Efficiency of Silver Nano Particles in Removing Pseudomonas Aeruginosa ATCC 27853 from Drinking Water Distribution Pipes

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Abstract

Silver nanoparticles prepared by the simple green method using Azadirachta indica (A. indica) leaf extract. The formulation was found to be effective in terms of reaction time as well as stability of the synthesized nanoparticles with the exception of external stabilizing agents/reducing agents. It has been shown to be an environmentally friendly and rapid synthetic approach that provides a low-cost, high-efficiency method for the synthesis of silver nanoparticles; this reaction direction matches all conditions of the green chemical process and with a high percentage. In addition to the benefits of using plant extracts in synthesis, it saves energy, protects human health and the environment, and provides safer products, as the manufactured silver nanoparticles showed effective activity against Pseudomonas aeruginosa ATCC 27853. AgNPs was biologically (green method) prepared using leaf extract of neem plant (A. indica). Experimental lab unite was designed. Two PVC pipes (3 meters length, 2.54 cm diameter) were used, each pipe partitioned into six section of 50cm length, the pipes were alternately coated with silver nanoparticles(250ppm,125ppm). Both coated and uncoated pipe parts were connected respectively to each other . Drinking water was artificially contaminated by 290 CFU Pseudomonas aeruginosa ATCC 27853 bacteria. The standard isolate of bacteria was obtained Pseudomonas aeruginosa ATCC 27853 used the (layered casting) method to find the effect of AgNPs prepared at different concentrations (125, 250) ppm and multiple periods (2, 5, 10, 15, 20) minutes. The result show that, the inhibition rate reached 100% at 250 ppm of AgNPs through the peiod of time15 and 20 minute,while with the concentration of AgNPs of 125 ppm, it did not achieve sufficient killing even after 20 minutes. UV-Vis spectrum, FTIR spectrum, XRD, FESEM and TEM, techniques were proved the success of the formation of silver nanoparticles. The pipes coated with silver nanoparticles were characterized using FE-SEM,TEM and XRD techniques.

Keywords: *Silver nano particles, Pseudomonas aeruginosa, Green synthesis, drinking water.*

1. Introduction

Obviously Nanotechnology has invaded various life infrastructure fields commencing from furniture industry [1] to aerospace needs [2] passing by health care [3] and beauty products [4], food industry [5] and food packaging[6], construction material [7] and road construction [8] and water disinfection and treatment such as drinking water [9], wastewater [10], ground water [11], and industrial wastewater [12].Certainly, AgNPs efficiency as drinking water antibacterial disinfectant was evaluated [13] since these AgNPs were used in the treatment of drinking water as disinfecting and decontaminating agent [14] and to help in sterilizing water from bacteria and viruses in addition to enhancing water quality to ensure water being safe for drinking and all home requirements. Since AgNPs were used in coating water filters and membranes, it was found that these methods have been shown to be much effective in destroying water bacteria species [15,16,17]. Interestingly, other studies have reported complete growth inhibition of both gram positive and negative bacteria [18, 19,20]

However, such AgNPs performance may be governed by environmental factors such as pH and temperature [21,22]. In addition, it may be affected by water dissolved organic matter [23], Ca and S [24], particle size [25]. Enterobacter Aerogenes are gram-negative bacteria that are classified as facultative anaerobes, which mean that they are able to thrive in both aerobic and anaerobic environments. E. Aerogenes are rod-shaped cells, not spore forming, and possess flagella and thus are motile, [26].

2. Methodology

2.1 Preparing silver nano particles.

Many methods were previously used for the synthesize of silver nanoparticles, which in short were chemical methods, physical methods, and the last of which was environmentally friendly manufacturing methods. Herein, the environmentally friendly green synthesis method, which is important in the following aspects:

1 . A method that does not include the use of chemical reducing agents that have poisoning effects if they are present with silver nanoparticles because the application is in contact with human uses [27].

2. The plant extracts act as natural weak reducing agents in addition to their work as dispersants that prevent the aggregation of silver nanoparticles and turn them into micro aggregates [28,29].

3. The plant extracts contain bio active substances that have the ability to inhibit the growth of bacteria, in addition to their ability to survive on the surface of the synthesized silver nanoparticles [30].

Azadirachta indica leaf extract was used to prepare silver nanoparticles for use in the coating of PVC pipes. AgNPs were characterization using ultraviolet–visible (UV-Vis) spectrum, Fourier Transform Infrared (FTIR,), X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscopy(FESEM) and Transmission

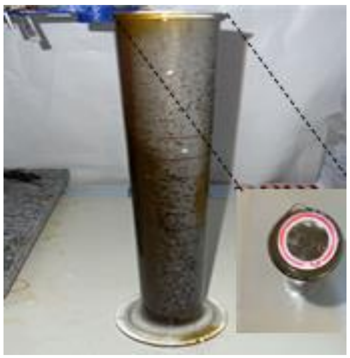
Electron Microscopy (TEM).

2.1.1. Coating PVC pipes by (AgNPs)

Initially, concentrations of AgNPs (125 and 250 ppm) were prepared to coat PVC pipes with a diameter of 2.54 (1 inch). The(3 m) pipe was divided into 6 parts of (50 cm) each. Three sections of the pipes were washed thoroughly with tap water, then rinsed with distilled water to clean the inner surfaces of the pipes, allowed to dry, and then re-washed with a solvent (thinner solution) to remove all oils and organic contaminants. Silver nanoparticles were uniformly dispersed using an ultrasound probe with a power of (150 W) for 5 min in order to determine the dispersion process of silver nanoparticles. Three separately parts dry sections of pvc pipes were immersed in a 125 ppm silver nanoparticle solution for 10 seconds as shown in figure 1 and dried with hot air for 25 seconds. This process was repeated ten times to confirm the coating of the inner surface of the PVC pipe parts with AgNPs. Finally, the silver-treated pipes were washed with tap water to remove undecorated silver nanoparticles.

The process was repeated in full detail using a concentration of 250 ppm silver nanoparticles for coating another 3 residul parts of PVC pipes.

Figure 1: PVC pipes were immersed in a 125 ppm silver nanoparticle solution



2.1.2. Designing the experimental set.

This set consists of the following:

- partitioned 50 cm PVC pipes of 2.54 cm diameter coated by 125 ppm AgNPs and similar other five PVC parts but coated by 250 ppm AgNPs as described above in addition to other uncoated 50 cm parts of PVC pipe. Both coated and uncoated parts were connected to each other respectively and linked by plastic container, Peristaltic pump and flow meter (Figure 2).
- plastic container.
- Peristaltic pump.
- Flow meter.

Figure 2: Experimental unite components.

2.2. A study of the effect of nanoparticles on the viability of P.aeruginosa ATCC 27853 bacteria

The pour plate method was carried out to study the effect of AgNPs prepared at different concentrations (0.0, 125, and 250) ppm of AgNPs to study its effects on isolates p. aeruginosa ATCC 27853, through the steps shown below.

1. Activate bacteria cells by culturing them in one liter of brain infusion broth (BIB) for 24 hours at 37 ºc with continuous shaking.

2. Collected the activated bacteria by centrifuged it at 5000rpm for 10 min, and washed the pellet by phosphate buffer saline for three times.

3. Adjusted the total bacterial count to reach 1x106 CFU/mL.

4. Add the activated bacteria to a water tank (75)liter tap water, connected to a distribution system as above details (paragraph 3.6), which have pipes coated with silver nanoparticles at different concentrations (0.0, 125, 250) ppm, then the contaminated water have been pumped into the distribution system at different times (0.0, 2, 5, 10, 15, 20) minutes.

5. After different exposure times, one milliliter of treated water was taken

and mix with a volume of 25 mL of Muller Hinton medium in liquid state and leave it for a quarter of an hour to solidify at sterile conditions.

All plates were incubated for 24 hours at a temperature of 37 ºC.

6. fter incubation time the grown colonies were counted the inhibition rate or (removal rate were calculated according the following equation:

Inhibition ratio = 100 - test reading / control reading x 100

3. Results and Discussion

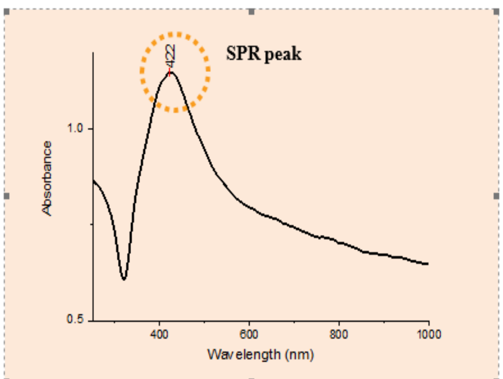
3.1 Characterization of synthesized silver nanoparticles

Silver nanoparticles characterization was determined by the following techniques:

3.1.1 UV-Vis spectrum

The UV-Vis spectrum of AgNPs showed the unique peak of silver nanoparticles at 422 nm (Figure 3), the SPR is typical of silver nanoparticles having absorbance values which were reported earlier ,therefore, this measurement proves the success of the formation of AgNPS [31,32]

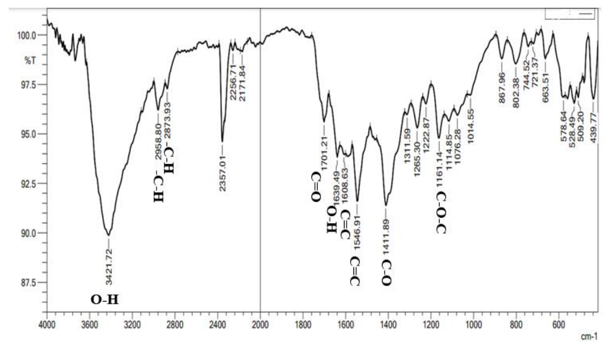
Figure 3: UV-Vis spectrum of green synthesized silver nanoparticles



3.1.2. Fourier Transform Infrared (FTIR spectrum)

identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components as shown in Figure 4. The measurement showed the presence of the following bands at 1161, 1411, 15461608, 1701, 2873,2958, which are attributed to the stretching vibration of C-O-C, C-O, C=C, C=O and -C-H groups, respectively. The presence of these functional groups confirms the presence of phenols, alkaloids, carboxylic acids, as well as aromatic compounds and aliphatic substitutes or aliphatic chains, this be compatible with [33] and [34].

Figure 4: FTIR spectrum of green synthesized silver nanoparticles



3.1.3. X-Ray Diffraction (XRD)

The XRD spectra for the AgNPs coating is shown in Fig.5 it is widely used for qualitative and quantitative chemical analysis, in particular, in electron microscopes. The X-ray results show that four main peaks at 38.190032, 44.440125, 64.740340 and 77.741242° which are the characteristic peaks of pure nano silver with FWHM values larger than 0.2458° (Table 1). These peaks belong to FCC silver because they correspond to crystal planes 111, 200, 220 and 311, respectively [35].

Figure 5: XRD measurement of green synthesized silver nanoparticles.

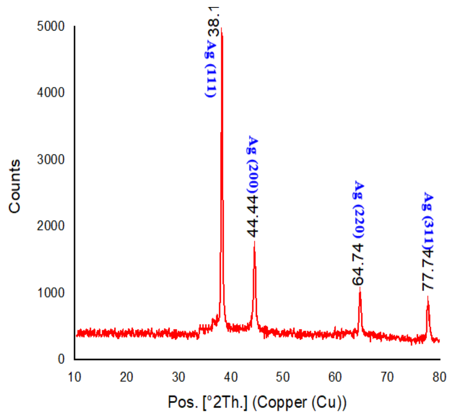


Table 1: XRD information and average particle size

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	D (nm)	D $_{average}$ (nm)	Index
38.190032	4374.01	0.294910	29.8000	31.8100	1 1 1
44.440125	1214.94	0.294910	30.4200		2 0 0
64.740340	670.14	0.245800	40.0000		2 2 0
77.741242	539.92	0.394300	27.0500		3 1 1

3.1.4. Field Emission Scanning Electron Microscopy (FESEM)

Field emission microscopy (FESEM) results in sharper images with less electrostatic distortion and a spatial resolution of 1 1/2 nm, thus determining its potential for use in the appropriate type of application, depending on the surface area generated by each geometry as well as the available porosity [36]. . The results were shown in Figure 6, which confirmed the presence of silver nanoparticles with spherical shapes and sizes ranging from (40-90) nm, and this is an important indicator of the success of the preparation method, which was proven by the particle size distribution chart in Figure 7.

Figure 6: FE-SEM measurement of green synthesized silver nanoparticle.

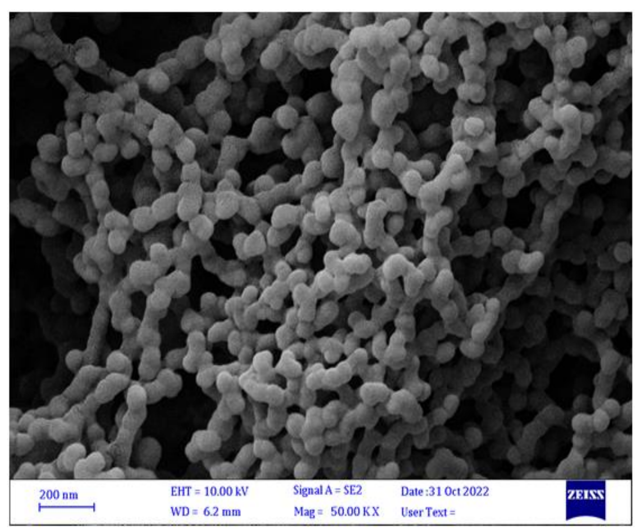
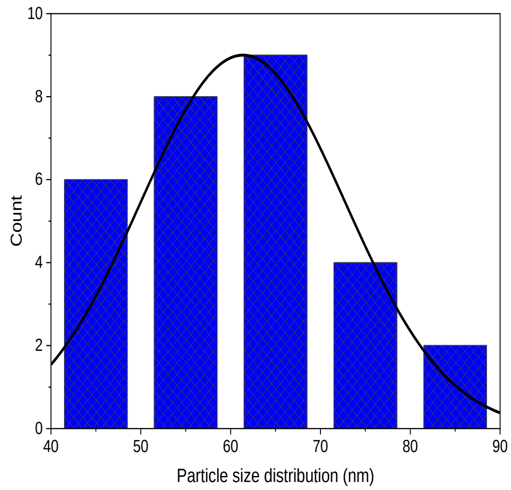


Figure 7: FESEM Particle size distribution of green synthesized silver nanoparticles.

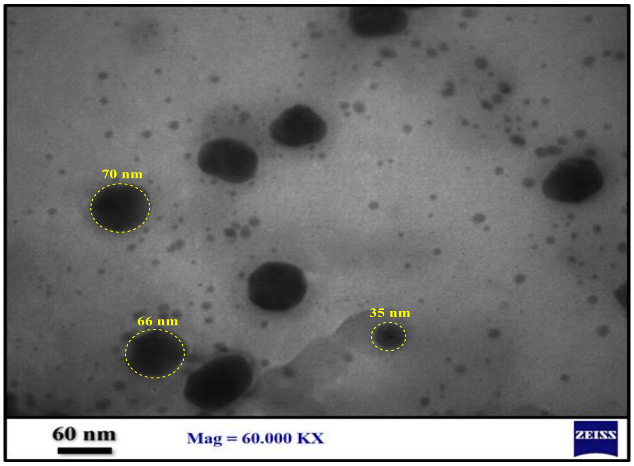


3.1.5 Transmission Electron Microscopy (TEM) measurement

This measure has been used to identify thesize,shape and morphology ,of nanoparticlesof In order to obtain the exact shape and size of the prepared AgNPs, as well as to assess the extent of the separation of the particles from each other; this measurement was required [37].

Figure 8 show that, the TEM measurement of AgNPs prepared using the bio reduction method . The measurement showed the presence of particles with irregular spherical shape and sizes ranging between 35 and 70 nm, which indicates the success of the used preparation method.

Figure 8: TEM of Ag NPs



3.2 Characterization of PVC pipes coated with AgNPs

3.2.1. FE-SEM

PVC pipes were coated with AgNPs using hot air current annealing technique, where concentrations (125 and 250) ppm were used to determine the sufficient concentration to kill A. aeruginosa ATCC 27853 and its relationship to the time factor, and these pipes were characterized using FE-SEM and XRD techniques. The results are shown in Figures 9 and 10. The measurement proved that the coating method was successful because the surface contains semi-spherical particles with sizes ranging from (24 to 32) nm at a concentration of 250 ppm, while it reached (22-29) nm for the pipe coated with a concentration of (125) ppm. As expected, with a lower concentration the particle size was relatively smaller.

Figure 9: Inner surface of PVC coated with silver nanoparticles (250 ppm)

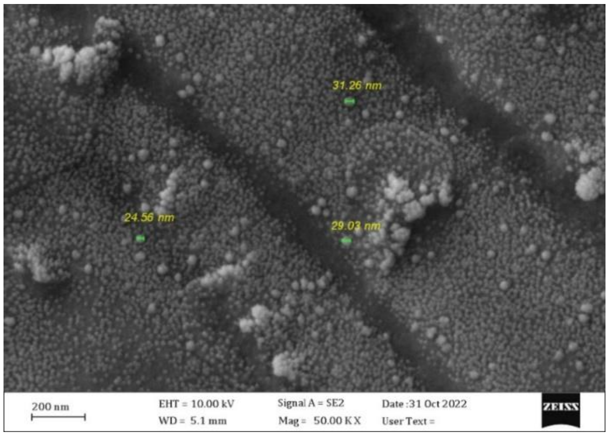
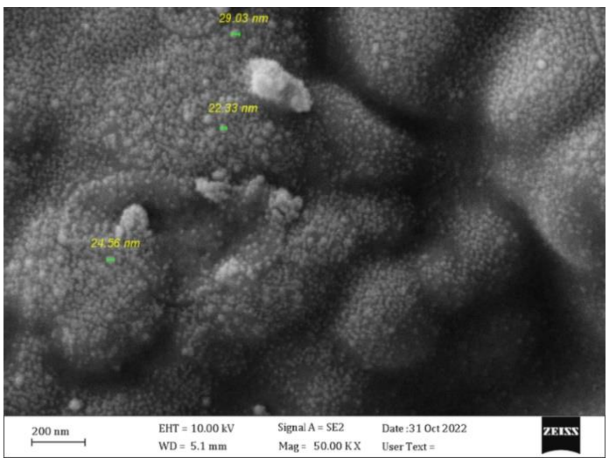


Figure 10: Inner surface of PVC coated with silver nanoparticles (125 ppm)



3.2.2. XRD

The XRD technique confirmed the presence of silver nanoparticles because it showed the characteristic peaks at 39.593, 47.497, 57.507 and 64.943 degrees, shifting to different positions than the silver nanoparticles alone, which indicates that the silver sticks interact on the surface of the tubes. In addition, the measurement shown in Figure 11 showed the presence of other peaks attributed to PVC [38]. The small size of the nanoparticles whose interaction was observed on the surface of the tube, where the particle size was calculated based on the characteristic peaks for silver only and amounted to 15.255 nm (Table 2), which is highly proportional to the results recorded in the FE-SEM measurement. The measurement proved that both tubes had a perfect match in the XRD measurement except for (intensity of silver peaks) due to the use of two different concentrations.

Figure 11: XRD measurement of PVC pipes coated AgNPs

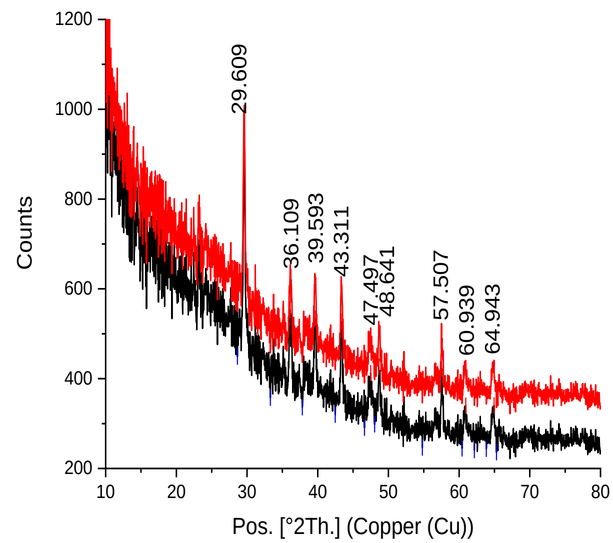


Table 2: XRD information and average silver particle size for the PVC coated silver nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	D (nm)	D $_{average}$ (nm)	Assignments
29.609	686.646	0.49023	-	15.2550	PVC

36.109	331.331	0.71686	-	PVC
39.593	311.89	0.69729	12.6600	Ag NPs
43.311	305.095	0.50815	-	PVC
47.497	190.549	1.03428	08.7700	Ag NPs
48.641	205.037	0.65772	-	PVC
57.507	200.633	0.4023	23.5400	Ag NPs
60.939	118.165	0.55432	-	PVC
64.943	118.647	0.6131	16.0500	Ag NPs

3.3. Pseudomonas aeruginosa ATCC 27853 bacteria examination

It is noticeable that, there is a clear decrease in the number of colonies growing on a medium(Muller Hinton),that relied mainly on the concentration of silver nanoparticles and the time of exposure, so the inhibition rate increased Significant increase at ($P<.05$) level when increasing the concentration and increasing the exposure time as shown in table 3 ,where it is observed zero bacterial growth was happened after 15 and 20 minutes at AgNPs concentration of 250 ppm

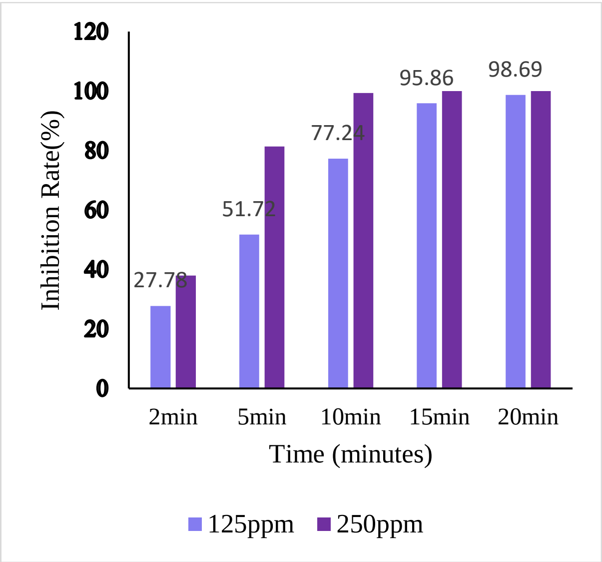
Table 3. Colony count of pseudomonas aeruginosa ATCC 27853 exposed to different concentration of Ag NPs and different exposure time

Concentration PPM	*pseudomonas aeruginosa* ATCC 27853 CFU/mL					
	Time (minutes) (50cm)					
	0.0	2	5	10	15	20
0.0	290±25	290±25	290±25	290±25	290±25	290±25
125	290±25	245±28*	140±11*	66±3*	12±0.5*	3.0±0.5*
250	290±25	180±12*	54±2*	2.0±0.02*	0.0*	0.0*

- * significant at $p<0.05$
- P value= 0.269858
- Each number represent (M±SD) for three replicates

In the set of 50 cm with a silver concentration of 250 ppm, the value of bacterial presence was 180 CFU/ml two minutes after the start of water passing within the pipes, which decreased to approximately four times after 5 minutes (54 CFU/ml), to continue the process of decreasing the bacterial presence until it reached zero after 15 minutes. From this we conclude that the system with 250 ppm has the larger ability to kill bacteria. Therefore, the inhibition rate reached 100% when the concentration was 250 ppm at the two times (15 and 20) minutes, but after 20 minutes for the AgNPs concentration of 125 ppm the inhibition rate to 98% as shown in figure 12 Which confirms the importance of the used silver concentration agent.

Figure12. Inhibition rate of pseudomonas aeruginosa ATCC 27853 CFU/mL



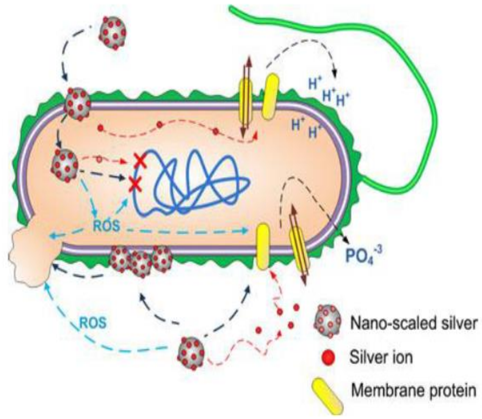
There are three mechanisms for the antimicrobial activities of AgNP :

a. It can stick to the cell membrane and disrupt the permeability and respiration functions of the cell and thus kill the cells;

B. Reactive oxygen species (ROS) can be generated on the surface of nanoparticles and cause DNA damage by exerting oxidative stress;

c. The silver ions released from AgNP can also disrupt ATP production and DNA replication.,This is consistent with previous studies [39], as shown in Figure 13.

Fig. 13: Mechanisms of interaction between AgNP and bacterial cells [39].



Metal and metal oxide nanoparticles have wide spectrum and non-resistant antimicrobial activity interacting, because of high surface area the nanoparticles can penetrate the bacterial cells and causes serious alteration in DNA, lysosomes, ribosomes, and enzymes structures that can lead to oxidative stress, electrolyte imbalances, and enzyme inactivation, gene expression, protein deactivation. Metallic nanoparticles have wide applications in environmental applications by inhibiting growth of p.aeruginosa and other enterobacter Promoting bacteriostatic and bactericidal decreasing waterborne disease. Work synergistically with other agents' reduction growth of p.aeruginosa and reducing the biofilm formation, and reducing resistance to antibiotics. From results that the Ag NPs have significant inhibition rate with safe use products in multi environmental applications.

4. Conclusion

Silver nanoparticles showed good results as a usable antibacterial agent in eliminating found in drinking water, which confirms the importance of the concentration of silver used and time, inally, concentration was seemed very clear effect on the time required to end the bacterial presence, due to the fact that the surface area was greater in the case of a higher concentration of silver

Reference

[1] Hendy, A.M. The Applications of Nanotechnology in Furniture Industry. Inter. J. of Scientific Research and Innovative Technology. 2020; 7(1): 25-36.

[2] Edwards, E.; Brantley, C and Ruffin, P.BOverview of Nanotechnology in Military and Aerospace Applications. In book. Nanotechnology Commercialization: Manufacturing Processes and Products. Edited by Mensah, T.O.; Wang, B.; Bothun, G.; Winter, J. and Davis, V.,2017; 1st publication.

[3] Patel, S.; Nanda, R. and Sahoo, S. Nanotechnology in Healthcare: Applications and Challenges. Medicinal chemistry.2015; 5(12): 528-533.

[4] Effiong, D.; Uwah, T.O.; Udofa, E. and Akpabio, A.E. Nanotechnology in Cosmetics: Basics, Current Trends and Safety Concerns-A Review. Advances in Nanoparticles.2020; 9(1): 1-22.

[5] Rashidi, L. and Khosray, KThe Applications of Nanotechnology in Food Industry. Critical Reviews in Food Science and Nutrition. 2011; 51(8): 723-730.

[6] Mylvaganam, H. and Rathnayake, P. Application of nanotechnology in food packaging: A review. J Food Technol Pres.2020; 4(4):1-4.

[7] Seevakan, K. and Sheeba, B. Applications of Nanotechnology and Nanomaterials in Construction. International Journal of Pure and Applied Mathematics.2018; 119 (12): 6275- 6286.

[8] Singh, A.K.; Kulshreshtha, A.; Banerjee, A. and Singh, B.R. Applications of Nanotechnology in Road Construction. J. of Physical Science Engineering and Technology.2019; 11(1): 2229-7111.

[9] Figoli, A.; Dorraji, M.S.S. and Amani-Ghadim, A.R. Application of nanotechnology in drinking water purification. Water Purification.2017: 119-167

[10] Abo Shmeis, R. Nanotechnology in Wastewater Treatment. In book: “Environmental nanotechnology: implications and applications” .2021;Publisher: Elsevier.

[11] Agarwal, A., and Jos, H. Application of nanotechnology in the remediation of contaminated groundwater. A short review. Recent Research in Science and Technology.1010 ; 2(6): 51-57.

[12] Hasham, A. Selected Nanotechnology Applications in Industrial Waste Water. Int. J. of Environmental Pollution & Environmental Modelling2018; 1(3): 71-76.

[13] Xiao, X.; He, E.; Lu, X.; Wu, L.; Fan, Y, and Yu, H. Evaluation of antibacterial activities of silver nanoparticles on culturability and cell viability of Escherichia coli. Science of The Total Environment.2021;. 794(1).

[14] Dutta, D.; Goswami, S.; Dubby, R.; Dwivedi, S. and Puzari, A. Antimicrobial activity of silver-coated hollow poly(methylmethacrylate) microspheres for water decontamination. Environmental Science Europe.2021 ; 33.

[15] Zhang, H.; Smith, J.A. and Oyanedel-Craver, V. Evaluation of the Disinfectant Performance of Silver Nanoparticles in Different Water Chemistry Conditions. Journal of Environmental Engineering.2012; 138(1):58-66.

[16] Khaydraov, R.R.; Khaydarov, R.A.; Gapurova, O. ;Garipov, I. Firdaus, M.L.

Chapter 18. Silver Nanoparticles as a Biocide for Water Treatment Applications. Advanced Research in Nanosciences for Water Technology.2019; DOI: 10.1007/978-3-030- 02381-2_18.

[17] Bhardwaj, A.K.; Sundaram, S.; Yadav, K.K., Arun Lal Srivastav, A.L. An overview of silver nano-particles as promising materials for water disinfection. Environmental Technology & Innovation.2021; 23, Article no. 101721.

[18] .Hwan, S,; Lee, H.; Ryu, D.; Choi, S. and Lee, D. Antibacterial Activity of Silver-Nanoparticles Against Staphylococcus Aureus and Escherichia coli. Korean Journal of Microbiology and Biotechnology.2010; 39(10):77-85.

[19] Mahsa, Y.; Abari, H. and Amir, A.A.. Antibacterial activity of silver-nanoparticles against Staphylococcus aureus. African Journal of Microbiology Research.2016; 10(25): 850-855.

[20] Maruthai, K.; Kommoin, V.; Ravibalan, N.; Philip, S.A ; Samrot, A.V. and Maruthai, M. Antibacterial Activity of the Silver Nanoparticles against Escherichia coli and Enterobacter sp. Progress in Bioscience and Bioengineering.2017; 1(1): 29-35.

[21] Zhang, Z.; Gao, P.; Li, M.; Cheng, J.; Liu, W. and Feng, Y. Influence of Silver nanoparticles on nutrient removal and microbial communities in SBR process after long-term exposure. Science of the Total Environment.2016; 569-570:234-243.

[22] Phuong, T.N.M.; Hai, N.T.T.; Thuy, N.T.; Phu, N.V.; Hong. N.T.; Nga, D.T.H.; Huy, H.X.Q. and Hoa, T.T. Factors affecting synthesis of silver-nanoparticles and antimicrobial applications. HUE university Journal of Science Natural Science.2020; 129(1D): 25-31

[23] Zhang, H.; Smith, J.A. and Oyanedel-Craver, V. The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers, Water Research.2011; 46(1): 691-699.

[24] Deshmukh, S.P.; Patil, S.M.; Mullani, S.B. and Delekar, (2019). Silver nanoparticles as an effective disinfectant: A review. Material Science and Engineering.2019; 97: 954-965.

[25] Dong, Y.; Zhu, H.; Shen, Y.; Zhang, W. and Zhang, L. Antibacterial activity of silver nanoparticles of different particle size against Vibrio Natriegens. 2019;Plos One. https://doi.org/10.1371/journal.pone.0222322.

[26] Kradin R. L. "Understanding Pulmonary Pathology: Applying Pathological Findings in Therapeutic Decision Making", Science Direct.2017 ; 157-242.

[27] Jasem, L. A., Hameed, A. A., Al-Heety, M. A., Mahmood, A. R., Karadağ, A., & Akbaş, H.. The mixture of silver nanosquare and silver nanohexagon: green synthesis, characterization and kinetic evolution. Materials Research Express.2019;6(8), 0850f9.

[28] Alheety, M. A., Al-Jibori, S. A., Ali, A. H., Mahmood, A. R., Akbaş, H., Karadağ, A., ... & Ahmed, M. H. Ag (I)-benzisothiazolinone complex: synthesis, characterization, H2 storage ability, nano transformation to different Ag nanostructures and Ag nanoflakes antimicrobial activity. Materials Research Express.2019; 6(12), 125071.

[29] Salih, B. D., Ali, A. H., Alheety, M. A., Mahmood, A. R., Karadağ, A., & Aydın, A. Biosynthesis of Ag nanospheres using waste phoenix dactylifera argonne: a

prospective anticancer and antibacterial. Materials Research Express.2019; 6(10), 105063.

[30] Alheety, N. F., Mohammed, L. A., Majeed, A. H., Sehgal, S., Aldahham, B. J., & Alheety, M. A. . The effect of addition Ag and MnO2 nanoparticles in the hydrogen storage of ethyl 2-((5-methoxybenzo [d] thiazol-2-yl) thio) acetate (organic: Inorganic nanohybrids). Journal of the Indian Chemical Society.2022;99(10), 100734.

[31] Bhui, D.K.; Bar, H.; Gobinda, P.S.; Sankar, P.S.; De., P. and Misra, A. Synthesis and Uv-vis spectroscopic study of silver nanoparticles in aqueous SDS solution. J. of Molecular Liquids.2009; 145(1): 33-37.

[32] Sikes, J. C., Niyonshuti, I. I., Kanokkanchana, K., Chen, J., Tschulik, K., & Fritsch, I. Single particle electrochemical oxidation of polyvinylpyrrolidone-capped silver nanospheres, nanocubes, and nanoplates in potassium nitrate and potassium hydroxide solutions. Journal of The Electrochemical Society.2022; 169(5), 056508.

[33] Barbhuiya, R. I., Singha, P., Asaithambi, N., & Singh, S. K. Ultrasound-assisted rapid biological synthesis and characterization of silver nanoparticles using pomelo peel waste. Food Chemistry.2022; 385, 132602.

[34] Aziz, S.B.; Hamasalih, G.H; Brza, M.A.; Jalal, S.; Abdulwahid, R.T.; Saeed, S.R. and Hassanzadeh, A. Fabrication of Interconnected Plasmonic Spherical Silver Nanoparticles with Enhanced Localized Surface Plasmon Resonance (LSPR) Peaks Using Quince Leaf Extract Solution. Nanomaterials.2019; 9 (11), DOI: 10.3390/nano9111557.

[35] Khandelwal, R.; Karchhawa, C.; Arora, S.K.; Ratan, J.K. and Garg, R. A sustainable method for synthesis of silver nanoparticles by using Glycyrrhiza glabra extract. Nanotechnology for Environment Engineering.2021; 6, Article number 58.

[36] Kahnouji, Y.A.; Mosaddegh, E. and Bolorizadeh, M.A. Detailed analysis of size-separation of silver nanoparticles by density gradient centrifugation method. Material Science Engineering .2019; C. Vol 103, https://doi.org/10.1016/j.msec.2019.109817.

[37] Tadjiki, S.; Montano, M.D.; Assemi, S.; Barber, A.; Ranville, J. and Beckett, R. Measurement of the Density of Engineered Silver Nanoparticles Using Centrifugal FFF-TEM and Single Particle ICP-MS. Anal Chem.2017; 89(11): 6056-6064.

[38] Taha, T. A. Optical properties of PVC/Al2O3 nanocomposite films. Polymer Bulletin.2019;76(2), 903-918.

[39] Zhang H. “Application of Silver Nanoparticles in Drinking Water Purification”, Open Access Dissertations.2013; Paper 29.