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Optical, Structural and morphological properties of Silver nanoparticles and their antimicrobial activity

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Abstract : Silver nanoparticles (Ag NPs) were synthesized by reducing silver nitrate with N,N-Dimethylformamide (DMF) under ultrasonic field where Poly-N-vinyl-2-pyrrolidone (PVP) was used as a stabilizing agent. As the volume of PVP increases with respect to silver nitrate, the size of the nanoparticles decreases. The nanoparticles were characterized by UV-vis, XRD, HRTEM, EDS and Particle size measurements. The appearance of surface plasmon band around 412 nm indicates the formation of Ag NPs. The nature of the prepared Ag NPs in the face centered cubic (fcc) structure are confirmed by the peaks in the XRD pattern corresponding to (111), (200), (220) and (311) planes. Ag NPs of different shapes (rods, triangular, pentagonal, hexagonal and spherical shape) were obtained and confirmed by HRTEM. The EDS measurement confirms the presence of silver nanoparticles. Antimicrobial activities of Ag NPs were performed for five different microbes. When Ag NPs were tested in Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, they effectively inhibited bacterial growth and highly sensitive against the Ag nanoparticles (1:10) than (1:2).The prepared Ag NPs have great promise as effective antimicrobial agents. Applications of Ag NPs based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial system.

Keywords: Silver nanoparticles, antimicrobial activity, ultrasonic field.

Introduction

Novel metal nanoparticles provide a more interesting research field due to their close lying conduction and valence bands in which electrons move freely. The free electron gives rise to a surface plasmon absorption band which depends on both the particle size and chemical surrounding[1]. Thus the color of the colloids varies depending on the method of preparation and the state of aggregation[2]. Among the metals, Ag NPs show tremendous applications in various fields such as environment, biomedicine, catalysis, optics and electronics[3]. The surface plasmon resonance and large effective scattering of individual Ag NPs makes them ideal candidates for molecular labeling, where phenomenon such as surface enhanced Raman scattering can be exploited[4].

Synthesis of highly dispersed nanoparticles is challenging and therefore many efforts have been made to prepare stable metal nanoparticles using various techniques[5] such as chemical reduction[6], electrochemical[7], γ-radiation[8], laser ablation[9], photochemical[10], sonochemical[11] and sputtering[12]. Since most of the organic reactions take place in organic solvents, it is desirable to develop synthetic methods that lead to the formation of different morphology in addition to the stabilization of metal nanoparticles in such solvents. In addition, if one can design synthetic method to prepare different morphological particles in the same medium without adding reductants from outside, this may have its own important implications[13]. It has been reported that the synthesis of Ag NPs with the variety of shapes ranges from spheres, triangular prisms, hexagons, pentagons and wires using DMF reduction method in the presence of PVP[14].

Among Me-NPs, Ag NPs have been known to have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro organisms. It can be expected that the high specific surface area and high fraction of surface atoms of Ag NPs, which allows them to interact closely with microbial membranes, will lead to high antimicrobial activity as compared with bulk silver metal[15].

In this present study, Ag NPs were synthesized by chemical reduction method using N, N-Dimethylformamide and Poly (N-vinyl-2-pyrrolidone) under ultrasonic field. And here we report that the prepared Ag NPs have great promise as effective antimicrobial agents. Applications of Ag NPs based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial system

Experimental

Materials

Silver nitrate ($AgNO_3$), Poly(N-vinyl-2-pyrrolidone) (PVP) and N,N-dimethylformamide (DMF) were analytical grade reagents and used as received from MERCK.

Preparation of Ag NP's

In a typical experiment, 30ml of 1% PVP and 10ml of 0.1M of $AgNO_3$ were separately dissolved in DMF. Initially the 2ml of PVP solution was kept in the ultrasonic field for 15 minutes at 60°C. Then 1ml of $AgNO_3$ was added rapidly and this solution was kept in the ultrasonic field for 1 hr at 60°C. The resultant solution was golden yellow in color. This procedure was repeated for various volume of PVP (3, 5, 7 and 10ml) with constant volume of $AgNO_3$ (1ml).

Characterization

Shimadzu UV-1700 UV-Visible spectrophotometer was used to carry out the optical measurements. The X-ray diffraction patterns were recorded on a X-ray diffractometer using Cu-Kα radiation (λ=0.1542nm) operated at 50kV and 100mA. The experiments were performed in the diffraction angle range of 2θ=20-80°. The size, composition and atomic structure of the NP's were analyzed by High Resolution Transmission Electron Microscopy (HR-TEM), and Energy Dispersive Spectroscopy (EDS) using a 200 KeV JEOL 2010F microscope with a NORAN Vantage DI+EDS system. The spherical (Cs) and chromatic aberration (Cc) coefficients of the objective lens were 0.5 mm and 1.1 mm respectively. The point to point resolution was 0.194 nm at Scherzer focus (-42 nm). The samples were made by depositing the Ag NP's on a carbon coated Cu grid, and size measurements were performed manually on HRTEM images.

Assay for antimicrobial activity of Ag NPs against microorganisms

The antimicrobial activity of Ag NPs was evaluated against *Staphylococcus aureus* (MTCC No. 96), *Bacillus subtilis* (MTCC No. 441), *Streptococcus mutans* (MTCC No. 497), *Escherichia coli* (MTCC No. 739) and *Pseudomonas aeruginosa* (MTCC No. 1934) by their agar disc diffusion method. Mueller Hinton agar plates were spread with 100 μL of actively grown broth cultures of the respective test bacteria and are allowed to dry for 10 minutes. The sterile readymade discs loaded with each Ag NPs individually (15 μL/ disc, 20 μL/ disc, 25 μL/ disc) were imposed on the inoculated plate. The plates were incubated for 48 hours at 37°C. The development of the inhibition zone around the extract loaded discs was recorded.

Results and Discussion

Optical studies

UV-visible spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in solution. Figure 1 shows the optical absorption spectra of Ag NP's in the region 275-575 nm. The absorption spectra show one prominent symmetric peak around 410 nm, which is due to the characteristic surface plasmon resonance of spherical Ag NPs[16]. As the volume of PVP increases, absorption peak is shifted to shorter wavelength (blue shift), indicates the Ag particle size is decreased. In addition to the surface plasmon resonance at 412 nm, a weak band was observed at 307 nm for the volume ratio of $AgNO_3$: PVP is 1:2. This weak band is due to the formation of various types of silver ions such as Ag^{2+} and Ag^{3+} due to clustering[17]. This weak band is disappeared as the volume of PVP increases. This is due to the fact that nitrogen hetero atom present in the polymer may coordinate with silver ions because of the vacancy in the d-orbital of the silver. The lone pair electron available in the nitrogen atom of PVP would tend to make a temporary chemical bond, thus reducing the number of silver ions and therefore broadened absorption patterns at about 307 nm due to suppressed surface plasmon resonance are observed. PVP is a polymer capable of complexing and stabilizing Ag NP's formed through the reduction of silver ions with DMF. PVP indicates the aggregation of Ag NP's to give a clear, stable dispersion of Ag NP's[5]. Such presence of silver ions leads to the better quality of Ag NP's.

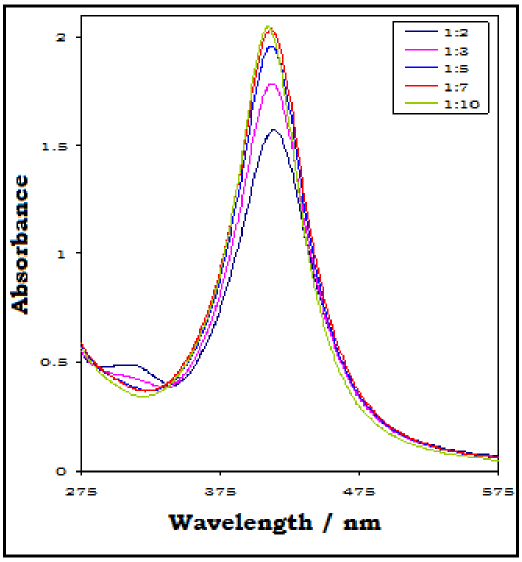

Figure 1. Optical absorption spectra of silver nanoparticles (1:2 represents 1ml of $AgNO_3$ in 2 ml of PVP)

As the concentration of PVP increased, keeping the concentration of $AgNO_3$ same, the much defined surface plasmon resonance band blue shifts, which indicate the particle size decreasing as the concentration of PVP increases. The peak width at half maxima (PWHM) is reported to be quite useful in understanding the particle size and their distribution within the medium. By adopting the concept[18,19], in the present work, the PWHM of the colloidal silver is 66 nm. It is understood that a PWHM of 66 nm is generally indicative of a narrow size distribution. Therefore, the present approach can be considered a suitable methodology for obtaining nano particle of silver with a narrow size distribution.

It is well known that the color of metal particles is caused by the sum of the effects of an absorption and scattering of visible light. Gustav Mie was the first to provide an explanation on the dependence of color on the metal particle size[20]. Surface plasmon resonance can be thought of as the collective oscillation of the conduction band electrons in the metals. This is due to the small size of the particle and surface property and is not exhibited by individual atoms or bulk materials. The radius of the Ag NPs has been calculated using Mie theory[20] and it is around 24 nm. This absorption band results from interactions of free electrons confined to small metallic spherical objects with incident electromagnetic radiation. Electronic modes in Ag NPs are particularly sensitive to their shape and size, leading to pronounced effects in the visible part of the spectrum. The observed plasmon band around 410 nm shows that the Ag NPs are spherical in shape[16]. According to Mie's theory small spherical nanoparticles should exhibit a single surface plasmon band, whereas anisotropic particles should exhibit two or three bands depending on their shape. The observed band at 410 nm is due to the out-of-plane dipole plasmon resonance[21].

Structural studies

XRD is a popular technique for determining phase purity of the materials. Small angle scattering is useful for evaluating the average interparticle distance while wide-angle diffraction is useful for refining the atomic structure of nanoclusters[22]. The width of the diffraction lines are closely related to the size and size distribution of, as well as defects and strain in nanoparticles.

Figure 2 shows the X-Ray diffraction pattern of the Ag NPs for $AgNO_3$: PVP volume ratio is 1:10. In the XRD pattern (Fig. 2), four diffraction peaks were observed at 2θ=37.85°, 44.0°, 64.2° and 77.2° which correspond to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg's reflections of the face centered cubic (FCC) structure of metallic silver respectively. All diffraction peaks are good agreement with the standard value (JCPDS card No. 04-0783).

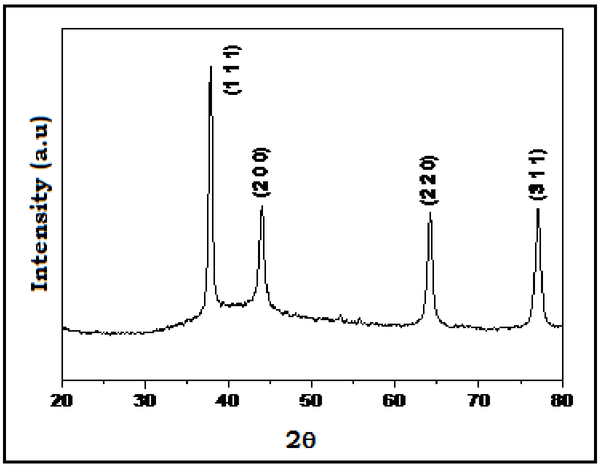

Figure 2. X-Ray diffraction pattern of the silver NPs for the volume ratio of $AgNO_3$: PVP is 1:10

The peak line width in the XRD spectra is broadened due to smaller particle sizes. The calculated average particle size is found in the range of 15-22 nm. The surface area was calculated from XRD pattern using the formula $S_{calc} = 6x10^4/td$ and it was about $30m^2/g$[17].

TEM is a high-spatial-resolution structural and chemical characterization tool and providing exact information about particle size and shape[24]. Figure 4 shows HRTEM image of Ag NPs for $AgNO_3$: PVP volume ratio is 1:10. The observation indicates the formation of Ag anisotropic nanoparticles. It can be seen, Ag NPs as rods, triangular, pentagonal, hexagonal and spherical shapes of different sizes. These anisotropic Ag NPs can act as suitable substrates for surface enhanced Raman scattering (SERS) spectroscopy using near IR laser source

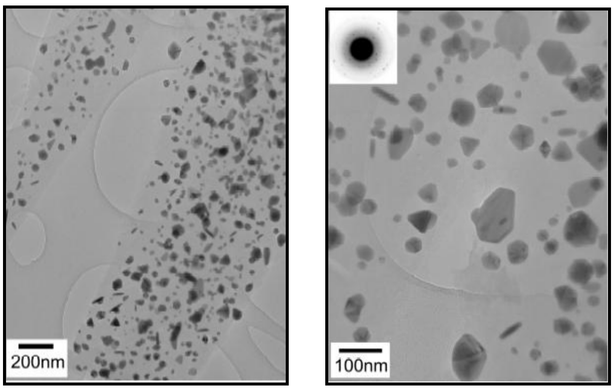

Figure 4. HRTEM images of the Ag NPs for the volume ratio of $AgNO_3$: PVP is 1:10 in different magnifications (inset: selected area electron diffraction pattern of AP NPs).

Figure 5 shows the energy dispersive spectra (EDS) of the prepared Ag NPs for $AgNO_3$: PVP volume ratio is 1:10. It confirms the presence of Ag NPs. The signal of Cu originated from the carbon coated Cu grid.

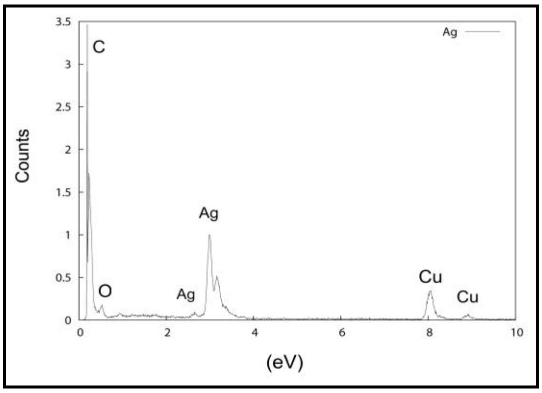

Figure 5. EDS Spectra of the Ag NPs for the volume ratio of $AgNO_3$: PVP is 1:10

Antimicrobial activity

In this study, to evaluate the antimicrobial effects against various microorganisms (gram positive and gram negative), Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa were used. There were distinct differences among them. The diameter of inhibition zones (in mm) produced by Ag NPs against these test strains are shown in Table 1. This Table 1 provides the evidence that Ag NPs synthesized in the ratio 1:10($AgNO_3$: PVP) have higher antibacterial activity than those synthesized in the ratio 1:2($AgNO_3$: PVP). This phenomenon is related to the size of colloidal Ag NPs which is lower for 1:10 than 1:2.

Table 1. Antimicrobial efficacy results of Ag NPs for $AgNO_3$: PVP volume ratio as 1:2 and 1:10

Organisms	Zone diameter (mm)					
	$AgNO_3$:PVP 1:2			$AgNO_3$:PVP 1:10		
	15µL/disc	20µL/disc	25µL/disc	15µL/disc	20µL/disc	25µL/disc
S. aureus	7	7	9	7	7	9
B. subtilis	9	11	13	17	17	19
S. mutans	7	7	7	7	7	9
E. coli	9	11	13	13	15	15
P. aeruginosa	7	9	11	9	11	11

Figure 6 shows the photographic image of an inhibition zone produced by Ag NPs prepared for the volume ratio of $AgNO_3$: PVP is 1:10 against a) *B. subtilis* and b) *E. col* (others not shown in fig). The protective mechanism of PVP in the electrochemical synthesis of Ag NPs is generally proposed on the basis of its structural features. PVP has a major role in steric stabilization upon Ag NPs[25]. As the concentration of PVP increase, the size of Ag NPs decreases. Ag NPs may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles are having larger surface area available for interaction will give more bactericidal effect than the larger particles[26].

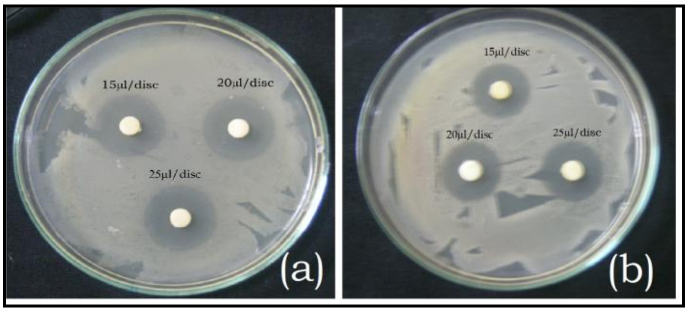

Figure 6. Photographic image of an inhibition zone produced by silver NPs prepared for the volume ratio of $AgNO_3$: PVP is 1:10 against a) *B. subtilis* and b) *E. coli.* (Photographs of other microbes are not shown)

When Ag NPs were tested in Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa they effectively inhibited bacterial growth. They are highly sensitive against the Ag nanoparticles (1:10) than (1:2). The inhibitory effect of Ag nanoparticles was very mild in Streptococcus mutans and no effect in Staphylococcus aureus as compared with other micro organisms. These results suggest that the antimicrobial effects of Ag NPs may be associated with characteristics of certain bacterial species. Gram positive and gram negative bacteria have differences in their membrane structure, the most distinctive of which is the thickness of the peptidoglycan layer. The lower efficacy of the Ag NPs against Staphylococcus aureus and Streptococcus mutans may derive from the difference as a point of membrane structure. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. To confirm this hypothesis, further comparative study between various gram-negative and gram-positive bacterial species is needed. If the antimicrobial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent[27].

In Proteomic and biochemical studies, nanomolar concentrations of Ag NPs have killed *E.Coli* cells with in minutes possibly due to immediate dissipation of the proton motive force[28]. Ag NPs thus seem to be more efficient than Ag^+ ions in performing antimicrobial activities. The effect of shape on the antibacterial activity of Ag NPs has only recently been reported. The Ag NPs of different shapes (triangular, spherical and rod) were tested against E. Coli[30]. The irreversible inhibition of bacterial growth is desirable to prevent bacterial colonization of silver- containing medical devices such as catheters, where bacteria- killing activity is required[26].

Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. The result of this study clearly demonstrated that the colloidal Ag NPs inhibited the growth and multiplication of the tested bacteria, including highly multi resistant bacteria such as Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. Such high anti bacterial activity was observed at very low total concentration of silver.

Conclusion

Silver NPs were synthesized by reducing silver nitrate with DMF under ultrasonic field where PVP is used as a stabilizing agent. As the volume of PVP increases with respect to silver nitrate, the size of the nanoparticles decreases. The nanoparticles were characterized by UV-vis, XRD, HRTEM, EDS and Particle size measurements. The nature of the silver nanoparticles is evident from HRTEM images and peaks in XRD pattern. The EDS measurement shows the formation of silver nanoparticles. When Ag NPs were tested in Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, they effectively inhibited bacterial growth. They are highly sensitive against the Ag nanoparticles (1:10) than (1:2). Applications of Ag NPs based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial system.

References

1. Kapoor, S., Preparation, Characterization, and Surface Modification of Silver Particles, Langmuir, 1998, 14, 1021- 1025.
2. De, S., Pal, A., Jana, N.R. and Pal, T. , Anion effect in linear silver nanoparticle aggregation as evidenced by efficient fluorescence quenching and SERS enhancement, J. Photochem. Photobiol. A , 2000, 131, 111-123.
3. Vo- Dinh, T., Yan, F. and Wabuyile, M.B., Surface-enhanced Raman scattering for medical diagnostics and biological imaging , J. Raman Spectrosc, 2005, 36, 640-647.
4. Wei, G., Zhou, H., Liu, Z. and Li, Z., A simple method for the preparation of ultrahigh sensitivity surface enhanced Raman scattering (SERS) active substrate Appl. Surface. Sci, 2005, 240, 260 – 267.
5. Sarkar, A., Kapoor, S. and Mukarjie, T., Synthesis of silver nanoprisms in formamide, J. Colloid and Interface Science, 2005, 287, 496 -500.
6. Pal, A., Shah, S. and Devi, S., Preparation of Silver, Gold and Silver-Gold Bimetallic Nanoparticles in w/o Microemulsion Containing TritonX-100, Colloids Surf. A: Physicochem. Eng. Aspects, 2007, 302, 483-487.
7. Reetz, M.T. and Helbig, W., Size-Selective Synthesis of Nanostructured Transition Metal Clusters, J.Am.Chem.Soc, 1994, 116, 7401 – 7402.

8. Choi, S.H., Zhang, Y.P., Gopalan, A., Lee, K.P. and Kang, H.D., Preparation of catalytically efficient precious metallic colloids by γ-irradiation and characterization Colloids Surf. A: Physicochem. Eng. Aspects, 2005, 256, 165- 170.
9. Willwohl, H., Wolfrum, H., Zumbach, V., Albers, P. and Seibold, K., Production and Characterization of Highly Dispersed Catalytic Active Platinum and Palladium Powders by Excimer Laser Photolysis, J.Phys.Chem.A, 1994, 98, 2244 -2247.
10. Li, Z., Li, Y., Qian, X.Y., Yin, J. and Chu, Z.K., A simple method for selective immobilization of silver nanoparticles, Appl. Surface Sci., 2005, 250, 109 -116.
11. Hayakawa, K. and Iwama, S., Preparation of ultrafine γ-Fe particles by microwave plasma processing, J. Cryst. Growth, 1990, 99, 188-191.
12. Murugadoss, A. and Chattopadhyay, A., A 'green' chitosan–silver nanoparticle composite as a heterogeneous as well as micro-heterogeneous catalyst, Nanotechnology, 2008, 19, 015603.
13. Wang, H., Qiao, X., Chen, J., Wang, X. and Ding, S., Mechanisms of PVP in the preparation of silver nanoparticles, Mater. Chem. Phys, 2005, 94, 449 -453.
14. Phumlane, Mdluli S., and Revaprasadu, N., An improved N,N-dimethylformamide and polyvinyl pyrrolidone approach for the synthesis of long silver nanowires, J. Alloys and Compounds., 2009, 469 519-528.
15. 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, Nanomedicine 2007, 3, 168-171.
16. Mock, J.J., Barbic, M., Smith, D.R., Schultz, D.A. and Schultz, S., Shape effects in plasmon resonance of individual colloidal silver nanoparticles, J. Chem. Phys. 2002, 116, 6755-6759.
17. Khanna, P.K., Singh, N., Kulkarni, D., Deshmukh, S., Charan, S., and. Adhyapak, P.V., Water Based Simple Syn- thesis of Re-Dispersible Silver Nano-Particles, Mater. Lett., 2007, 61, 3366 – 3370.
18. Brown, K.R., Walter, D.G., and Natan, M., Seeding of colloidal Au nanoparticle solutions 2. Improved control of particle size & shape, J., Chem. Mater. 2000, 12, 306-313.
19. Link, S. and El-Sayed, M.A., Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals, Int. Rev. Phy. Chem., 2000, 19, 409- 456.
20. Bohren, C.F., and Huffman, D.F., Absorption and scattering of light by small particles, 1983, Wiley, New York,.
21. Jin, R.C., Cao, Y.W., Chad, A.M., Kelly, K.L., George, C.S. and Zheng, J.G., Photoinduced Conversion of Silver Nanospheres to Nanoprisms, Science, 2001, 294, 1901 -1903.
22. Alivisatos, A.P., Semiconductor clusters, Naocrystals and quantum dots, Science, 1996, 27, 933-937.
23. Wang, Z.L., Transmission Electron Microscopy of Shape-Controlled Nanocrystals and Their Assemblies, J. Phys. Chem. B, 2000, 104, 1153- 1175.
24. Yogamalar, R., Srinivasan, R., Vinu, A., Ariga, K. and Bose, A.C., X-ray peak broadening analysis in ZnO nanoparticles, Solid State Communications 2009, 149, 1919-1923
25. Petica, A., Gavriliu, S., Lungu, M., Buruntea, N. and Panzaru, C., Colloidal silver solutions with antimicrobial properties, Mat. Sci. eng. B, 2008, 152, 22-27.
26. Ales, P., Silver Colloid Nanoparticles: Synthesis, Characterization, and Their Antibacterial Activity, J.Phys.Chem. B, 2006, 110, 16248-16253.
27. Jun Sung K., Antimicrobial effects of silver nanoparticles, Nanomedicine 2007, 3, 95-101.
28. Lok, C.N., Ho, C.M., Che R., He, Q.Y., Yu, W.Y. and Sun, H., Proteomic analysis of the mode of antibacterial action of silver nanoparticles, J.Proteome Res 2006, 5, 916 – 924.
29. Dibrov, P., Dzioba, J., Gosinl, K.K. and Hase, C.C., Chemiosmotic Mechanism of Antimicrobial Activity of Ag+ in Vibrio cholera, Antimicrob Agents Chemother, 2002, 46, 2668- 2670.
30. 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.

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