

PAPER • OPEN ACCESS

Antimicrobial activity of silver nanoparticles synthesized using honey and gamma radiation against silver-resistant bacteria from wounds and burns

To cite this article: A M S Hosny et al 2017 Adv. Nat. Sci: Nanosci. Nanotechnol. 8 045009

View the article online for updates and enhancements.

You may also like

- <u>Preparation and characterization of</u> graphene nanosheet doped with silver nanoparticles A H Mohammed and A N Naje
- Effect of biosynthesised silver nanoparticles as sterilant on physiological and biochemical characteristics in micropropagation of *Musa sapientum* L. Siriporn Phongtongpasuk, Thitikamon Liamnimit, Thanyaporn Buakaew et al.
- Increasing the accumulation of aptamer AS1411 and verapamil conjugated silver nanoparticles in tumor cells to enhance the radiosensitivity of glioma Jing Zhao, Dongdong Li, Jun Ma et al.

IOP Publishing

Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 (2017) 045009 (7pp)

Antimicrobial activity of silver nanoparticles synthesized using honey and gamma radiation against silver-resistant bacteria from wounds and burns

A M S Hosny¹, M T Kashef¹, S A Rasmy¹, D S Aboul-Magd² and Z E El-Bazza²

 ¹ Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt
² Drug Radiation Research Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, 11787, Egypt

E-mail: mona.kashef@pharma.cu.edu.eg

Received 22 June 2017 Accepted for publication 31 August 2017 Published 26 September 2017

Abstract

Silver nanoparticles (AgNPs) are promising antimicrobial agents for treatment of wounds and burns. We synthesized AgNPs using honey at different pH values or with different gamma irradiation doses. The resulting nanoparticles were characterized by UV–vis spectroscopy, TEM, DLS and FTIR. Their antimicrobial activity, against standard bacterial strains and silver-resistant clinical isolates from infected wounds and burns, was evaluated *in vitro* through determination of their minimum inhibitory concentration (MIC).

AgNPs prepared using 30 g of honey exposed to 5 kGy gamma radiation had the best physical characters regarding stability and uniformity of particle size and shape. They recorded the lowest MIC values against both the standard and silver-resistant isolates. In conclusion, honey and gamma radiation can be used in synthesis of highly stable pure AgNPs, without affecting the physico-chemical and antimicrobial activity of honey. This offered an advantage in terms of inhibition of silver-resistant bacteria isolates.

Keywords: antimicrobial, honey, gamma radiation, silver nanoparticles, silver-resistant infection

Classification numbers: 2.00, 2.05, 4.02, 5.08

1. Introduction

Different silver products have been used topically as antimicrobial compounds, such as silver nitrate and silver sulfadiazine [1]. They exert broad-spectrum bactericidal activity by binding with the bacterial thiol (–SH) group resulting in complete blocking of respiration, electron transfer, enzyme and transport systems, transcription and replication of both RNA

Original content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. and DNA of the bacteria leading to cell death [2]. These properties make silver compounds effective in treatment of chronic wounds and burns, which are often poly-microbial with both Gram-positive cocci and Gram-negative bacteria [3]. In addition, blood flow alteration associated with these wounds minimizes the effect of systemic antibiotics and so, its use will be of little or no effect [4]. Unfortunately, the increased use of such silver compounds has led to the occurrence of bacterial silver resistance and consequently has compromised its medical utility [5].

Advances in nanotechnology have opened new platforms to modify the important properties of silver allowing



its synthesis in the nanoparticle form. Silver nanoparticles (AgNPs) have greater efficiency regarding their microbial inhibitory activity in comparison with other silver compounds [6, 7]. The synthesis of Ag in its nanostructure was done by using several physico-chemical protocols, such as metal ablation [8], micro emulsion [9], chemical reduction [10] and electrochemical method [11], which involve the use of hazardous materials that adversely affect human health and environment. Other safer techniques were proposed such as using natural polymers [12], microorganisms [13] and plant extracts [14] in production of AgNPs.

Honey has also been used in the green synthesis of AgNPs. It contains ingredients such as glucose and fructose that allowed its use as both capping and reducing agent in the synthesis process. In addition, it has extraordinary healing properties that are attributed mainly to its acidic pH and the presence of hydrogen peroxide. However, the reduction of silver ions into AgNPs, using honey, occurred only at alkaline pH [15] where these valuable properties of honey were lost.

Gamma radiation has also been used in the synthesis of metallic NPs; such as silver, nickel and copper [16–18]. It has several important advantages, including: (i) controlled reduction of metal ions without using excess reducing agent or producing any undesired oxidation products from the reductant, (ii) reducing agent is produced uniformly in medium, (iii) provides metal NPs in fully reduced, pure and highly stable form, (iv) no interfering impurities like metal oxides, (v) synthesis can be carried-out at ambient conditions [16, 19].

In this study AgNPs were synthesized using honey, as capping and reducing agent, in combination with different doses of gamma radiation. The antimicrobial activity, of the resulting nanoparticles, was evaluated against standard bacterial strains as well as against different silver-resistant clinical bacterial isolates obtained from wounds and burns.

2. Experimental

2.1. Chemicals

Silver nitrate (AgNO₃, cat. no. 209139) was obtained from Sigma-Aldrich (USA). Sodium hydroxide (NaOH, cat. no. ICN1534955) was obtained from MP Biomedicals, Inc. (USA). Tryptic soy broth (TSB, cat. no. 146318) was obtained from Merck Millipore (Germany). The used honey in the study was from the Egyptian commercial market.

2.2. Microorganisms

Standard bacterial strains, namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213, were obtained from VACSERA, Cairo, Egypt. Also, 150 clinical bacterial isolates were obtained from different wound sepsis and burns. They were collected from the Central Laboratories, EL-Demerdash Hospital, Ain-Shams University, Cairo, Egypt. These bacterial isolates were identified as *S. aureus* (n = 38), *E. coli* (n = 22), *Klebsiella pneumoniae* (n = 32), *P. aeruginosa* (n = 21), *Acinetobacter* spp. (n = 14), *Enterobacter* spp. (n = 8) and

A M S Hosny et al

Proteus spp. (n = 15). A total of 19 bacterial isolates were silver-resistant with a silver nitrate minimum inhibitory concentration (MIC) $\ge 512 \ \mu g \ ml^{-1}$.

2.3. Synthesis of AgNPs

2.3.1. Using honey at different pH. According to Haiza *et al* [15], 20g of honey was dissolved in 80 ml of deionized water. Then 15 ml of this honey solution was added to 20 ml of AgNO₃ solution (1 mM) and stirred well for 1 min. To initiate the reduction of Ag ions, the pH of the mixture was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 using NaOH. The same procedure was repeated using different honey weights (10g, 30g, 40g and 50g). Changing the color of the system from transparent to yellow indicated the formation of AgNPs.

2.3.2. Using honey combined with different gamma radiation doses. The radiation took place in cobalt-60 (Co^{60}) 220 gamma cell, Canada Co. Ltd., located at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. The dose rate was 1.774 kGy h⁻¹ at the time of experiment.

The synthesis was done using the same above procedure; however, the reduction was initiated by exposure to different gamma radiation doses (1, 5, 10, 15, 20, 25 and 30 kGy). The same above procedure was repeated using different weights of honey (10g, 30g, 40g and 50g). The original pH of honey was kept throughout the preparation at 3.9. After irradiation, the formation of AgNPs was indicated by changing the solution color from transparent to yellow.

2.4. Characterization of AgNPs

The physical properties of the synthesized AgNPs were characterized using:

2.4.1. Ultraviolet-visible (UV-vis) spectroscopy. The optical properties of the AgNPs were determined by measuring the absorbance in the range from 190 to 600 nm at a resolution of 1 nm using UV-vis spectrophotometer (JASCO-Japan, model V-560). The honey solution was used as a blank.

2.4.2. Transmission electron microscope (TEM). The produced AgNPs' structure was analyzed using JEOL 1010 transmission electron microscope, at accelerating voltage 80kV and 120kV. The TEM samples were prepared by drop-casting the reaction mixtures on carbon-coated grids and allowed to dry at room temperature, prior to measurement.

2.4.3. Dynamic light scattering (DLS). The particle size, the size distribution and the peak intensity of the AgNPs was determined by DLS using Zetasizer nano series Nano ZS, Malvern Instrument, UK. The DLS measurements were carried-out for size ranges from 0.1 to 100 nm.

2.4.4. Fourier transform infrared (FTIR). For the identification of the possible biomolecules responsible for the capping and stabilization of the nanoparticles in the honey solution;



Figure 1. Synthesis of AgNPs using different honey weights at different pH values and gamma radiation doses. (a) 20 g of honey at pH = 10; (b) 30 g of honey at pH = 9.5 and 10; (c) 40 g of honey at pH = 9, 9.5 and 10; (d) 30 g of honey at different gamma radiation doses; (e) 40 g of honey at different gamma radiation doses.

measurements were carried-out using FTIR (FTIR spectrum one, Perkin Elmer, Germany). The spectra were scanned in the $400-4000 \text{ cm}^{-1}$ range at a resolution of 4 cm^{-1} .

2.5. Determination of the antimicrobial activity of AgNPs

The antimicrobial activity of the different AgNPs preparations, that were confirmed to have pure, fully reduced and uniform properties, was tested against the standard bacterial strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923) and against the nineteen 19 clinical silver-resistant bacterial isolates. AgNPs solutions were prepared to give a concentration range of 0.84–108 μ g ml⁻¹. Their MIC was determined using broth macro-dilution method [20]. The MIC was recorded as the lowest concentration yielding no visible growth.

3. Results

3.1. Synthesis of AgNPs

3.1.1. Using honey at different pH. It was found that the synthesis of AgNPs was achieved only at alkaline pH = 9, 9.5 and 10. However, no AgNPs were produced at lower pH values. The increase in the used honey concentration allowed the production of AgNPs at the pH = 9 and 9.5. Thus, the color change, and hence the production of AgNPs, was observed with the following combinations of honey/pH values: 20 g/pH 10, 30 g/pH 9.5, 30 g/pH 10, 40 g/pH 9, 40 g/pH 9.5 and 40 g/pH 10 (figures 1(a)–(c)). While no color change was observed when using either 10 g or 50 g of honey at any tested pH.

3.1.2. Using honey combined with different doses of gamma radiation. Exposure to different gamma radiation doses stimulated the reduction of Ag^+ ions to AgNPs. The color change, and hence AgNPs production, was observed when 20g of honey combined with 25 kGy and when weights of 30g and 40g were combined with gamma radiation doses of 5, 10, 15, 20 and 25 kGy (figures 1(d) and (e)). However, no AgNPs

Table 1.	UV-vis analysis of the AgNI	Ps synthesized using honey
solution	at different pH and gamma ra-	diation doses.

Tested solution ^a	Wavelength (nm)	Absorbance (nm)	Peak shape
20 g/pH 10	400	2.801	Sharp
	460	3.373	Sharp
30 g/pH 9.5	430	0.511	Broad
30g/pH 10	415	3.031	Sharp
	435	9.999	Sharp
	455	1.963	Sharp
40 g/pH 9.5	435	1.486	Broad
40 g/pH 10	400	2.336	Sharp
	415	2.880	Sharp
	435	1.798	Sharp
	450	3.391	Sharp
20 g/25 kGy	440	0.844	Broad
30g/5 kGy	415	9.999	Sharp
30g/10 kGy	415	2.195	Broad
30g/15 kGy	415	1.555	Broad
40 g/20 kGy	415	1.873	Sharp
40 g/25 kGy	400	1.923	Sharp
	420	3.379	Sharp

^a Honey weight (g)/pH or gamma radiation dose (kGy).

were produced using either 10 g or 50 g of honey at any radiation dose tested.

3.2. Characterization of AgNPs

3.2.1. Ultraviolet–visible spectroscopy studies. The result of the UV–vis spectroscopic analysis revealed that the AgNPs prepared using 20 g, 30 g and 40 g of honey at pH = 10 produced absorption spectra with sharp peaks (surface plasmon resonance, SPR) at 400 and 460 nm; 415, 435, 455 nm; and 400, 415, 435, 450 nm, respectively. While in case of AgNPs, prepared using gamma radiation, the strong sharp peaks SPR appeared only with the following combination of honey/ gamma radiation; 30 g/5 kGy, 40 g/20 kGy and 40 g/25 kGy at 415 nm, 415 nm, and 400 and 420 nm, respectively. The peak



Figure 2. UV-vis spectrum of the AgNPs prepared using 30 g of honey: (a) reduced using gamma radiation of 5 kGy and (b) reduced using pH = 10.



Figure 3. TEM image of the AgNPs prepared using 30 g of honey: (a) reduced using gamma radiation of 5 kGy and (b) reduced using pH = 10.

intensity of the UV–vis spectra was higher in case of the preparations using gamma radiation compared to that prepared at different pH. Other NPs formulae showed broad peaks indicating the formation of low-yield AgNPs with different particle sizes (table 1). The AgNPs prepared using 30g of honey irradiated with gamma radiation dose of 5kGy produced a very sharp peak with the highest intensity (9.999) indicating the highest yield of AgNPs with a highly uniform particle size (figure 2).

3.2.2. Transmission electron microscope studies. The transmission electron microscope (TEM) analysis revealed that the AgNPs prepared with 30g of honey irradiated with 5 kGy gamma radiation yielded the highest well-dispersed spherical NPs over the honey matrix. The TEM image of its honey matrix clearly showed the absence of agglomeration with roughly-spherically shaped NPs and smooth edges (figure 3(a)) compared with that prepared using the same honey weight 30 g/pH 10 (figure 3(b)).

3.2.3. Dynamic light scattering studies. The results of dynamic light scattering (DLS) analysis revealed that AgNPs prepared with 30 g of honey irradiated at gamma radiation dose of 5 kGy had the best properties regarding their particle size range and predominant particle size (table 2). They had a particle size range from 2.69–10.10 nm with most predominant particle size of 4.187 nm and good size distribution intensity (figure 4(a)). However, AgNPs prepared using the same honey weight at pH = 10 had a particle size range of 7.5–21 nm with most predominant particle size of 11.7 nm (figure 4(b)).

3.2.4. Fourier transform infrared (FTIR) studies. The infrared (IR) spectrum of the different AgNPs produced bands at 3444, 2974, 2885, 1640, 1634, 1412, 1348, 1237 and 1057 cm^{-1} indicating the presence of stabilizing and capping agent with the NPs. Only the IR-spectrum of the AgNPs synthesized

Table 2. Particle size range and average particle size of AgNPs synthesized using honey solution at different pH and gamma radiation doses.

Tested solution ^a	Particle size range (nm)	Most predominant size (nm)
20g/pH 10	4.849-21.04	7.531
30g/pH 10	7.531-21.04	11.70
40 g/pH 10	13.54-37.84	18.17
30 g/5 kGy	2.696-10.10	4.187
40 g/20 kGy	10.10-24.36	13.54
40 g/25 kGy	4.849-15.69	6.503

^a Honey weight (g)/pH or gamma radiation dose (kGy).

using 30 g of honey irradiated at a dose of 5 kGy gamma radiation showed an additional peak at 1750 cm^{-1} (figure 5).

The presence of a band at 3444 cm^{-1} corresponds to O–H stretching vibration and indicated the presence of alcohol and phenols. The bands at 2947 and 2885 cm⁻¹ region arising from C–H stretching of aromatic compounds were observed. The band at 1750 cm^{-1} assigned for C=O stretching mode of the carboxylic acid group of the gluconic acid. Also, bands due to the C=N, C=C and N–H bend of protein amines were found as prominent IR bands at 1640 and 1634 cm⁻¹. The bands at 1412 and 1057 cm⁻¹ also assigned for the N–H and C–N (amines) stretching vibration of the proteins, respectively. The band at 1349 cm^{-1} amplifies the N=O symmetry stretching typical of the nitro compound. Finally, the band at 596 cm⁻¹ region corresponds to C–N stretching of the amines [21].

3.3. Determination of the antimicrobial activity of AgNPs

The MIC of different AgNPs preparations tested against the standard bacteria and the different silver-resistant bacterial isolates (n = 19) indicated that the AgNPs synthesized using 30g of honey irradiated with gamma radiation at 5



Figure 4. DLS graph of AgNPs prepared using 30 g of honey: (a) reduced using gamma radiation of 5 kGy and (b) reduced using pH = 10.

kGy had the best activity against all tested organisms. The MIC ranged from 1.6875 to 3.375 μ g ml⁻¹ and 1.6875 to 6.25 μ g ml⁻¹ against the standard bacteria and tested clinical isolates, respectively (table 3). It was also revealed that the synthesized AgNPs were more active against the tested Gramnegative bacteria than Gram-positive ones.

4. Discussion

The synthesis of AgNPs was successful only at alkaline pH. This was in accordance with that reported by Haiza *et al* [15] where NaOH solution was used as a pH regulator. The addition of NaOH increased the pH of the solution. At the same time, it allowed the production of more gluconic acid from glucose resulting in a rapid reduction of Ag ions and formation of a large number of small NPs. This accounted for the decrease in the size of produced AgNPs with NaOH addition and pH increase.

The use of gamma radiation allowed successful production of AgNPs, in presence of honey and maintained its acidic pH. AgNPs were produced at gamma radiation doses ranging from 5 to 25 kGy, depending on the used honey weight. This was in accordance with the study of Rao et al [16] where AgNPs were successfully produced using gamma radiation in a range 1-24 kGy along with gum acacia. Also, our results indicated that the use of higher honey weight led to smaller size of AgNPs. According to Spinks and Woods [22], the exposure of aqueous solutions to γ -radiolysis allowed the production of the solvated electrons and H. atoms, which act as strong reducing agents. They effectively can reduce Ag⁺ ions, which is the main process in the formation of NPs. Therefore, gamma radiation's use allowed the maintenance of the acidic honey pH, which is highly advantageous for the healing and antimicrobial activities of honey.

The physical characterization of AgNPs proved the superiority of AgNPs prepared using 30 g of honey at 5 kGy radiation dose. In the UV–vis spectrophotometric analysis, the colloidal AgNPs exhibit absorption peak in the wavelength from 400–450 nm range depending on the complex dielectric constant of the metal, the cluster size and the environment [16]. Also, the spectroscopical range depends strongly on the size, shape and functionalization of the metallic NPs [6]. The AgNPs synthesized using 30 g honey irradiated with gamma rays at 5 kGy exhibited a sharp peak at 415 nm only with the highest intensity. This indicated the formation of a uniformly sized AgNPs in spherical or nearly spherical shape with highest yield, compared to other tested preparations.



Figure 5. FTIR spectrum of AgNPs prepared using 30 g of honey at a dose of 5 kGy gamma radiation.

The superiority of the physical characters of the AgNPs prepared using honey at 5 kGy gamma irradiation dose was confirmed using the TEM and DLS. TEM confirmed the absence of agglomeration of AgNPs in this preparation. Also, DLS results revealed the homogeneity and small particle size of the produced nanoparticles. Sizes and shapes of NPs are influenced by number of factors including pH, precursor concentration, reductant concentration and time of incubation, temperature as well as method of preparation [23]. The superiority of this AgNPs preparation indicated that the use of gamma radiation allowed the production of AgNPs with superior physical properties compared to those produced using the same amount of honey at elevated pH.

The results of FTIR indicated the importance of honey ingredients in stabilizing the produced nanoparticles where different types of honey biomolecules were responsible for the stabilization and capping of the AgNPs within the matrix. The stabilization of 30 g/S kGy occurred through both the free amine groups and the $-\text{COO}^-$ (carboxylate ion) groups of amino acids residues with free carboxylate groups in the protein. On the other hand, other AgNPs preparations were stabilized only through the—COO⁻ (carboxylate ion) groups of amino acids residues with free carboxylate groups in the protein. The binding of honey proteins to AgNPs using both the free amine groups and carboxylate ion of the amino acid residue was previously reported [15, 24, 25].

Also, the AgNPs synthesized using 30g of honey irradiated at 5 kGy gamma irradiation dose recorded the highest antimicrobial activity against both the standard and the silverresistant clinical bacterial strains obtained from wounds and burns. This can be accounted for in terms of the superior physical properties of this preparation where the effect of AgNPs is highly influenced by their size, shape and concentration [7]. The smaller particle size the larger surface area to volume 0 11 00

- 1000

Table 3.	MIC (μ g ml	¹) of different A	AgNPs preparation	s against the	standard bacterial	strains and clinica	l silver-resistant	bacterial isolates
----------	------------------	-------------------------------	-------------------	---------------	--------------------	---------------------	--------------------	--------------------

AgNPs\Bacteria	20g/pH 10	30g/pH 10	40 g/pH 10	30 g/5 kGy	40 g/20 kGy	40 g/25 kGy
<i>E. coli</i> ATCC 25922	3.375	6.75	3.375	1.6875	3.375	3.375
P. aeruginosa ATCC 27853	6.75	6.75	3.375	1.6875	6.75	3.375
S. aureus ATCC 25923	6.75	13.5	13.5	3.375	13.5	6.75
E. coli 205	3.375	3.375	6.25	1.6875	6.25	1.6875
<i>E. coli</i> 263	3.375	6.25	13.5	3.375	6.25	3.375
K. pneumoniae 176	3.375	6.25	13.5	3.375	6.25	3.375
K. pneumoniae 197	3.375	3.375	6.25	1.6875	6.25	1.687
K. pneumoniae 199	6.25	6.25	6.25	3.375	6.25	3.375
K. pneumoniae 215	3.375	3.375	13.5	3.375	6.25	3.375
K. pneumoniae 465	6.25	6.25	13.5	3.375	13.5	3.375
K. pneumoniae 724	6.25	6.25	13.5	3.375	13.5	3.375
K. pneumoniae 793	6.25	6.25	13.5	3.375	13.5	3.375
P. aeruginosa 314	3.375	6.25	13.5	1.6875	6.25	3.375
P. aeruginosa 646	6.25	6.25	13.5	3.375	13.5	6.25
Acinetobacter spp. 407	3.375	3.375	6.25	1.6875	6.25	1.687
Acinetobacter spp. 457	3.375	3.375	6.25	3.375	6.25	3.375
Enterobacter spp. 285	3.375	3.375	3.375	1.6875	6.25	3.375
Enterobacter spp. 367	3.375	3.375	6.25	1.6875	6.25	3.375
S. aureus 447	13.5	6.25	13.5	6.25	6.25	6.25
S. aureus 579	6.25	13.5	13.5	6.25	6.25	6.25
S. aureus 586	13.5	13.5	13.5	6.25	13.5	6.25
S. aureus 601	6.25	6.25	13.5	3.375	6.25	6.25

ratio and the higher the antimicrobial activity [26]. Its superiority can also be attributed to the presence of honey at acidic pH. Where, it is well-known that the antimicrobial activity of honey depends mainly on its acidic pH [27] and the use of gamma radiation doses in a range from 5 to 15 kGy keeps the physical and chemical properties of honey unchanged, especially its low pH [28]. Therefore, the use of gamma radiation is highly advantageous in allowing the production of AgNPs with the smallest particle size (4.187 nm) and maintaining the antimicrobial activity of the honey. In accordance with previous studies, the synthesized AgNPs were more active on Gram negative bacteria than Gram positive ones [29, 30]. This difference in activity may be attributed to the difference in the cell structure between the Gram positive and negative bacteria, where the thicker cell wall of Gram positive allow less silver entry to the cytoplasmic membrane than the Gram negative [31].

Our green synthesized AgNPs had superior properties compared to other green synthesized ones [32-41]. They had the smallest particle size (4nm) that showed a high intensity (9.999) at a short wavelength (415 nm) by UV-vis spectroscopy; indicating the synthesis of a high yield of monodispersed spherical AgNPs. Their antimicrobial properties, in terms of their MIC, were superior to those recorded in other studies.

5. Conclusion

In conclusion, the green synthesis of AgNPs using honey and gamma radiation allowed the production of nanoparticles with a uniform small particle size in a highly-stable state without using harsh conditions. This method kept the physico-chemical and antimicrobial properties of honey unchanged. These AgNPs were highly active against the different silver-resistant bacterial isolates obtained from wounds sepsis and burns. Therefore, they could be used as an effective treatment in resistant wound sepsis and burn infections.

References

- [1] Rai M K, Deshmukh S D, Ingle A P and Gade A K 2012 J. Appl. Microbiol. 112 841
- [2] Rao P, Chandraprasad M S, Lakshmi Y N, Rao J, Aishwarya P and Shetty S 2014 Int. J. Multidisciplin. Curr. Res. 2 165
- [3] Bertesteanu S, Triaridis S, Stankovic M, Lazar V, Chifiriuc M C, Vlad M and Grigore R 2014 Int. J. Pharm. 463 119
- [4] Bradshaw C E 2011 Biosci. Horizons 4 61
- [5] Randall C P, Gupta A, Jackson N, Busse D and O'neill A J 2015 J. Antimicrob. Chemother. 70 1037
- [6] Cavassin E D et al 2015 J. Nanobiotechnol. 13 64
- [7] Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G and Galdiero M 2015 Molecules 20 8856
- [8] Abid J P, Wark A W, Brevet P F and Girault H H 2002 Chem. Commun. 2002 792
- [9] Zhang W, Qiao X, Chen J and Wang H 2006 J. Colloid Interface Sci. 302 370
- [10] Khan Z, Al-Thabaiti S A, Obaid A Y and Al-Youbi A O 2011 Colloids Surf. B 82 513
- [11] Reicha F M, Sarhan A, Abdel-Hamid M I and El-Sherbiny I M 2012 Carbohydr. Polym. 89 236
- [12] Huang H, Yuan Q and Yang X 2004 Colloids Surf. B 39 31
- [13] Sharma V K, Yngard R A and Lin Y 2009 Adv. Colloid. Interface Sci. 145 83
- [14] Song J Y and Kim B S 2009 Bioprocess Biosyst. Eng. 32 79

- [15] Haiza H, Azizan A, Mohidin A H and Halin D S C 2013 Nano Hybrids 4 87
- [16] Rao Y N, Banerjee D, Datta A, Das S K, Guin R and Saha A 2010 Rad. Phys. Chem. 79 1240
- [17] Rao V M, Castano C H, Rojas J and Abdulghani A J 2013 Rad. Phys. Chem. 89 51
- [18] Hori T, Nagata K, Iwase A and Hori F 2014 Jpn. J. Appl. Phys. 53 05FC05
- [19] Shameli K, Bin Ahmad M, Yunus W M Z W, Ibrahim N A, Gharayebi Y and Sedaghat S 2010 Int. J. Nanomed. 5 1067
- [20] Clinical and Laboratory Standard Institute (CLSI) 2013 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement (Wayne, PA: Clinical and Laboratory Standards Institute) CLSI Document M100-S23
- [21] Jyoti K, Baunthiyal M and Singh A 2016 J. Rad. Res. Appl. Sci. 9 217
- [22] Spinks J W T and Woods R J 1990 Introduction to Radiation Chemistry 3rd edn (New York: Wiley) p 243
- [23] Umoren S A, Obot I B and Gasem Z M 2014 J. Mater. Environ. Sci. 5 907
- [24] Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W and He N 2007 Nanotechnology 18 105104
- [25] Philip D 2010 Spectrochim. Acta A Mol. Biomol. Spectrosc. 75 1078
- [26] Van Phu D, Quoc Le A, Duy N N, Lan N T K, Du B D, Luan Le Q and Hien N Q 2014 Nanoscale Res. Lett. 9 162
- [27] Liponatti C, Soledad V and Marina B 2014 J. Microbiol. Antimicrob. 6 51
- [28] Saxena S, Panicker L and Gautam S 2014 Int. J. Food Sci. 2014 1

- [29] Grigor'eva A, Saranina I, Tikunova N, Safonov A, Timoshenko N, Rebrov A and Ryabchikova E 2013 *Biometals* 26 479
- [30] Kim J S et al 2007 Nanomed. Nanotech. Biol. Med. 3 95
- [31] Egger S, Lehmann R P, Height M J, Loessner M J and Schuppler M 2009 Appl. Environ. Microbiol. 75 2973
- [32] Kumar V A, Uchida T, Mizuki T, Nakajima Y, Katsube Y, Hanajiri T and Maekawa T 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 015002
- [33] Ebrahiminezhad A, Bagheri M, Taghizadeh S M, Berenjian A and Ghasemi Y 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 015018
- [34] Chandrappa C P, Govindappa M, Chandrasekar N, Sarkar S, Ooha S and Channabasava R 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 025016
- [35] Govindappa M, Farheen H, Chandrappa C P, Channabasava R, Rai R V and Raghavendra V B 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 035014
- [36] Kumari R M, Thapa N, Gupta N, Kumar A and Nimesh S 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 045009
- [37] Rao B and Tang R C 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 015014
- [38] Ajayi E and Afolayan A 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 015017
- [39] Prasannaraj G and Venkatachalam P 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 025001
- [40] Erjaee H, Rajaian H and Nazifi S 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 025004
- [41] Mittal J, Jain R and Sharma M M 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 025011