

Different anisotropic silver nanocrystals show different antibacterial activities – an effect of different prominent crystallographic orientations in different shapes

Sumit Sarkar¹, Biraj Sarkar², Sukhendu Mandal² and Ratan Das^{1,*}

¹Nano-Physics and Nanotechnology Research Laboratory, Department of Physics, Tripura University, Suryamaninagar 799 022, India

²Department of Microbiology, University of Calcutta, Kolkata 700 019, India

The antibacterial activity of silver (Ag) nanoparticles is well established and various researchers have provided different explanations for the same. We have tested the activity of similar-sized anisotropic Ag nanocrystals. Silver nanocubes and nanohexagons were prepared and their antibacterial activity was tested against a few bacteria such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*. It was found that the two shapes were active against all these bacteria. However, the plot of cell density of different bacterial pathogens against the concentration of silver nanocrystals was found to be different for these two shapes. Moreover, half maximal inhibitory concentration value and minimum bactericidal concentration value were also different for the two shapes. XRD analysis showed that both the nanocrystals were crystalline in nature, but their crystallographic orientation was different. So, it can be inferred from this study that some crystallographic planes are probably more active towards reaction with different bacterial compositions and hence, responsible for stronger antibacterial activity.

Keywords: Antibacterial activity, anisotropic silver nanocrystals, crystallographic planes, half maximal inhibitory concentration, minimum bacterial concentration.

NOWADAYS, nanomaterials have become important in the fast-developing field of nanomedicine. Therefore, knowledge of the properties of nanoparticles and their effect on different microbes is essential for different clinical application purposes. Due to their unique physico-chemical properties, silver nanoparticles (AgNPs) have recently gained considerable attention among researchers¹⁻³. AgNPs show high surface reactivity with greater versatility for technological applications, especially in the field of nano-biotechnology. Nonspherical nanoparticles such as hexagons, cubes, triangles, prisms and several types of

silver nanocrystals exhibit many important properties, which makes them useful in sensing and imaging, biomedical labelling, photonics and plasmonics among other applications, including medical purposes⁴⁻⁸.

Antibiotic resistance is a major problem nowadays in medical science and hence, has received considerable attention. Nanotechnology offers several nanomaterials which can be used as nanomedicine; these nanomaterials show better results compared to their respective bulk counterpart. In healthcare, AgNPs are being widely used in nanomedicine, with an annual production of more than 500 tonnes worldwide⁹⁻¹³.

Many researchers have reported that silver nanocrystals show better antimicrobial activity against multidrug resistant pathogenic microorganisms. Hence, these have been used in different medical devices^{2-3,14}, and also in different consumer products such as filters, food containers and textiles¹⁵⁻¹⁷. It is also reported that these nanocrystals show little cytotoxicity¹⁸⁻²⁰, but mechanisms of this toxicity need further studies.

Recently, AgNPs have been tested against bacteria of Gram-positive as well as Gram-negative type by various researchers and found to be non-toxic at low concentration levels. AgNPs also show inhibition of bacterial growth at very low concentration compared to that of antibiotics, with no report of side effects as of now²¹⁻²⁴.

Interestingly, like size effect on many unique properties of silver nanocrystals, its antimicrobial activity has also been reported to be dependent on size²⁵⁻²⁸. Nevertheless, research on shape-dependent antibacterial activity is on-going.

With the aim to study of shape effect on antibacterial activity, we prepared silver nanohexagons and silver nanocubes of almost the same size, which were tested for their antibacterial activity against seven different types of bacteria. The study showed that antibacterial activity of silver nanocrystals was shape-dependent. Many researchers have reported that anisotropic shapes play a major role in biocidal activity. Pal *et al.*²⁹ reported that silver nanocrystals of truncated triangular shape showed much better antibacterial efficiency than spherical and

*For correspondence. (e-mail: dasratanphy@gmail.com)

rod-shaped ones. Similarly, Dong *et al.*³⁰ reported that sharp edge and sharp vertex triangular silver nanoprisms showed better antiseptic performance. Such high antibacterial activity can be attributed to the plane with high-atom-density {111} facets, that may act as a high-reactivity site.

The main aim of this study was to examine the antibacterial effect of different shapes of silver nanocrystals of almost the same size against seven types of bacteria. We synthesized the cubic and hexagon-shaped silver nanocrystals by chemical reduction method and further characterized them using transmission electron microscopy (TEM) for morphological analysis, X-ray diffraction (XRD) for structural analysis, followed by UV/Vis absorption spectroscopy. The antibacterial properties of these anisotropic silver nanocrystals were studied against *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Vibrio parahaemolyticus* using disk diffusion method. The antibacterial activity of these anisotropic silver nanocrystals was determined by half maximal inhibitory concentration (IC₅₀) value and minimum bactericidal concentration (MBC) value for positive and negative strains.

Experiment

Synthesis of anisotropic silver nanocrystals

Anhydrous ethylene glycol (EG; 5 ml) was stirred at 160°C for almost 1 h, and 1.5 ml of 0.285 M silver nitrate solution (AgNO₃) and 0.5 ml 0.177 M polyvinylpyrrolidone (PVP) solution were simultaneously added to it, according to the synthesis method³¹. Prior to this, 80 µl of 3 mM solution of Na₂S was injected into the EG solution. During stirring, temperature was kept constant whereas rate of stirring was fixed at 500 rpm. Silver nanocubes were prepared using this technique. Further, changing the temperature and molar concentration, we can prepare the 50 nm silver nanohexagons^{32,33}.

Collection of microbial strains

Both types of bacteria (Gram-positive and Gram-negative) were collected from the Department of Microbiology, University of Calcutta, and maintained in Luria broth medium.

Methodology for antimicrobial studies

Freshly grown overnight seed culture of a few bacterial strains was used for the study of inhibitory effect of silver nanocubes and silver nanohexagons against *B. cereus*, *E. coli*, *S. typhi*, *S. epidermidis*, *V. parahaemolyticus*,

P. aeruginosa and *K. pneumoniae* using the agar well diffusion technique. All these bacteria were inoculated on nutrient agar broth. Here, mainly the IC₅₀ and MBC values have been determined and compared for all the bacteria.

Characterization

UV/Vis spectroscopy (Shimadzu-1800, Japan) was used for absorption spectral studies, in which deuterium–tungsten–halogen lamp was the light source. For the XRD study (Rigaku Miniflex X-ray diffractometer, Japan), Cu-K α wavelength used was 1.54056 Å. TEM (JEM-2100, Japan) model operated at 200 kV was used for morphological analysis in which accelerating voltage of the instrument was 200 kV. Finally, a FTIR spectrophotometer (Perkin Elmer, USA) was used for recording FTIR spectra in the range 800–4000 cm⁻¹.

Results and discussion

UV/Vis spectroscopic analysis

Absorption spectroscopy is based on the matter–radiation interaction, which determines the nature of the substances. Silver nanocrystals strongly absorb visible light due to surface plasmon resonance (SPR), which is defined as coherent excitation of free electrons present in the sample on interaction with light. Hence, silver nanocrystals show SPR peak in the visible range, which depends on their size and shape. Larger particles as well as anisotropic nanocrystals show redshift of the SPR peak compared to smaller and spherical particles. Here, absorption spectra of all the samples were studied using UV/Vis spectrophotometer (Figure 1).

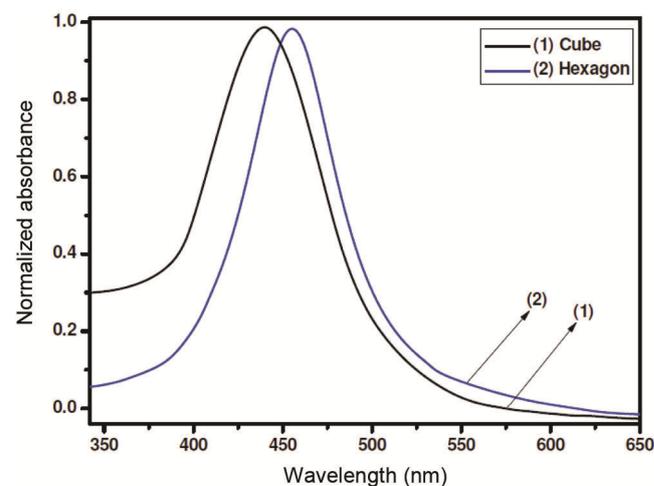


Figure 1. Absorption spectra of (1) silver nanocubes and (2) silver nanohexagons.

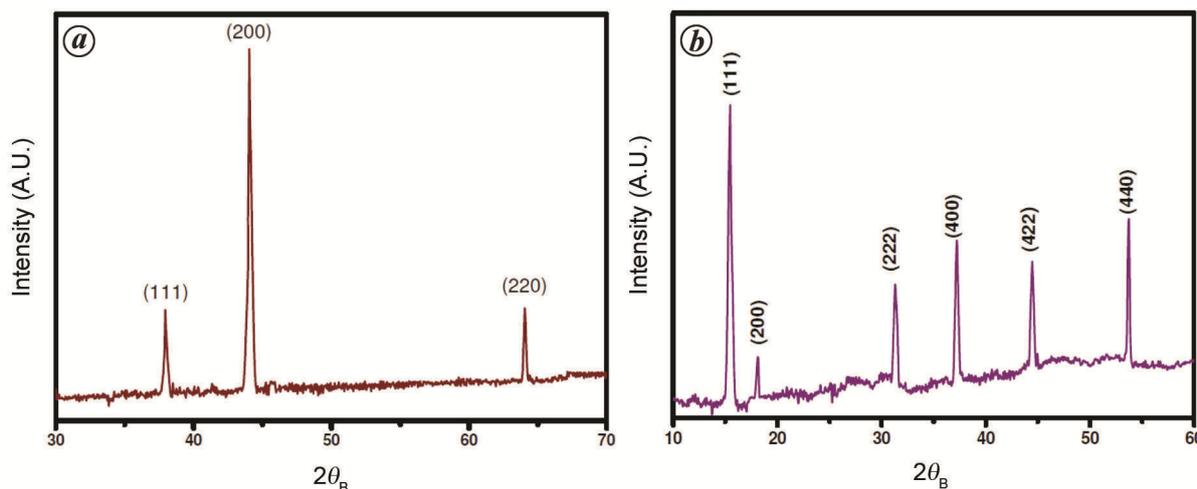


Figure 2. X-ray diffraction pattern of (a) silver nanocubes and (b) silver nanohexagons.

Figure 1 shows the SPR peak position at 438 and 454 nm wavelength for silver nanocube and silver nano-hexagon solutions. Therefore, shift of the SPR peak position with shape of the nanocrystals may result from changes in the lattice constant³⁴. As lattice constant in nanocrystals is different from that of its bulk, therefore, surface or interface stress is produced in the nanocrystals³⁵.

X-Ray diffraction analysis

The XRD pattern of silver nanocrystals confirmed the crystalline nature of the sample (Figure 2 a and b).

Three sharp peaks appeared at 37.95°, 44.02° and 63.95° which could be assigned to the (111), (200) and (220) planes for silver nanocubes. Similarly, for silver nano-hexagons six peaks appeared at 15.37°, 17.94°, 31.41°, 37.23°, 44.47° and 53.78°, which could be assigned to the (111), (200), (222), (400), (422) and (440) planes. All the peaks were found to correspond to the face centred cubic (fcc) symmetry of silver nanocrystals. Average size of the respective sample was calculated using the Debye–Scherrer formula: $D = k\lambda/\beta\cos\theta$, where $\lambda = 0.1541$ nm is wavelength of X-rays used (Cu-K α radiation), β the full width at half maxima and θ is the diffraction angle. From this formula, considering the (111) plane, the respective average size was found to be 51.4 and 52.3 nm, which matched well with that obtained from the TEM study.

Transmission electron microscopic analysis

TEM analysis provides a good way to view the size and shape of the nanocrystals. For this, a single drop of silver nanocrystal was taken on a carbon-coated copper grid. Figure 3 a and b shows TEM images of PVP-capped silver nanocubes and silver nano-hexagons with average size of nearly 50 nm.

Fourier transform infrared spectral analysis

FTIR analysis was carried out to confirm the capping of silver nanocrystals by PVP molecules. Figure 4 a–c shows the FTIR spectra of silver nanocubes, PVP and silver nano-hexagons.

Figure 4 b shows three vibrational bands of pure PVP at 1647, 1443 and at 1087 cm^{-1} corresponding to C=O stretching of PVP polymer, CNC stretching vibrations and C–N bond respectively.

In Figure 4 a, PVP-capped silver nanocubes show absorption peaks at 2954 and 1642 cm^{-1} due to the C–H and C=O stretching vibrations respectively. In addition, peaks at 1464, 1096 and 882 cm^{-1} correspond to $-\text{CH}_2$ absorption, C–N stretching and C–C vibration in ring of PVP respectively. These peaks were also observed in the FTIR spectra of PVP-capped silver nano-hexagons. On comparing the spectra, it was found that the peak at 1647 cm^{-1} for C=O stretching vibration in PVP, shifted to 1642 cm^{-1} for PVP-capped silver nanocrystals. Such decrease in wavenumber occurs because of weakening of bonds as well as bond formation with the surface Ag atoms of the nanocrystals. The C–N bond in the PVP peak at 1087 cm^{-1} also shifted to higher wavenumber at 1096 cm^{-1} due to the chemical coordination of the bond with Ag atoms. From these results, it has been confirmed that PVP caps the nanocrystals by forming a bond with the surface, and thereby stabilizes the nanoparticles.

Antibacterial activity of anisotropic silver nanoparticles

As there is continuous increase of resistance to multiple antibiotics by different bacteria, many researchers are trying to develop effective and resistance-free antimicrobial reagents. Silver nanocrystals have been found to be an effective resistant-free antimicrobial agent. Here,

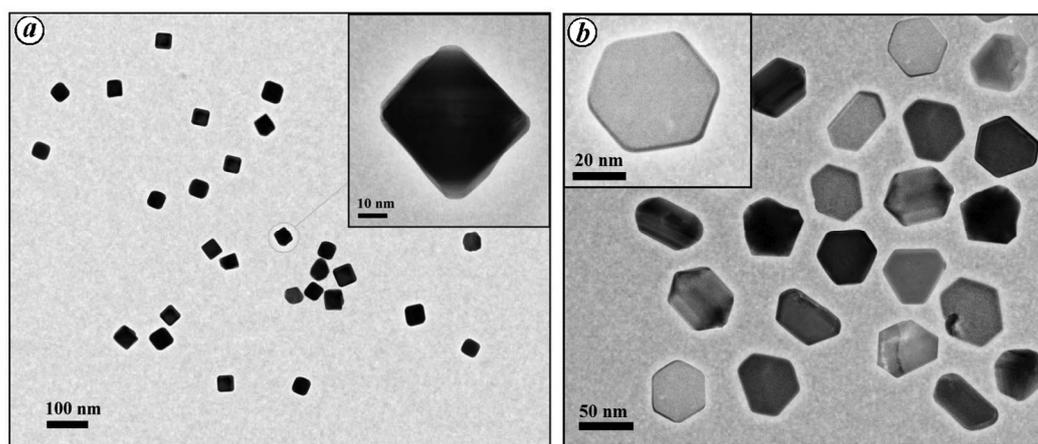


Figure 3. Transmission electron microscopy photomicrograph of (a) silver nanocubes and (b) silver nanohexagons with the respective HRTEM images given in the inset.

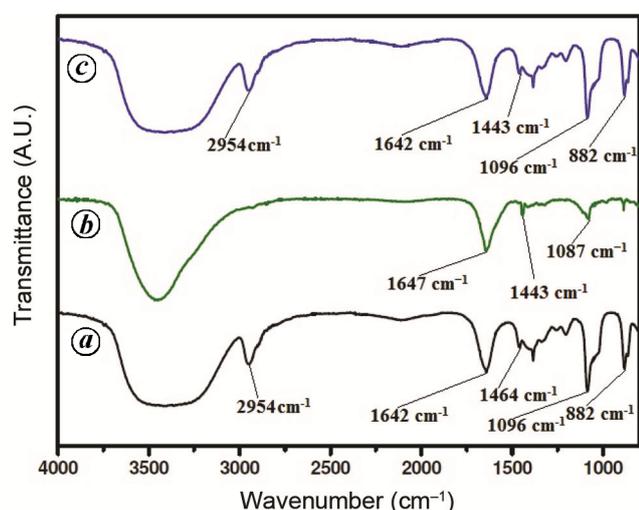


Figure 4. FTIR spectra of (a) polyvinylpyrrolidone, (b) Ag nanocubes and (c) Ag nanohexagons.

prepared anisotropic Ag nanoparticles of almost the same size have been tested against different Gram-positive and negative bacteria for the study of the effect of shape of the nanoparticles on the antibacterial activity.

Bacterial culture: The individual pathogenic bacteria were grown in a shaking incubator overnight at 37°C in Luria broth (LB) medium which contains 1% of both NaCl and tryptone, 0.5% yeast extract of pH 7.0, at shaking condition of 180 rpm as seed culture. From the seed culture, 1% of each inoculum was transferred to fresh medium and distributed in 100 µl volume in each well of a 96-well plate. Among the 96-well plate with freshly grown seed culture of different bacterial strains, solution of silver nanohexagons with the concentration of 0.234 µg/ml ($\sim 4.26 \times 10^9$ nos/ml), 0.936 µg/ml ($\sim 1.70 \times 10^{10}$ nos/ml), 3.75 µg/ml ($\sim 6.84 \times 10^{10}$ nos/ml), 14.4 µg/ml ($\sim 26.26 \times 10^{10}$ nos/ml) and 60 µg/ml ($\sim 109.44 \times$

1010 nos/ml) hexagon nanocrystals has been added to study its effect. Again, the effect of cubic nanocrystals was assessed at various concentrations, i.e. 0.31 µg/ml ($\sim 3.92 \times 10^9$ nos/ml), 1.25 µg/ml ($\sim 1.65 \times 10^{10}$ nos/ml), 5 µg/ml ($\sim 6.73 \times 10^{10}$ nos/ml), 20 µg/ml ($\sim 26.13 \times 10^{10}$ nos/ml) and 80 µg/ml ($\sim 109.41 \times 10^{10}$ nos/ml). The control set, which contains only saline and free LB, was also assessed. Each measurement was repeated thrice. The plate was then incubated in 37°C at 180 rpm shaking condition overnight. After overnight incubation, the density of each bacterial cell was recorded at 600 nm wavelength by the 96-well plate reader (EON Microplate Spectrophotometer, BioTek Instruments, Inc.). The obtained data were used to calculate mean and standard deviation, and they were plotted against the respective similar concentration of hexagon and cubic nanocrystals. The inhibitory effect of the nanocrystals was observed from the growth curve obtained, along with the calculation of IC_{50} and MBC values. MBC represents the minimum bacteriocidal concentration of any antimicrobial compound (here, hexagonal and cubic silver nanocrystals) which kills all the bacteria, whereas IC_{50} represents the concentration that can kill 50% of the bacteria. Absorbance of bacterial cultures treated with different concentrations of the nanoparticles was measured and plotted against concentration (µg/ml) of the nanocrystals. In these plots for each bacterial strain that concentration of nanoparticles is considered as IC_{50} value for which the cell density decreases to 50% of its value at control as it signifies the fact of killing of 50% of the bacterial population at that concentration.

Antibacterial activity of anisotropic silver nanocrystals on a few bacterial pathogens: The antibacterial activity of the prepared silver nanocubes and silver nanohexagons was studied against various bacteria. The antibacterial activity takes place because silver nanocrystals attack the cell membrane and penetrate into the bacteria, thereby

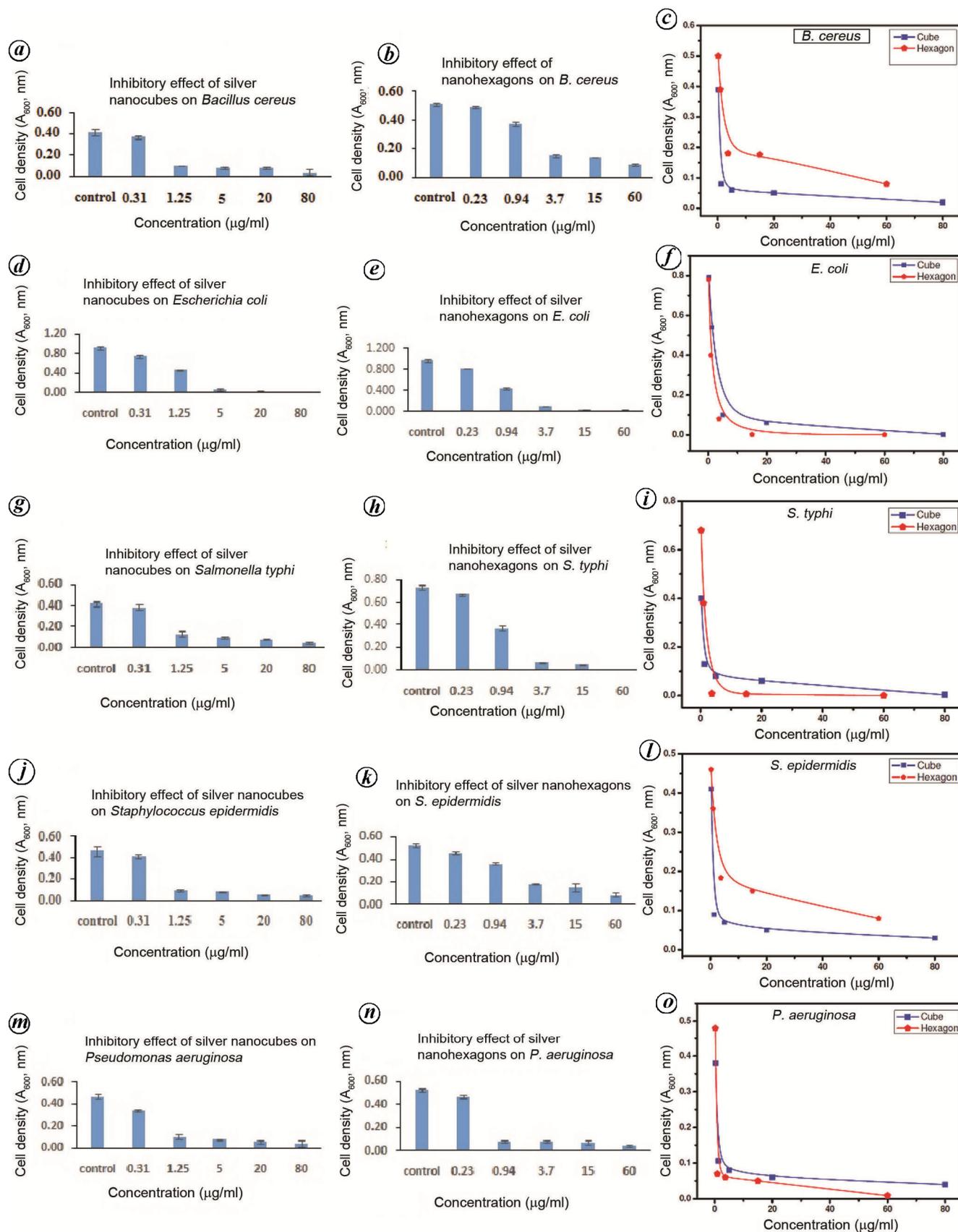


Figure 5. (Contd)

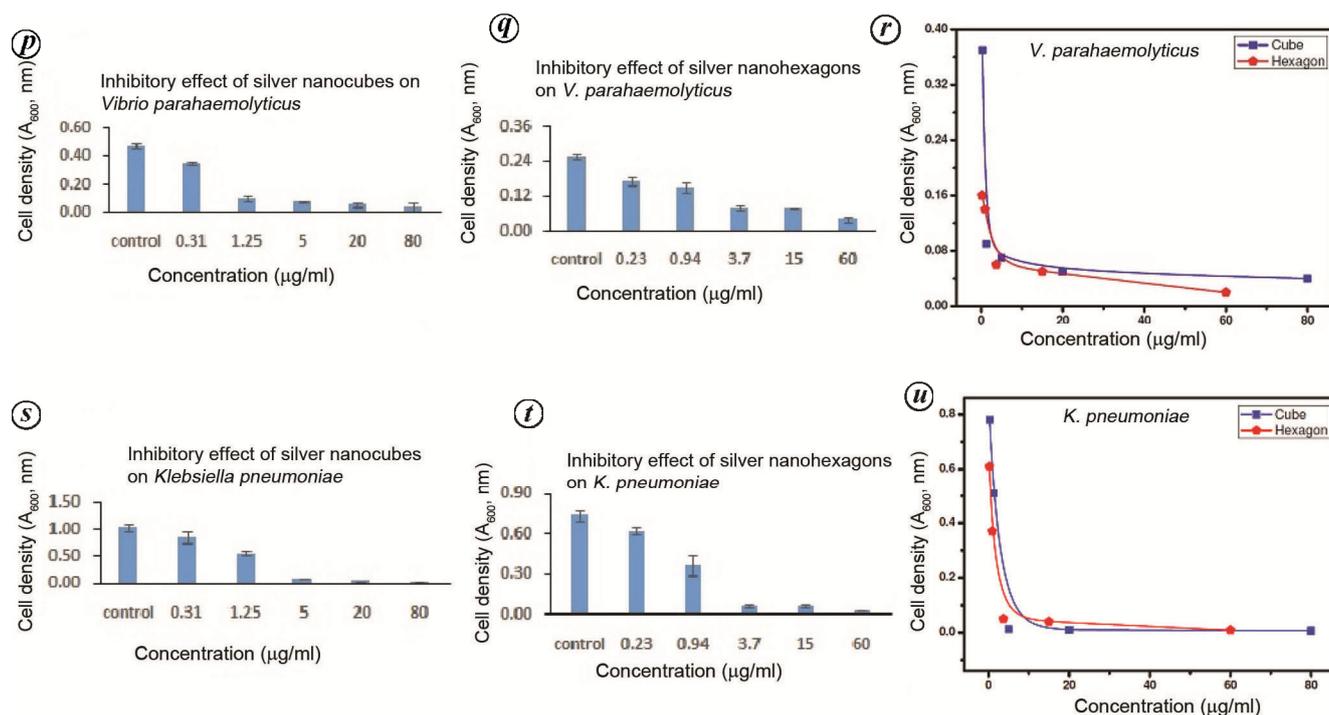


Figure 5. Cell density of different bacterial pathogens against the concentration of silver nanocubes (*a*, *d*, *g*, *j*, *m*, *p* and *s*) and silver nano-hexagons (*b*, *e*, *h*, *k*, *n*, *q* and *t*) along with cell density versus concentration graph (*c*, *f*, *i*, *l*, *o*, *r* and *u*) for the respective bacteria.

interacting with different proteins and DNA in the cell membrane; whereas inside the bacteria, nanoparticles specifically attack the respiratory system and hinder cell division, which ultimately leads to cell death^{36,37}. According to the literature^{38,39}, these prepared silver nanocrystals show different inhibitory effects in different bacteria, because of their different composition of capsular and cell wall, as well as S-layer thickness or their combination. Figure 5 shows that for almost the same concentration of nanoparticles for silver nanocubes and nano-hexagons, different shapes show different inhibitory effects in the same bacteria, which also has been confirmed from the plot of cell density of different bacterial pathogens against the concentration of silver nanocrystals.

The IC_{50} and MBC values were found to be different for different shapes of the silver nanocrystals (Table 1). As the size is almost the same, this study clearly shows the shape effect of silver nanocrystals on antibacterial activity. This shape effect can be explained on the basis of different crystallographic orientations in different shapes. Many researchers have reported another antibacterial activity of nanocrystals, which is related with crystallographic orientation^{40–42}. Chemical reactivity of anisotropic nanocrystals is different because of their different surface atom arrangement and surface energy. From the XRD study of silver nanocubes and silver nano-hexagons, crystallographic orientation can be inferred. For silver nanocubes, XRD pattern showed three intense peaks corresponding to (111), (200) and (220) diffraction planes (Figure 2*a*), whereas for silver nano-hexagons, it

showed eight intense peaks corresponding to (111), (200), (222), (400), (331), (422), (333) and (440) diffraction planes (Figure 2*b*). So, presence of extra planes in the nano-hexagons may be the reason for their different antibacterial activity compared to silver nanocubes, as confirmed from their IC_{50} and MBC values (Table 1). This shows that the atomic arrangement of different crystallographic facets plays an important role. Silver has a fcc crystal structure. For the cubes (Figure 2*a*), XRD pattern gives a major peak from the (200) plane, thereby indicating that silver nanocubes are primarily composed of {100} planes (Figure 2*b*), whereas for hexagons, intense peak is obtained from the (111) diffraction, showing that they are primarily composed of {111} planes (Figure 2*b*). Based on the analysis of chemical activities of different surfaces, it can be predicted that the silver nano-hexagons with {111} facet are more active than the silver nanocubes with {100} plane as the {111} plane is more energetic than the {100} plane. Further, it is clear from the graphs (*c* and *l*) in Figure 5 that the difference in the activity of silver nanocubes and silver nano-hexagons is large against *B. cereus* and *S. epidermidis*, which are Gram-positive type. But that difference is small for Gram-negative bacteria like *P. aeruginosa*, *E. coli*, *S. typhi*, *K. pneumoniae* and *V. parahaemolyticus*. This difference in the activity is clearly an effect of shape of the nanoparticles, and this shape effect again depends on the type of bacteria. As silver nanocubes and silver nano-hexagons have different crystallographic orientations, this may play an important role in antibacterial activity. It

Table 1. Results of half maximal inhibitory concentration (IC₅₀) and minimum bactericidal concentration (MBC) for both silver nanocrystals

| Pathogens | Silver nano-hexagons (µg/ml) | | Silver nano-cubes (µg/ml) | |
|-----------------------------------|------------------------------|------|---------------------------|------|
| | IC ₅₀ | MBC | IC ₅₀ | MBC |
| <i>Bacillus cereus</i> | 16.58 | 60.0 | 30.96 | 80.0 |
| <i>Escherichia coli</i> | 10.21 | 3.70 | 13.69 | 5.00 |
| <i>Salmonella typhi</i> | 09.09 | 3.70 | 16.32 | 1.25 |
| <i>Klebsiella pneumonia</i> | 10.81 | 15.0 | 40.30 | 1.25 |
| <i>Pseudomonas aeruginosa</i> | 10.40 | 0.94 | 17.23 | 1.25 |
| <i>Vibrio parahaemolyticus</i> | 13.25 | 3.70 | 15.16 | 1.25 |
| <i>Staphylococcus epidermidis</i> | 16.17 | 60.0 | 14.41 | 1.25 |

is reported that antibacterial activity of a particular type of nanocrystal is different for Gram-positive and Gram-negative bacteria, because the cell structure and outer membrane are different for these bacteria^{43,44}. The outer layer of Gram-positive bacteria is made up of lipopolysaccharides⁴⁵, whereas for Gram-negative bacteria there is a thick peptidoglycan outer layer⁴⁶. The large difference in the activity of silver nanocubes and silver nano-hexagons against Gram-positive bacteria in this study, attributed to different crystallographic planes present in the silver nanocubes and silver nano-hexagons, which show different activities against lipopolysaccharides and hence, depend on the shape of the nanocrystals. Again, these crystallographic orientations are not effective against the thick peptidoglycan layer of Gram-negative bacteria. So, the result confirms that different crystallographic orientation in nanocrystals of different shapes is responsible for the antibacterial activity, especially against Gram-positive bacteria.

Conclusion

In this study, silver nano-hexagons and silver nanocubes have been prepared chemically, with PVP as a capping agent. TEM analysis confirmed that the prepared nanocrystals were cubic and hexagonal in shape having similar size (50 nm), whereas UV/Vis spectroscopy showed the respective SPR peaks at 438 and 454 nm. Antibacterial activity of these anisotropic silver nanocrystals was studied on a number of bacteria, which confirmed a shape effect, i.e. silver nanocrystals of different shapes have different antibacterial activities, because of different crystallographic orientations, as confirmed from XRD analysis. The difference in antibacterial activity of silver nanocrystals may be attributed to different crystallographic planes.

Conflict of interest: The authors declare that they have no conflict of interest.

1. Taton, T., Mirkin, C. and Letsinger, R., Scanometric DNA array detection with nanoparticle probes. *Science*, 2000, **289**, 1757–1760.

- Schultz, S., Smith, D., Mock, J. and Schultz, D., Single-target molecule detection with nonbleaching multicolor optical immunolabels. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 996–1001.
- Yguerabide, J. and Yguerabide, E., Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications. *Anal. Biochem.*, 1998, **262**, 157–176.
- Sadeghi, B., Garmaroudi, F. S., Hashemi, M., Nezhad, H. R., Nasrollahi, A., Ardalan, S. and Ardalan, S., Comparison of the antibacterial activity on the nanosilver shapes: nanoparticles, nanorods and nanoplates. *Adv. Powder Technol.*, 2012, **23**, 22–26.
- Pal, S., Tak, Y. K. and Song, J. M., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol.*, 2007, **73**, 1712–1720.
- Das, R., Saha, M., Hussain, S. A. and Nath, S. S., Silver nanoparticles and their antimicrobial activity on a few bacteria. *BioNanoScience*, 2013, **3**, 67–72.
- Priyadarshini, S., Gopinath, V., Meera Priyadarshini, N., Mubarak Ali, D. and Velusamy, P., Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. *Colloids Surf. B*, 2013, **102**, 232–237.
- Singh, P., Kim, Y. J., Singh, H., Mathiyalagan, R., Wang, C. and Chun Yang, D., Biosynthesis of anisotropic silver nanoparticles by *Bhargavaea indica* and their synergistic effect with antibiotics against pathogenic microorganisms. *J. Nanomater.*, 2015, **2015**, 10; doi:10.1155/2015/234741.
- Hsueh, Y. H., Lin, K. S., Ke, W. J., Hsieh, C. T., Chiang, C. L., Tzou, D. Y. and Liu, S. T., The antimicrobial properties of silver nanoparticles in *Bacillus subtilis* are mediated by released Ag⁺ ions. *PLoS ONE*, 2015; doi:10.1371/journal.pone.0144306.
- Gottschalk, F., Sonderer, T., Scholz, R. W. and Nowack, B., Modeled environmental concentrations of engineered nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for different regions. *Environ. Sci. Technol.*, 2009, **43**, 9216–9222; doi: 10.1021/es9015553.
- Mueller, N. C. and Nowack, B., Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.*, 2008, **42**, 4447–4453.
- Shipelin, V. A., Gmshinski, I. V. and Khotimchenko, S. A., Risk assessment of silver nanoparticles. *IOP Conf. Ser.: Mater. Sci. Eng.*, 2015, **98**, 012010; doi:10.1088/1757-899X/98/1/012010.
- Fabrega, J., Luoma, S. N., Tyler, C. R., Galloway, T. S. and Lead, J. R., Silver nanoparticles: behaviour and effects in the aquatic environment. *Environ. Int.*, 2011, **37**, 517–531.
- Yang, Y., Matsubara, S., Xiong, L., Hayakawa, T. and Nogami, M., Solvothermal synthesis of multiple shapes of silver nanoparticles and their SERS properties. *J. Phys. Chem. C*, 2007, **111**, 9095–9104.
- Agnihotri, S., Mukherji, S. and Mukherji, S., Immobilized silver nanoparticles enhance contact killing and show highest efficacy:

- elucidation of the mechanism of bactericidal action of silver. *Nanoscale*, 2013, **5**, 7328–7340; doi:10.1039/C3NR00024A.
16. Bhardwaj, A. K. *et al.*, Power and time dependent microwave assisted fabrication of silver nanoparticles decorated cotton (SNDC) fibers for bacterial decontamination. *Front. Microbiol.*, 2017, **8**, 330; doi:10.3389/fmicb.2017.00330.
 17. Deshmukha, S. P., Patila, S. M., Mullani, S. B. and Delekar, S. D., Silver nanoparticles as an effective disinfectant: a review. *Mater. Sci. Eng. C*, 2019, **97**, 954–965.
 18. Ning, C. *et al.*, Concentration ranges of antibacterial cations for showing the highest antibacterial efficacy but the least cytotoxicity against mammalian cells: implications for a new antibacterial mechanism. *Chem. Res. Toxicol.*, 2015, **28**, 1815–1822.
 19. Kim, Y. J., Yang, S. I. and Ryu, J. C., Cytotoxicity and genotoxicity of nano-silver in mammalian cell lines. *Mol. Cellular Toxicol.*, 2010, **6**, 119–125.
 20. Gupta Mukherjee, S., Claonadh, N., Casey, A. and Chambers, G., Comparative *in vitro* cytotoxicity study of silver nanoparticle on two mammalian cell lines. *Toxicol. in vitro*, 2012, **26**, 238–251.
 21. Chopra, I., The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? *J. Antimicrob. Chemother.*, 2007, **59**, 587–590.
 22. Gemmell, C. G., Edwards, D. I., Frainse, A. P., Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the UK. *J. Antimicrob. Chemother.*, 2006, **57**, 589–608.
 23. Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R. and Minaian, S., Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomed.: Nanotechnol., Biol. Med.*, 2007, **3**, 168–171.
 24. Rai, M., Yadav, A. and Gade, A., Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.*, 2009, **27**, 76–83.
 25. Agnihotri, S., Mukherji, S. and Mukherji, S., Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *RSC Adv.*, 2014, **4**, 3974–3983.
 26. Raza, M. A., Kanwal, Z., Rauf, A., Sabri, A. N., Riaz, S. and Naseem, S., Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. *Nanomaterials*, 2016, **6**, 74; doi:10.3390/nano6040074.
 27. Rajiv, P., Rajeshwari, S. and Venckatesh, R., Bio-fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens. *Spectrochim. Acta Part A*, 2013, **112**, 384–387.
 28. Helmlinger, J., Sengstock, C., Groß-Heitfeld, C., Mayer, C., Schildhauer, T. A., Köller, M. and Epple, M., Silver nanoparticles with different size and shape: equal cytotoxicity, but different antibacterial effects. *RSC Adv.*, 2016, **6**, 18490–18501.
 29. Pal, S., Kyung Tak, Y. and Myong Song, J., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? a study of the Gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol.*, 2007, **73**, 1712–1720.
 30. Dong, P. V., Ha, C. H., Binh, L. T. and Kasbohm, J., Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. *Int. Nano Lett.*, 2012, **2**, 1–9.
 31. Kodiyath, R., Malak, S. T. and Combs, Z. A., Assemblies of silver nanocubes for highly sensitive SERS chemical vapor detection. *Mater. Chem. A*, 2013, **1**, 2677–2928.
 32. Das, R. and Sarkar, S., X-ray diffraction analysis of synthesized silver nanohexagon for the study of their mechanical properties. *Mater. Chem. Phys.*, 2015, **167**, 97–102.
 33. Sarkar, S. and Das, R., Presence of chlorpyrifos shows blue shift of the absorption peak of silver nanohexagons solution – an indication of etching of nanocrystals and sensing of chlorpyrifos. *Sensor. Actuat. B*, 2018, **266**, 149–159.
 34. Sarkar, S. and Das, R., Shape effect on the optical properties of anisotropic silver nanocrystals. *J. Lumines.*, 2018, **198**, 464–470.
 35. Liu, X., Huang, R. and Zhu, J., Functional faceted silver nanohexapods: synthesis, structure characterizations, and optical properties. *Chem. Mater.*, 2008, **20**, 192.
 36. Zhan, H., Zhou, X., Cao, Y., Jagtiani, T., Changa, T. L. and Liang, J. F., Anti-cancer activity of camptothecin nanocrystals decorated by silver nanoparticles. *J. Mater. Chem. B*, 2017, **5**, 2692–2701.
 37. Ghosh, T., Das, A. B., Jena, B. and Pradhan, C., Antimicrobial effect of silver zinc oxide (Ag–ZnO) nanocomposite particles. *Front. Life Sci.*, 2015, **8**, 47–54.
 38. Ibrahim, H. M. M., Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J. Radiat. Res. Appl. Sci.*, 2015, **8**, 265–275.
 39. Tran, Q. H., Nguyen, V. Q. and Le, A. T., Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, 2013, **4**, 033001.
 40. Ren, J., Wang, W., Sun, S., Zhang, L., Wang, L. and Chang, J., Crystallography facet-dependent antibacterial activity: the case of Cu₂O. *Ind. Eng. Chem. Res.*, 2011, **50**, 10366–10369.
 41. Yu, J. *et al.*, Synthesis, characterization, antimicrobial activity and mechanism of a novel hydroxyapatite whisker/nano zinc oxide biomaterial. *Biomed. Mater.*, 2015, **10**, 015001.
 42. Durán, N., Marcato, P. D., De Conti, R., Alves, O. L., Costa, F. T. M. and Brocchi, M., Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. *J. Braz. Chem. Soc.*, 2010, **21**, 949–959.
 43. Erridge, C., Bennett-Guerrero, E. and Poxton, I. R., Structure and function of lipopolysaccharides. *Microbes Infect.*, 2002, **4**, 837–851.
 44. Fang, G. *et al.*, Differential Pd-nanocrystal facets demonstrate distinct antibacterial activity against Gram-positive and Gram-negative bacteria. *Nature Commun.*, 2018, **9**, Article number 129; doi:10.1038/s41467-017-02502-3.
 45. Clifton, L. A. *et al.*, Effect of divalent cation removal on the structure of Gram-negative bacterial outer membrane models. *Langmuir*, 2015, **31**, 404–412.
 46. Willey, J. M., Sherwood, L. M. and Woolverton, C. J., *Microbiology*, McGraw-Hill, 2008, 7th edn; ISBN 978-0-07-299291-5.
- ACKNOWLEDGEMENTS. We thank Sophisticated Analytical Instrumental Facility, North Eastern Hill University, Shillong, for providing the TEM images and the Department of Chemistry, Tripura University, Suryamaninagar for FTIR analysis.

Received 30 January 2019; revised accepted 10 December 2019

doi: 10.18520/cs/v118/i12/1903-1910