

ORIGINAL ARTICLE

One-pot microwave-assisted synthesis of size-dependent L-glutathione-capped spherical silver nanoparticles suitable for materials with antibacterial properties

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Capping agent, glutathione size dependent, microwave synthesis, silver nanoparticles.

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Abstract

In the last years, there has been an alarming increase in antibiotic resistance by pathogenic microbes, which has become a major public health concern. There is a great interest in developing new antimicrobial for reducing the impact. Silver nanoparticles (AgNPs) as antibacterial agents are currently being studied to be used to fight these pathogenic microbes. The aim of the present study was to synthesize AgNPs of different sizes through the use of microwave and determine their antimicrobial activities. Synthesis of size-dependent L-glutathione-capped spherical nanoparticles through one-pot microwave synthesis was achieved, and their antimicrobial properties were determined. Different sizes of AgNPs between 5-10, 15-35, and 50-80 nm were made by varying the concentration of silver nitrate and using sodium borohydride (NaBH₄) as a reducing agent. L-glutathione was used to stabilize the AgNPs to prevent them from aggregation in the colloidal solution. The synthesized AgNPs showed ultraviolet absorption at around 400 nm with high concentration of AgNO₃ having sharp peaks. The formed particles were crystalline in nature with uniform spherical shape. The formed AgNPs were of crystalline size of 9.94, 18.45, 34.96, 52.40, and 58.50 nm. Fourier transform infrared analysis confirmed conjugation of glutathione as a capping agent to AgNPs as the result of the formed spectra showing the absence of -SH stretch. The high temperature generated by microwave helped to synthesize nanoparticles within a short time and by varying the concentration of AgNO₃ helped obtain the desired particle size. Glutathione conjugated well with AgNPs as a result of interaction of negative thiol resulting to colloidal stabilization and reduced aggregation. The antibacterial activity of AgNPs was found to be size dependent with the smaller size of 9.94 nm being more efficient than 18.45, 34.96, 52.40, and 58.50 nm against the tested strains *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Salmonella* spp. (ATCC 700623), and *Staphylococcus aureus* (ATCC 25923). Of the four stains, *E. coli* was found to be the least affected by all three different particle sizes of the synthesized AgNPs.

Introduction

Nanotechnology involves synthesis, identification, study, and application of very small particles (1-100 nm) in the field of science to solve various problems (Mehr et al., 2012). Nanoparticles exhibit improved physical, biological, and chemical properties and functionality. The field of nanotechnology has found application in the area of medicine in drug delivery (Franci et al., 2015), in diagnosis of diseases, in treatment of current diseases such as cancer and diabetes (De Matteis et al., 2018), in catalysis (Joseph and Mathew, 2014), and in making sensors (Zhang et al., 2015). Nanotechnology is also a promising area in provision of solutions to antimicrobial resistance (Khatoun et al., 2011; Nyamu et al., 2018). Nanoparticles with different sizes and dimensions can be prepared by chemical method (Raza et al., 2016), physical method, bio-based method (Phanjom et al., 2015; Sunita and Palaniswamy, 2017), green synthesis method (Atta et al., 2014) polyol mediated (Dzido et al., 2015), or the use of microwave (Chen et al., 2008; Pal et al., 2009; Aitenneite et al., 2016).

The use of microwave to synthesize nanoparticles is a promising method as it results to consistently uniform particle sizes, higher degree of crystallization, and narrower size distributions preventing agglomeration of particles (Iravani et al., 2014). Other than the good yields of nanoparticles, microwave-assisted synthesis helps to reduce reaction time and chemical waste as it supports solvent-free reaction (Ombaka et al., 2014; Saloga et al., 2018).

Due to the problem of aggregation of nanoparticles brought about by their high surface energy (Pal et al., 2013), capping agents and dispersing agents are used to stabilize them. Selection of the capping/stabilizing agents is important as it contributes to determination of their stability, solubility, dispersibility, reactivity, and shape and size of silver nanoparticles (AgNPs) during the synthesis (Suriati et al., 2014). A number of capping agents such as poly acrylic acid, poly vinyl pyrrolidone, poly vinyl alcohol, polysaccharide, and L-glutathione have been reported to stabilize nanoparticles (Wang et al., 2010; Atta et al., 2014). A capping agent works by restricting nanoparticles from growing, resulting to agglomeration; the steric forces of this capping agent keep ligands separated from each other. If a capping agent is not used, nanoparticles will still be formed, but they will grow uncontrollably, and in the process, the nucleated particles join other surrounding particles becoming non-uniform. Such non-uniform and

agglomerated particles are not good for applications that need mono-dispersed particles such as cell separation and biomedical and information storage. A dispersing agent creates an active polymer when added to the suspensions, preventing clumping of the particles in the colloidal solution.

Glutathione (Fig. 1) is a tripeptide that contains an -SH group and an acetyl group -COO . This peptide can be easily adsorbed onto the surface of metal nanoparticles; the free-cysteiny l thiol moiety has a strong binding affinity for silver (Murariu et al., 2014; Consumption A, 2018). The -SH group and -COO of glutathione form a weak bond with the surface of AgNPs due to its positive charge. This brings colloidal stabilization because the positive AgNPs are surrounded on the surface by the negatively charged acetyl group and the thiol. (Figure 2)

Statement of the Problem and Justification

The treatment of infectious diseases is facing challenges due to the emergency of bacteria that are resistant to antibiotics that are currently in use. Mutation and changes in biochemical activities in the microbes may prevent antibiotic from fighting the microbes or change the target of antibiotic (WHO, 2014). Drug resistant by bacteria have been reported, from single to multi-drug resistance. Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* resistant to cephalosporins and fluoroquinolones, and *Shigella* species resistant to fluoroquinolones are good examples of multi-drug-resistant strains (Consumption A, 2018). Current studies are being geared towards incorporation of nanoparticles in medicine to fight the microbes. Studies performed have shown that silver nanomaterials have antimicrobial properties; however, the debate on the topic that how the size and shape of nano-sized entities influence the antimicrobial activities is still ongoing. This work reports on easily achieved way for one-pot

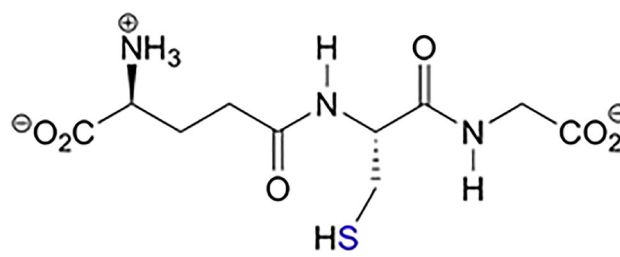


Figure 1. Glutathione.

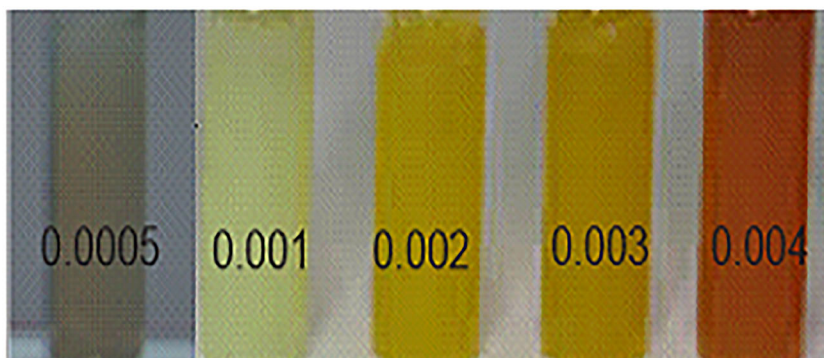


Figure 2. Color of the formed silver nanoparticle solution as a function of silver nitrate concentration.

synthesis of AgNPs of different particle sizes by the help of microwave irradiation and determination of antimicrobial properties. The AgNPs are synthesized by the use of sodium borohydride, which is a reducing agent, and stabilized using L-glutathione as a capping agent.

Materials and Methods

The following analytical grade chemicals, sodium borohydride (99%), L-glutathione (98%), and silver nitrate (99%), were all acquired from Sigma Aldrich company (Germany). The bacterial strains *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 25922), *Salmonella* spp. (ATCC 700623), and *S. aureus* (ATCC 25923) were obtained from KEMRI Centre for Microbiology Research.

All glass wares were first soaked in a solution of chromic acid for a night and then washed with a detergent. A final rinse was performed in alcoholic KOH and then in de-ionized water and then dried in oven before use.

Synthesis of silver nanoparticles

Five samples of nanoparticles were prepared by varying concentration of AgNO_3 , while that of NaBH_4 were maintained constant for all reactions as shown in Table 1. Using a burette, 30 mL of 0.002-M NaBH_4 was transferred in a 250-mL conical flask followed by addition of 4 mL of 0.004-M AgNO_3 dropwise while stirring. The mixture was then placed in microwave and irradiated for 30 s at high power until a yellow color was formed, which indicated formation of AgNPs. The formed AgNPs colloidal solution was allowed to cool to around 50°C ; then 10 mL of 0.01-M L-glutathione was added while stirring for 3 min for stabilization of AgNPs. The colloidal solution was then placed in 100-mL plastic bottles and named solution A. The same procedure was repeated for preparation of sample solutions B, C, D,

Table 1. Various concentration of silver nitrate and sodium borohydride and their volumes that were reacted.

Solution	AgNO_3 (M)	Amount (mL)	NaBH_4 (M)	Amount (mL)
A	0.004	4	0.002	30
B	0.003	4	0.002	30
C	0.002	4	0.002	30
D	0.001	4	0.002	30
E	0.0005	4	0.002	30

and E by varying the concentration of AgNO_3 . The formed AgNPs were confirmed using ultraviolet (UV)-visible spectra and also analyzed using Fourier transform infrared, scanning electron microscope (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD).

Microwave oven (Domestic Model RM 240, output 700 W) was used as a heating source in order to take advantage of rapid synthesis of AgNPs under microwaves irradiation. The UV-visible spectra of the nanoparticles were determined by UV/Vis spectrophotometer model (Specord-200). The samples were measured against distilled water as reference. The XRD spectra were recorded in a D2 Phaser Bruker Diffractometer (with $\text{Cu K}\alpha$ radiation, λ being 0.154056). The diffracted intensities were recorded from the range between 10° and 80° 2θ angles.

Antimicrobial assay

The antimicrobial activities of the synthesized AgNPs of different sizes were determined using the following pathogens bacteria: *B. subtilis*, *Salmonella* spp., *E. coli*, and *S. aureus*. The AgNPs particles were dispersed

in autoclaved water by ultrasonication before preparing the required concentration.

Disc diffusion method

The antimicrobial assay was performed following the standard method NCCLS (Eleonor and Tendencia, 2004). Nutrients broth/agar were used to cultivate bacteria. The prepared agar was placed in the petri disc and left for 45 min to solidify. Then after 45 min, the fresh four different cultures of inoculum that had been prepared and left overnight were spread on the agar plates. Sterilized filter paper discs of diameter 6 mm were immersed in 50 mg/L of the prepared different size AgNPs solution. The standard antibiotic containing ciprofloxacin disc (30 μ g) that serves as a positive control was also placed onto the cultured agar plate. Cultured plates with the AgNPs and the controls were placed inside the incubator at 37°C for a period of 24 h. The zone of inhibition was later determined against the different bacteria and compared with the accepted inhibitory zones.

Results and Discussion

Ultraviolet-visible spectroscopy

The characteristic change of color of the solution during formation of AgNPs may be due to the excitation brought about by shrinking of lattice of nanoparticles during formation causing surface plasmon vibrations Figure 2. We observed spectra of silver colloids of different concentrations within the range of 250 to 600 nm with the use of UV-visible spectroscopy. A well-defined plasmon band was observed at around 400 nm.

Increased concentration of AgNO₃ gave a sharp peak as compared with low concentration that gave a broad peak as shown in Figure 3. Comparing the results with that performed for XRD, a sharp peak/narrow band at 398 nm for UV-Vis was associated with smaller nanoparticles, while a broad peak/wide band that had shifted to 424 nm symbolized increased size of nanoparticles.

X-ray diffraction analysis

The XRD spectra of the synthesized AgNPs had the following diffraction peaks at $2\theta = 38^\circ$, 20° , 44.40° , 64.59° , and 77.50° . These corresponded well with planes of a face center cubic lattice of Ag (111), (200), (220), and (311), respectively. This suggests that the synthesized AgNPs were in agreement with reference standard cubic Ag° of Joint Committee on Powder Diffraction Standards - JCPDS file no. 00-001-1167

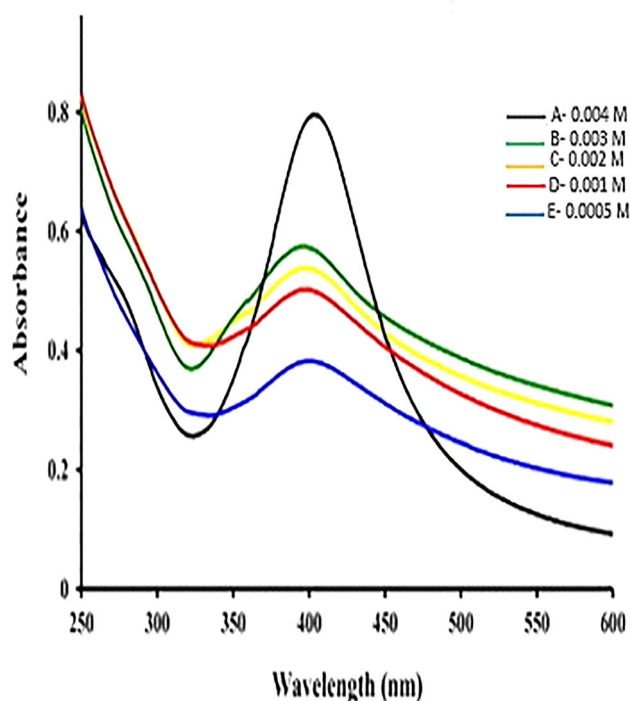


Figure 3. Ultraviolet-visible spectra of silver nanoparticles under different concentrations of AgNO₃.

Table 2. Sample of AgNPs, their peak numbers, diffraction angles, and full width at half maximum height of the peak.

AgNPs	Peak no.	2θ	FWHM
A	1	38.2043	0.9213
	2	44.4012	0.9127
	3	64.5907	0.9993
	4	77.5012	0.9997
B	1	38.2901	0.6223
	2	44.5583	0.6397
	3	64.8185	0.6033
	4	77.0231	0.6682
C	1	38.3271	0.4100
	2	43.9720	0.2557
	3	64.2381	0.2575
	4	76.9932	0.2341
D	1	38.4832	0.1433
	2	44.7825	0.1534
	3	64.8185	0.1734
	4	77.8326	0.1634
E	1	38.4831	0.1444
	2	44.7825	0.1534
	3	64.8180	0.1727
	4	77.8330	0.1637

AgNPs, silver nanoparticles; FWHM, full width at half maximum.

(Mehta et al., 2017). Diffraction peak angles together with full width at half maximum height of the diffraction peaks for the synthesized nanoparticles are shown in Table 2.

58.50 nm (as shown in Figs 4-8, respectively) determined from transmission electron microscopy images using ImageJ software.

Transmission electron microscopy and scanning electron microscopy analyses

The formed AgNPs particles were crystal-like in nature with uniform spherical shape. The formed particles were of average sizes 9.94, 18.45, 34.96, 52.40, and

Fourier transform infrared analysis

Fourier transform infrared was useful in providing the chemical composition of the nanoparticles and the environment of the stabilizing agent. The characteristic band of glutathione used as the stabilizing agent before and after conjugation with AgNPs is shown in Figure 9. A

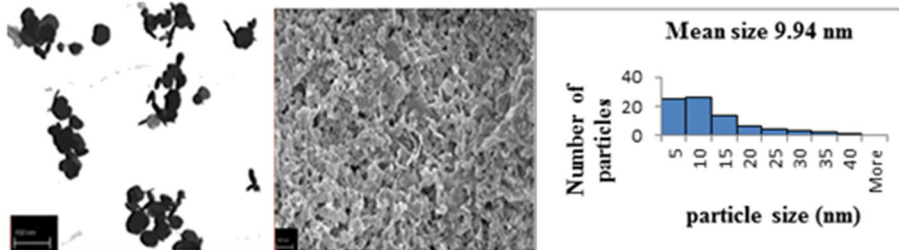


Figure 4. Transmission electron microscopy image, scanning electron microscopy micrograph, and histogram of particle size of A.

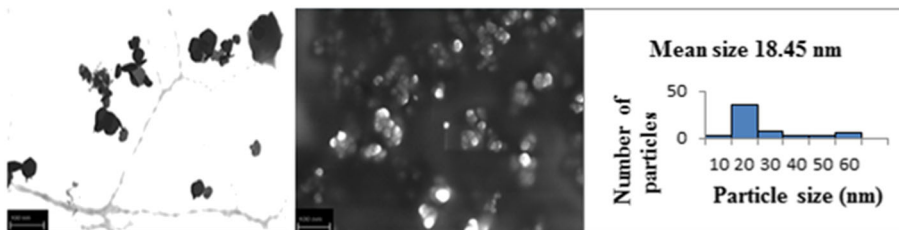


Figure 5. Transmission electron microscopy image, scanning electron microscopy micrograph, and histogram of particle size of B.

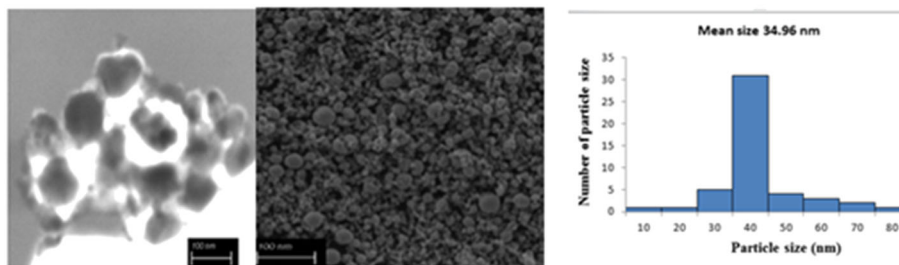


Figure 6. Transmission electron microscopy image, scanning electron microscopy micrograph, and histogram of particle size of C.

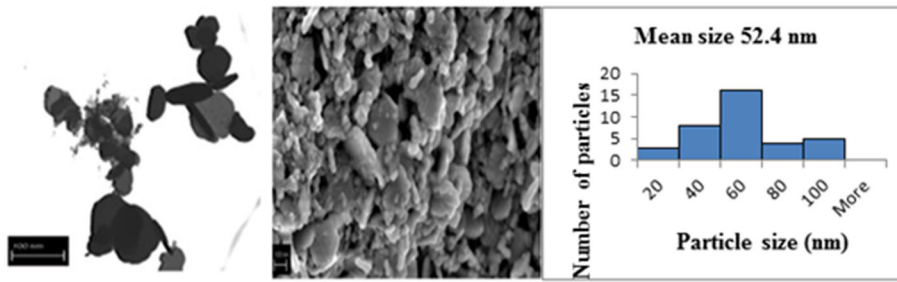


Figure 7. Transmission electron microscopy image, scanning electron microscopy micrograph, and histogram of particle size of D.

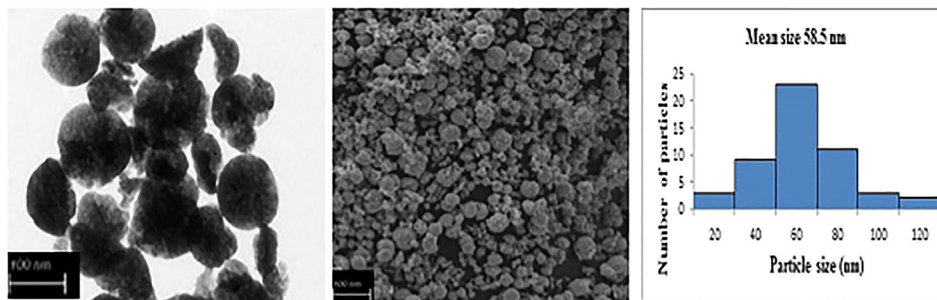


Figure 8. Transmission electron microscopy image, scanning electron microscopy micrograph, and histogram of particle size of E.

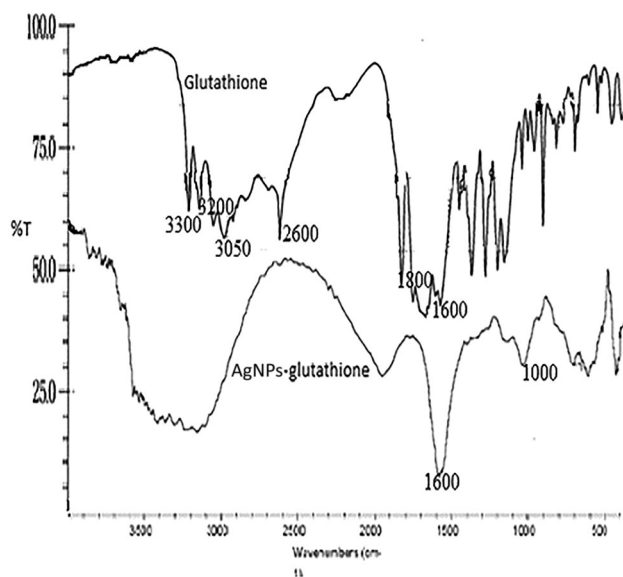


Figure 9. Fourier transform infrared spectra of glutathione and silver nanoparticles (AgNPs) conjugated to glutathione.

band at 2600 cm^{-1} for glutathione spectra represented SH stretch, which is a thiol group in glutathione. A band at 1600 cm^{-1} represented a COO^- , and a peak at

3300 cm^{-1} signified NH_3^+ stretches, which are all characteristic functional groups for glutathione.

There was no shift of peak for NH_3^+ and COO^- stretch after inclusion of glutathione as a capping agent in the synthesized AgNPs. This indicated that there is no interaction between these functional groups and the AgNPs, therefore helping it not to aggregate. However, the spectra for conjugation of glutathione with AgNPs showed the absence of SH peak, indicating that the thiol had taken part in interaction with the surface of AgNPs. The SH group of glutathione forms a weak bond with the surface of AgNPs, which is positively charged. This brings colloidal stabilization because the positive charged AgNPs are surrounded on the surface by the negatively charged thiol, thus reducing chances of aggregation.

Disc diffusion test of silver nanoparticles

Effects of different particle sizes of AgNPs 9.94 (A), 18.45 (B), 34.96 (C), 52.40 nm (D), and 58.50 (E) on bacteria were performed. Table 3 shows the zone of inhibition treated with different sizes of AgNPs.

The analyzed AgNPs of sizes 9.94, 18.45, 34.96, 52.40, and 58.50 nm showed antibacterial activity

Table 3. Antimicrobial activities of size-dependent AgNPs with control ciprofloxacin.

Microbes	Zone of inhibition in millimetres				
	AgNP size (nm)	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Salmonella</i> spp.	<i>Staphylococcus aureus</i>
	9.94	17.0 ± 0.20 ^{†1,†3,†3,†4,†2,†5,†6,†3}	10.0 ± 0.30 ^{†1,†3,†3,†4,†5,†6,†3}	13.5 ± 0.2 ^{†1,†3,†3,†4,†5,†6,†3}	21.0 ± 0.10 ^{†1,†3,†3,†4,†5,†6,†3}
	18.45	13.5 ± 0.30 ^{†1,†3,†5,†6,†1}	8.0 ± 0.10 ^{†1,†3,†5,†6}	9.5 ± 0.50 ^{†1,†3,†5,†6}	16.5 ± 0.35 ^{†1,†3,†4,†5,†6,†1}
	34.96	12.2 ± 0.16 ^{a,3,f}	7.7 ± 0.20 ^{†1,†3}	8.8 ± 0.25 ^{†1,†3,†5,†6}	14.7 ± 0.10 ^{†1,†3,†6}
	52.40	11.0 ± 0.50 ^{†1,†3}	7.5 ± 0.35 ^{†1,†3}	6.0 ± 0.10 ^{†1,†3}	13.0 ± 0.20 ^{†1,†3,†6}
	58.50	10.3 ± 0.24 ^{†1,†3}	7.0 ± 0.15 ^{†1,†3}	6.0 ± 0.20 ^{†1,†3}	11.4 ± 0.35 ^{†1,†3}
Ciprofloxacin (30 µg/disc)		39.0 ± 0.15	24.0 ± 0.30	28 ± 0.30	45.0 ± 0.65

The values were expressed as mean ± standard error of the mean (n = 3); analysis was carried out with one-way analysis of variance followed by Tukey's test with post hoc multiple comparisons. The negative control (water) showed no antimicrobial activity. AgNP, silver nanoparticle.

¹Compared with the positive control (ciprofloxacin).

²Compared with 9.94-nm particle size.

³Compared with 18.45-nm particle size.

⁴Compared with 34.96-nm particle size.

⁵Compared with 52.50-nm particle size.

⁶Compared with 58.50-nm particle size.

¹P < 0.0.

²P < 0.01.

³P < 0.000.

against the selected strains *B. subtilis*, *Salmonella* spp., *E. coli*, and *S. aureus*. Ciprofloxacin was used as the positive control, while distilled water was used as the negative control. The zone of inhibition of the AgNP with a particle size of 58.50 nm was significantly different when compared with that of a particle size of 9.94 nm for the tested bacteria *B. subtilis*, *Salmonella* spp., *E. coli*, and *S. aureus* ($P < 0.05$). The zone of inhibition for a particle size of 9.94 nm was significantly different to that of 52.40 and 34.96 nm against all the tested bacteria ($P < 0.05$). All the tested bacteria in all different AgNPs particle sizes were significantly different from that of the positive control (ciprofloxacin, 30 µg/disc) ($P < 0.05$).

In general, AgNPs of small sizes showed higher antibacterial properties against all the strains of bacteria in terms of the zone of inhibition as compared with that of other large particle sizes. This may be a result of small particles having good penetration in the cell membrane of bacteria as compared with the large particles.

Conclusions

The use of microwave-mediated synthesis of nanoparticles helps increased kinetic of reaction due to rapid initial heating resulting to high temperatures. This helped to synthesize AgNPs with uniform spherical shape with desired particle size. The size of AgNPs was found to strongly depend on the concentration of AgNO₃ used. Glutathione conjugated well with AgNPs as a result of interaction of negative thiol bringing colloidal stabilization and reduced aggregation. From the results of microbial assay, we can conclude that AgNPs have antibacterial activity against the analyzed strains of bacteria. This activity varies with particle size with the smaller particles being more efficient; this can be included to make materials with antibacterial properties.

Acknowledgments

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Conflict of Interest

None declared.

REFERENCES

- Aitenneite, H., Abboud, Y., Tanane, O., Solhy, A., Sebti, S., and El Bouari, A. **2016**. Rapid and green microwave-assisted synthesis of silver nanoparticles using aqueous Phoenix Dactylifera L. (date palm) leaf extract and their catalytic activity for 4-Nitrophenol reduction. *J Mater Environ Sci* 7(7):2335-2339.
- Atta, A. M., Al-Lohedan, H. A., and Ezzat, A. O. **2014**. Synthesis of silver nanoparticles by green method stabilized to synthetic human stomach fluid. *Molecules* 19(5):6737-6753.
- Chen, J., Wang, J., Zhang, X., and Jin, Y. **2008**. Microwave-assisted green synthesis of silver nanoparticles by carboxymethyl cellulose sodium and silver nitrate. *Chem Mater Phys* 108(2-3):421-424.
- Consumption A. WHO Report on Surveillance. **2018**.
- De Matteis, V., Cascione, M., Toma, C., and Leporatti, S. **2018**. Silver nanoparticles: synthetic routes, in vitro toxicity and theranostic applications for cancer disease. *Nanomaterials* 8(5):319 Available from: <http://www.mdpi.com/2079-4991/8/5/319>.
- Dzido, G., Markowski, P., Małachowska-Jutysz, A., Prusik, K., and Jarzębski, A. B. **2015**. Rapid continuous microwave-assisted synthesis of silver nanoparticles to achieve very high productivity and full yield: from mechanistic study to optimal fabrication strategy. *J Nanopart Res* 17(1):27.
- Eleonor, A., and Tendencia, T. **2004**. Disk diffusion method. pp. 13-29. in *Laboratory Manual of Standardized Methods for Antimicrobial Sensitivity Tests for Bacteria Isolated from Aquatic Animals and Environment*. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines.
- Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., and Galdiero, M. **2015**. Silver nanoparticles as potential antibacterial agents. *Molecules* 20(5):8856-8874.
- Iravani, S., Korbekandi, H., Mirmohammadi, S. V., and Zolfaghari, B. **2014**. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci* 9(6):385-406.
- Joseph, S., and Mathew, B. **2014**. Synthesis of silver nanoparticles by microwave irradiation and investigation of their catalytic activity. *Res J Recent Sci* 3:185-191.
- Khatoun UT, Rao KV, Rao JVR, Aparna Y. Synthesis and characterization of silver nanoparticles by chemical reduction method. *Int Conf Nanosci Eng Technol (ICONSET 2011)* 2011;9(05):97-9. Available from: <http://ieeexplore.ieee.org/lpdocs/epic03/wrapper.htm?arnumber=6167920>
- Mehr, F. P., Khanjani, M., and Vatani, P. **2012**. Synthesis of nano-Ag particles using sodium borohydride. *Orient J Chem* 31(3):1831-1833 Available from: <http://www.orientjchem.org/vol31no3/synthesis-of-nano-ag-particles-using-sodium-borohydride/>.
- Mehta, B. K., Chhajlani, M., and Shrivastava, D. **2017**. Green synthesis of silver nanoparticles and their characterization by XRD. *Front Phys Plasma Sci IOP Publ IOP Conf Ser J Phys Conf Ser* 836:012050. <https://doi.org/10.1088/1742-6596/836/1/012050/pdf>.
- Murariu, M., Stoica, I., Gradinaru, R., Drochioiu, G., and Mangalagiu, I. **2014**. Glutathione-based silver nanoparticles with dual biomedical activity. *Rev Roum Chim* 59(10):867-874.
- Nyamu, S. N., Ombaka, L., Masika, E., and Ng, M. **2018**. Antimicrobial photodynamic activity of phthalocyanine derivatives. *Adv Chem* 2018:2598062.
- Ombaka, L. M., Ndungu, P. G., Omondi, B., and Nyamori, V. O. **2014**. Mechanochemical synthesis and spectroscopic properties of 1,1'-ferrocenyldiacrylonitriles: the effect of *para*-substituents. *J Coord Chem* 67(11):1905-1922.
- Pal, A., Shah, S., and Devi, S. **2009**. Microwave-assisted synthesis of silver nanoparticles using ethanol as a reducing agent. *Mater Chem Phys* 114(2-3):530-532.

- Pal, J., Deb, M. K., and Deshmukh, D. K. **2013**. Microwave-assisted synthesis of silver nanoparticles using benzo-18-crown-6 as reducing and stabilizing agent. *Appl Nanosci* 4(4):507-510. <https://doi.org/10.1007/s13204-013-0229-6>.
- Phanjom, P., Ahmed, G., Bhimba, B. V., Gurung, S. B., and Nandhini, S. U. **2015**. Marine fungus (*Aspergillus oryzae*) mediated biosynthesis of silver nanoparticles. *Int J ChemTech Res* 5(1):68-72. Available from. <https://www.scopus.com/inward/record.uri?eid=2-s2.0-84937579331&partnerID=40&md5=d8ed07daefc65271343fd02236de6d3e>.
- Raza, M., Kanwal, Z., Rauf, A., Sabri, A., Riaz, S., and Naseem, S. **2016**. Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. *Nanomaterials* 6(4):74 Available from: <http://www.mdpi.com/2079-4991/6/4/74>.
- Saloga, P. E. J., Kästner, C., and Thünemann, A. F. **2018**. High-speed but not magic: microwave-assisted synthesis of ultra-small silver nanoparticles. *Langmuir* 34(1): 147-153.
- Sunita, P., and Palaniswamy, M. **2017**. Size dependent application of biologically synthesized silver nanoparticles against bacterial skin pathogens. *Asian J Pharm Clin Res* 10(10):192 Available from: <https://innovareacademics.in/journals/index.php/ajpcr/article/view/19718>.
- Suriati, G., Mariatti, J., and Azizan, A. **2014**. Synthesis of silver nanoparticles by chemical reduction method: effect of reducing agent and surfactant concentration. *Int J Automot Mech Eng* 10(December):1920-1927 Available from: http://ijame.ump.edu.my/images/Volume_10/9_Suriati_et_al.pdf.
- Wang, B., Zhuang, X., Deng, W., and Cheng, B. **2010**. Microwave-assisted synthesis of silver nanoparticles in alkaline carboxymethyl chitosan solution. *Engineering* 02(05):387-390. Available from: <http://www.scirp.org/journal/doi.aspx?eng.2010.25050>. <https://doi.org/10.4236/eng.2010.25050>.
- WHO. **2014**. Antimicrobial resistance. *Bull World Health Organ* 61(3):383-394.
- Zhang, J., Yuan, Y., Wang, Y., Sun, F., Liang, G., Jiang, Z., and Yu, S. H. **2015**. Microwave-assisted synthesis of photoluminescent glutathione-capped Au/Ag nanoclusters: a unique sensor-on-a-nanoparticle for metal ions, anions, and small molecules. *Nano Res* 8(7):2329-2339.