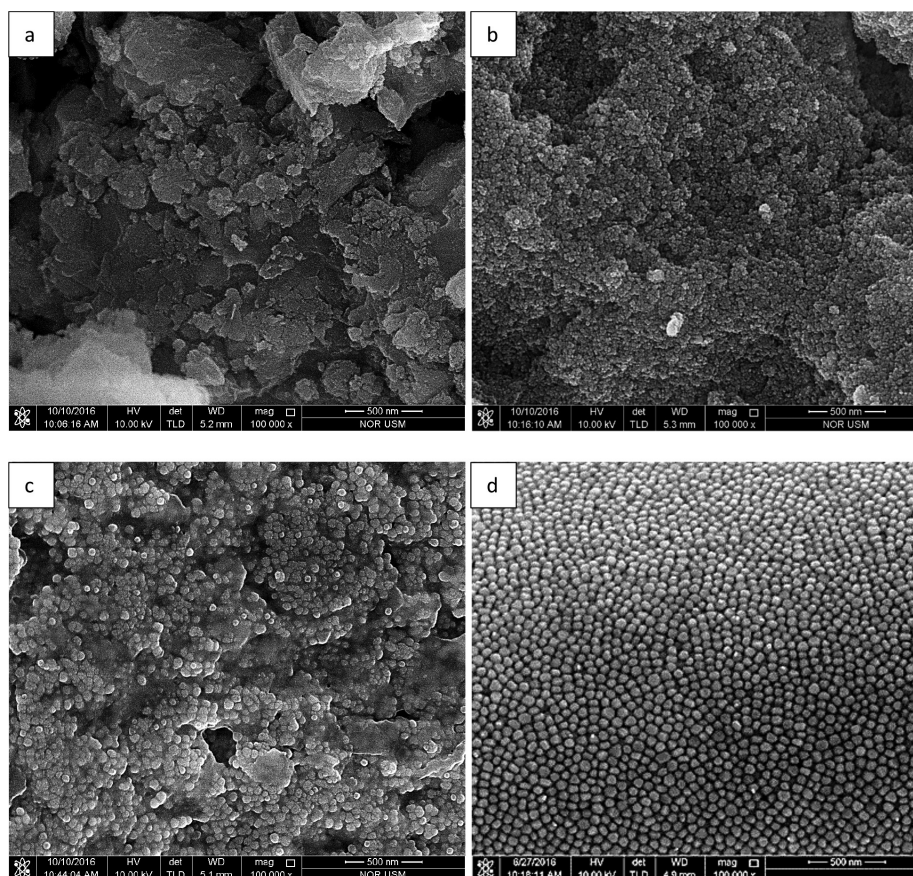


FIGURE 6. FESEM micrographs of AgNPs synthesised using various reaction time (a) 6 h, (b) 12 h, (c) 18 h and (d) 24 h using molar ratio AgNO_3 : PVP (1:5) at 160°C

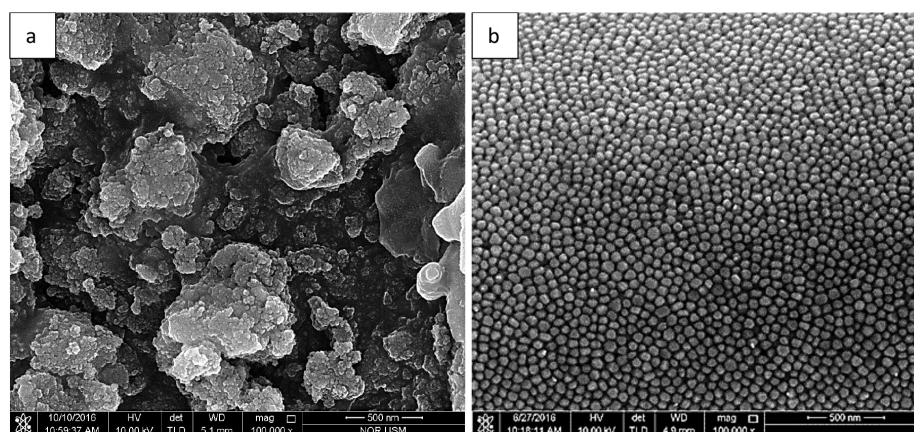


FIGURE 7. FESEM micrographs of AgNPs synthesised using different temperatures (a) 140°C and (b) 160°C

remained stable. Both factors would provide an ability to generate active reducing and binding sites and the polymer will promote the formation of crystals (Mahmudin et al. 2016).

ANTIMICROBIAL ACTIVITIES OF AgNPs

Silver nanoparticles are generally known for their antimicrobial activities on microorganisms.

Hydrothermally synthesised AgNPs using different AgNO_3 :PVP molar ratios were tested against Gram negative bacteria (*E.coli*) and Gram positive bacteria (*B.subtilis*) using the disc diffusion assay. Choloramphenicol and PVP were included into the assay as the control samples. The obtained zone of inhibition (ZOI) results (Figure 10) for all samples were recorded and summarised in Table 1. As shown in Table 1, all the AgNPs samples generally exhibited antibacterial activities that were stronger

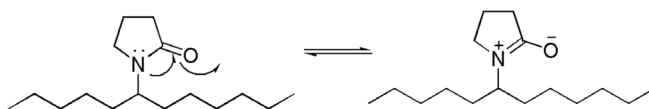
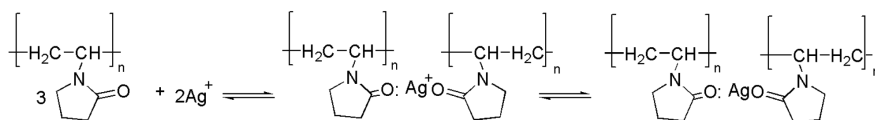
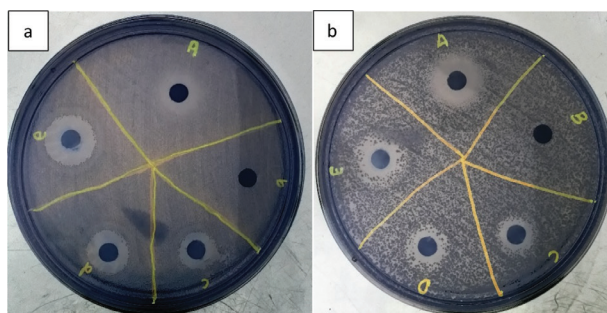


FIGURE 8. Partial electron donation of nitrogen to oxygen

FIGURE 9. Reduction and bond between Ag^+ ions and PVP moleculesTABLE 1. Average zon of inhibition (ZOI) against *E. coli* and *B. subtilis*

Samples	Average zon of inhibition (mm)	
	<i>E.Coli</i>	<i>B.subtilis</i>
A (Cholaramphenicol)	10.3 ± 0.57	11.3 ± 0.57
B (PVP)	-	-
C (1:1 AgNP)	11.3 ± 0.57	9.3 ± 0.57
D (1:3 AgNP)	12.3 ± 0.57	11.0 ± 1.0
E (1:5 AgNP)	14.3 ± 0.57	12.3 ± 0.57

FIGURE 10. Antimicrobial test (disc diffusion assay) of synthesised AgNPs under different molar ratio AgNO_3 :PVP (A:Cholaramphenicol, B:PVP, C:1:1 AgNP, D:1:3 AgNP and E: 1:5 AgNP) against (a) *E. coli* and (b) *B. subtilis*

against Gram-negative than compared to Gram-positive bacteria. The diameter of the ZOI increased from 11.3 ± 0.6 mm to 14.3 ± 0.6 mm with the growing of AgNO_3 :PVP molar ratios from 1:1 to 1:5, respectively. This result was expected, because at high molar ratio, the particle size of AgNPs reduced with increased surface area. This also enhanced the driving force of silver ion diffusion to the agar medium where it promoted the interaction of AgNPs with bacteria (Yu et al. 2011). The difference in antibacterial activity against different bacteria may be due to the structural dissimilarity between Gram-positive and Gram-negative bacteria. Gram-positive bacteria are expected to have a thicker peptidoglycan layer at the outer cell compared to Gram-negative bacteria. This also stated in an earlier study which reported that AgNP retained an antibacterial effect which is stronger against *E. coli* than *B. subtilis* due to the

thinner peptidoglycan layer of Gram-negative bacteria. Thus, *B. subtilis* bacteria might be protected from the attack of AgNPs due to its thicker peptidoglycan layer at outer cell (Chook et al. 2012).

As the actual mechanism of AgNPs is still under debate, there are few proposed mechanisms of AgNPs which can be mentioned here. Generally, the bacteria's permeability and respiration ability were disturbed during the interaction between AgNPs and the surface of the bacteria's membrane. Also, the viability of bacterial cell was affected as AgNPs reacted with sulphur containing proteins inside or outside the cell membrane. Furthermore, as the AgNPs release the Ag ions into the bacteria, it can cause damage on bacterial main components such as the DNA, peptidoglycan and protein resulting in its disability to replicate or induce cell death in bacteria (Hebeish et al. 2011; Yen & Mashitah 2013)

CONCLUSION

From this investigation, it can be concluded that it is possible to synthesise silver nanoparticles *via* simple toxic free hydrothermal method using silver nitrate (AgNO_3) as the precursors with water as the solvent, and polyvinylpyrrolidone (PVP) as the stabiliser or reducing agent. Corroborative results from FESEM, HRTEM, XRD and UV-Vis spectroscopy showed the composition and crystalline structures, as well as the size and distribution of the Ag nanoparticles. The morphology of AgNPs can be controlled by varying the reactant composition, time and temperature of the process. Well-dispersed and uniform AgNPs were successfully synthesised using 1:5 AgNO_3 :PVP molar ratio at 160°C for 24 h. Antimicrobial tests of AgNPs synthesised using several molar ratio (AgNO_3 :PVP) were conducted against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*B. subtilis*) using the disc diffusion assay. All the AgNPs samples generally exhibited antibacterial activities that were stronger against Gram-negative compared to Gram-positive bacteria. From the antimicrobial tests, AgNPs synthesised using the molar ratio 1:5 of AgNO_3 :PVP showed the highest zone of inhibition for both *E. coli* (14.3 ± 0.6 mm) and *B. subtilis* (12.3 ± 0.6 mm). This study delineates that well-dispersed and uniform AgNPs have excellent antimicrobial properties and can be synthesised by green chemistry approaches.

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