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Structural, Morphological and Antimicrobial Study of ZnO/Ag Nanoparticles

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Metal oxide nanoparticles gain attention in the field of biomedical applications because of their unique physico-chemical properties and emerging out as an alternative to antibiotics. The major cause of most of the human diseases is the bacterial infection. However, antibiotics used in the cure show other complications to human health. Therefore, the purpose of the present work is to investigate the antibacterial properties of ZnO/Ag nanoparticles on the test bacterial strains, *Escherichia coli* (*E. coli*). ZnO/Ag nanoparticles are synthesized using surfactant mediated route in a single step and double step procedure. Here, CTAB and hydrazine hydrate used as a surfactant and reducing agents respectively. The synthesized nanoparticles are characterized by *x*-ray diffraction, scanning electron microscopy and energy dispersive *x*-ray spectroscopy for structure, morphology and compositional properties. The antibacterial activities of these nanoparticles are also studied using the agar-well diffusion technique. The result analysis shows that synthesized nanopaticles are spherical in shape, having particles of the size 6 nm and 13 nm in the desired elemental composition. ZnO/Ag nanoparticles possessed a strong antibacterial effect against *E. coli*. This study signifies that ZnO/Ag metal oxide nanoparticles exhibit stronger antimicrobial activity against pathogen bacteria *E. coli* which may works effectively on the antibacterial and antifungal infections.

Keywords: ZnO/Ag nanoparticles, surfactant mediated route, Structural properties, antibacterial activity.

With the advancement in nanotechnology, the metal oxide nanoparticles have gained research interest in the field of biomedical applications because of their distinctive physico-chemical features, large surface to volume ratio and strong intra material interactions[1, 2]. Among different type of metal oxides, ZnO is well a known material because of its effective photocatalytic response, chemical stability, high oxidation capacity, non-toxic nature and low-cost behaviour[3, 4], which makes it an important material for biological as well as for other applications including photocatalysis, gas sensing, electronics, cosmetics, etc.,[5-9]. At present different ZnO nanostructures are widely investigated because of their high excitation energy (60 meV), high optical band gap

(3.3 eV) at room temperature and stable hexagonal (wurtzite) structure [10, 11]. The doping of metal ions in the pure ZnO matrix increases the surface area by reducing particle size, enhancing the light absorption and fluorescence property by changing the concentration of defects[12]. Several metals like Sb, Ag, Cu, Fe, etc., have been utilized to doped in ZnO structure in order to improve their structural, optical, electrical and catalytic properties[13-16]. It has been observed that doping of silver (Ag) and gold (Au) metals in ZnO are responsible to enhances photocatalytic activity by changing the charge separation and reducing the recombination of electron-hole pair[17, 18]. Nowadays, different bacterial infections become the main reason of the large number of diseases. Antibiotics have been frequently preferred for their treatment. However, different studies show that the extensive use of antibiotics cause many complications to public health[19]. So, as an alternative to antibiotics, metal oxide nanoparticles are establishing a promising approach to solve this problem. These nanoparticles efficiently work for the rapid neutralization of the surface electric charge of the bacterial membrane and change its penetrability which leads to bacterial death[20, 21]. Recently, Kim et. al have reported the antimicrobial effects of silver nanoparticles while Manyasree et. al have reported antibacterial activity of ZnO nanoparticles of size 35 nm against gram-negative and gram-positive organisms[20, 21]. However, the antibacterial effect of Ag doped ZnO on gram-positive bacteria are seldomly explored till date.

Hence, in the present work, we are trying Ag metal as a dopant in ZnO material using single step and double step processes. The high solubility, minimum orbital energy and large ionic radius[18] of Ag metal may help to improve the functionality of ZnO material. The antibacterial activity of ZnO/Ag nanoparticles is investigated against gram-negative pathogenic bacteria, *Escherichia coli* (*E. coli*).

MATERIALS AND METHODS

Chemicals

To pursue the work, analytical grade chemicals, purchased from S.D. Fine Chem. Limited and Hi Media Laboratories Pvt. Ltd. India, were used without any further purification. Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and silver nitrate ($AgNO_3$) were used as zinc and silver ions sources respectively whereas CTAB used as a surfactant and hydrazine hydrate as a reducing agent. All the solutions for experimentation were prepared using the deionized water.

Synthesis of ZnO/Ag nanoparticles

ZnO/Ag nanoparticles were synthesized by the surfactant mediated route using single step and double step procedure.

Single step procedure for the synthesis of ZnO/Ag nanoparticles

ZnO/Ag nanoparticles were prepared by single step procedure as: initially 0.576 gm of $ZnSO_4.7H_2O$, 20 mg of $AgNO_3$ and 0.74 gm of CTAB were dissolved in 100 ml of deionized water individually to prepare 0.01 M solution of each reagent. All the solutions were then mixed, sonicated for 1 h and kept at continuous stirring for 1 h using a magnetic stirrer. Finally, ammonia solution (~78 ml) was added which maintain the solution *p*H at ~10. The whole solution was kept under continuous stirring and left to complete the precipitation process at room temperature. The residue of this chemical reaction was filtered and washed using deionized water three times. The precipitates in the powder form are collected and dried for 2 h at temperature 75 °C using a microwave oven. This powder is then re-dispersed in 20 ml deionized water containing 0.2 ml of 1 M hydrazine hydrate under vigorous stirring. During this reaction process, Ag nanoparticles were deposited on the surface of ZnO nanoparticles, which leads to the formation of ZnO/Ag precipitates (AZO-1). Finally, this powder was collected by filtration, washed with deionized water successively and dried well again in the microwave oven at 75 °C for 2 h.

Double step procedure for the synthesis of ZnO/Ag nanoparticles

In this procedure, ZnO nanoparticles were prepared in the step-1 and then Ag mixing was done in step-2 to obtain ZnO/Ag nanoparticles.

Step-1: Preparation of ZnO Nanoparticles

For the preparation of ZnO nanoparticles, initially, 4 ml of $ZnSO_4$ (1 M) and 4 ml of CTAB (1 M) were dissolved in 400 ml of deionized water so that the final concentration of both become 0.01 M. The whole solution is then sonicated for 30 minutes at temperature of 42 °C. Finally, 6 ml of ammonia solution is added to this solution to maintain *p*H =

10. The solution became cloudy slowly due to the formation of $Zn(OH)_2$ and then the precipitation occurs. The white precipitates were filtered and washed with deionized water consecutively. The obtained precipitates were dried at 75 °C for 2 h in a microwave oven.

Step-2: Preparation of ZnO/Ag Nanoparticles

The process of mixing Ag with ZnO nanoparticles to form ZnO/Ag nanoparticles consists of dissolution of 0.1 g of ZnO in 10 ml of deionized water. The sonication of the solution was done for ~30 min. Then, a solution of 0.17 g of $AgNO_3$ in 10 ml of water (0.01 M) was prepared. The two solutions were mixed together under continuous stirring to make precipitates, which were collected and washed. After that, the precipitates were re-dispersed in 20 ml of water and 0.2 ml of 1 M hydrazine hydrate and vigorously stirred. Ag nanoparticles were deposited on the surface of ZnO nanoparticles, which results in the formation of ZnO/Ag precipitates (AZO-2). Finally, this powder was collected, washed with deionized water repeatedly and dried well again in the microwave oven at 75 °C for 2 h.

Finally, both the synthesized samples (AZO-1 and AGO-2) were annealed in a muffle

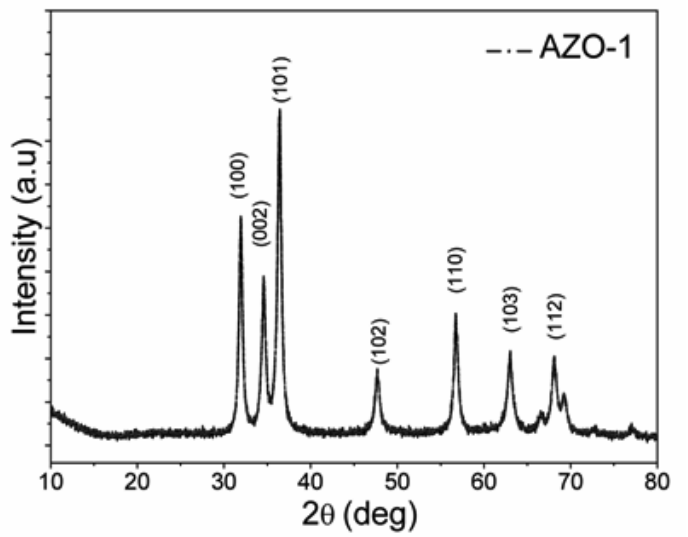

Fig. 1(a). XRD spectra for ZnO/Ag nanoparticles (AZO-1) synthesized by single step procedure

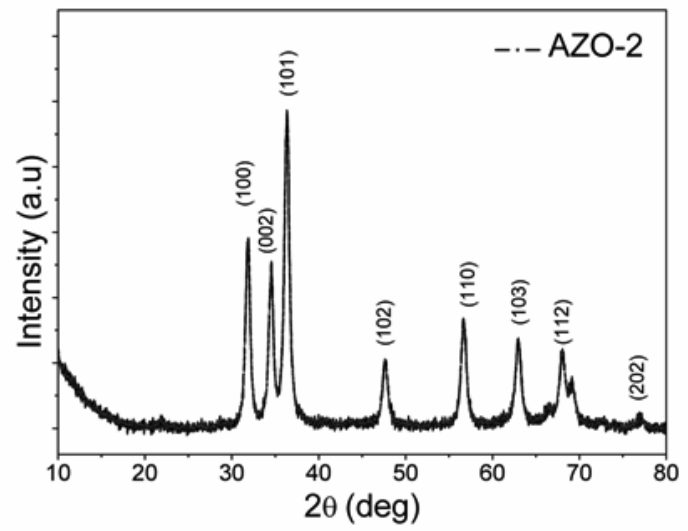

Fig. 1(b). XRD spectra for ZnO/Ag nanoparticles (AZO-2) synthesized by double step procedure

furnace at 500 °C for 1 hour before further characterizations.

Characterization techniques

The structural and phase identification of ZnO/Ag nanoparticles were analyzed from the XRD spectra obtained by Panalytical *x*-ray diffractometer in the 2*è* range of 10° to 80°. The surface morphology was observed by field effect scanning electron microscope (FE-SEM; Jeol JSM-6100). The elemental composition of nanoparticles identified using Bruker Quantax energy dispersive *x*-ray analyzer (EDX).

Antibacterial activity by agar well diffusion method

The antibacterial activity of the ZnO/Ag nanoparticles was done on *E. coli* bacterial test strains using agar-well diffusion technique. This method involves the inoculation of *E. coli* in agar plate with the synthesized ZnO/Ag nanoparticles. The plate was prepared with nutrient broth (medium used to grow various types of bacteria and contains many nutrients needed for the bacterial growth) and nutrient agar (also a medium used to grow various types of bacteria and contains many nutrients needed for the bacterial growth and is obtained from algae). The plates were then incubated for 24 h at 37 °C and the growth was observed.

RESULTS AND DISCUSSION

XRD analysis of ZnO/Ag nanoparticles

The crystallinity and phase of the synthesized nanoparticles AZO-1 and AZO-2 were

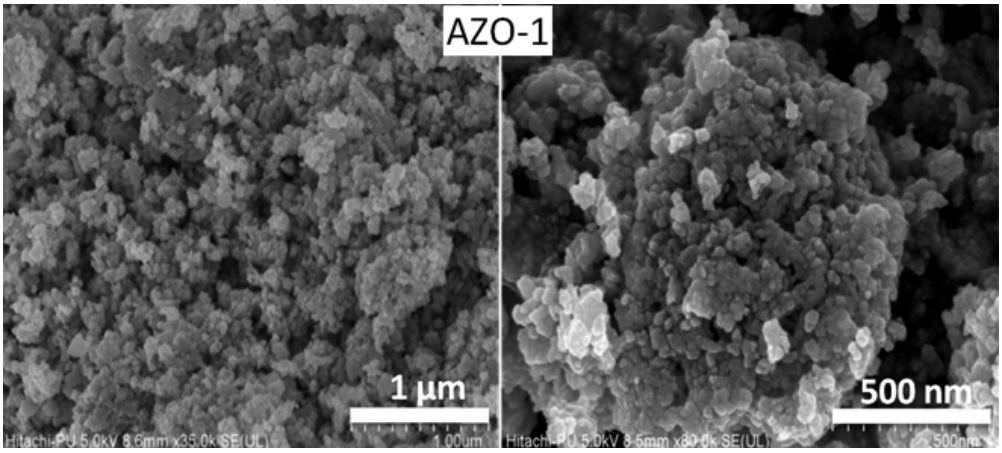

Fig. 2(a). FE-SEM images for ZnO/Ag nanoparticles (AZO-1) synthesized by single step procedure at the scale of 1 um and 500 nm

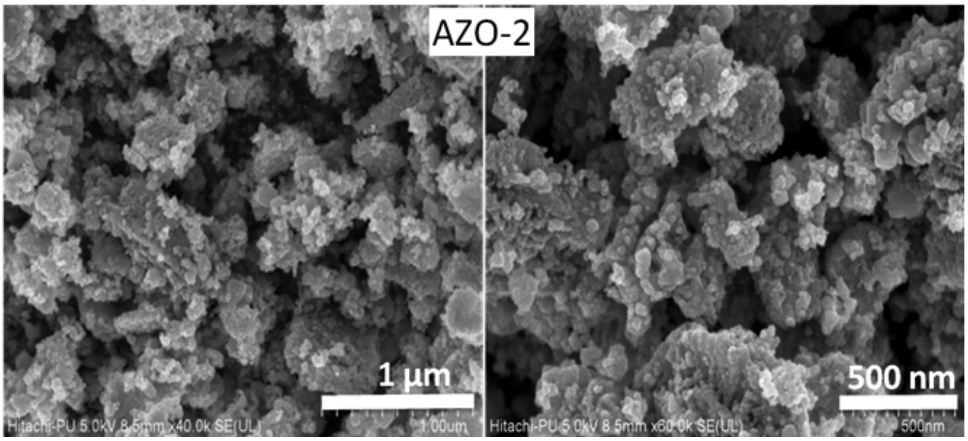

Fig. 2(b). FE-SEM images for ZnO/Ag nanoparticles (AZO-2) synthesized by double step procedure at the scale of 1 µm and 500 nm

analyzed from XRD spectra (Fig. 1a and Fig. 1b) respectively.

Fig. 1(a) and 1(b) clearly displayed well defined XRD peaks positioned at 2*è* values of 31.9°, 34.5°, 36.3°, 47.6°, 56.6°, 63.0°, 68.1°, 69.2° and 77.4° which belongs to (100), (002), (101), (102), (110), (103), (112), (201) and (202) crystal planes of wurtzite (hexagonal) phase of ZnO respectively. These XRD observations were matched with the standard *jcpds* card # 36-1451 and reported values for the ZnO synthesized by facile hydrothermal process[22]. A little variation in 2*è* values for AZO-1 and AZO-2 may be due to the difference in the synthesis procedure which results in particle size variation. The average particle size (*D*) of the ZnO/Ag nanoparticles has been determined using full-width at half maximum (*â*) of the XRD peaks by using Debye-Scherer equation[23]

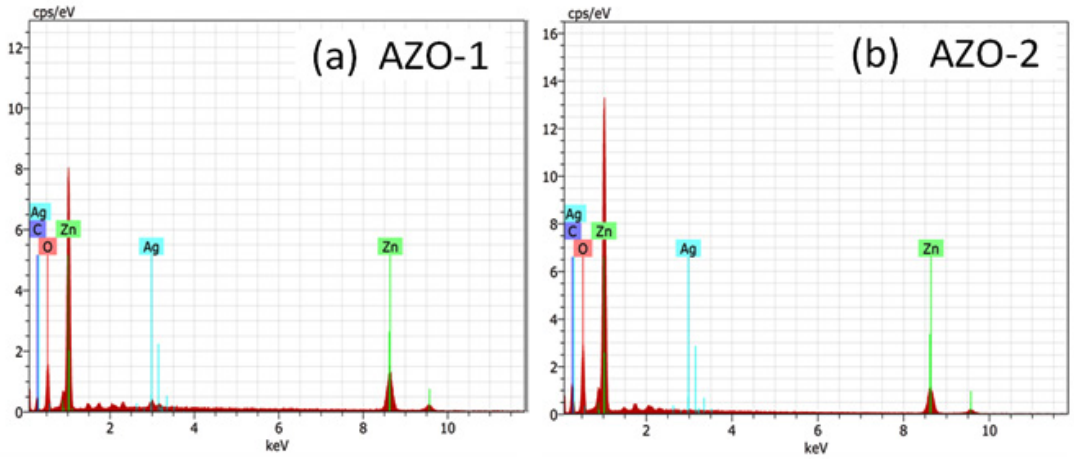

Fig. 3. EDX images for ZnO/Ag nanoparticles synthesized by (a) single step procedure (AZO-1) and (b) double step procedure (AZO-2)

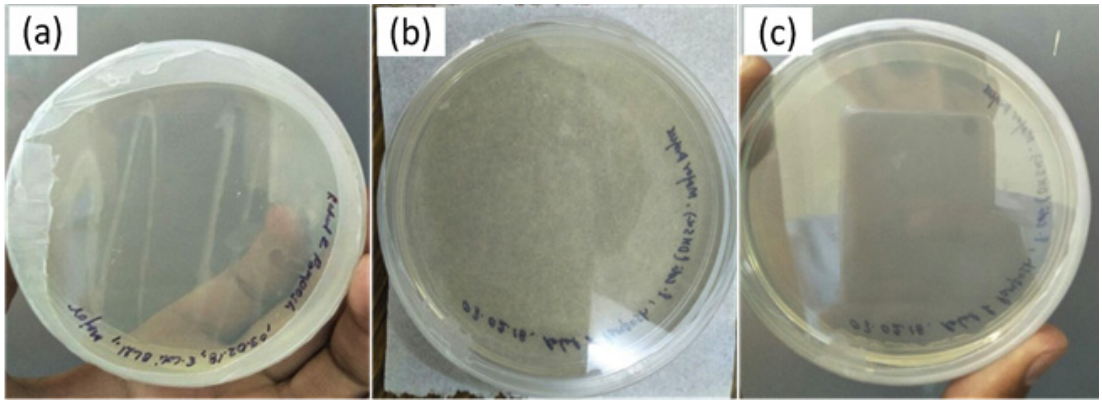

Fig. 4(a). Plate 1 **Fig. 4(b).** Plate 2 **Fig. 4(c).** Plate 3

Table 1. The Composition of the plates for the testing of antibacterial activity

Plates with AGO nanoparticles	Nutrient broth (gm)	Nutrient agar (gm)	Water (ml)	Dose of nanoparticles (ml)
Plate 1 (no AGO)	0.65	1.65	50	-
Plate 2 (AZO-1)	0.65	1.65	50	0.5
Plate 3 (AZO-2)	0.65	1.65	50	0.5

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where K (= 0.89) is the shape parameter, *ë* is the *x*-rays wavelength and *è* is the Bragg angle. The average size of particle is estimated to be 13.4 nm and 6.9 nm for AZO-1 and AZO-2 respectively. However no distinguish peak for Ag detected in the XRD spectra which may be probably due to the small quantity of Ag nanoparticles in ZnO structure.

FE-SEM analysis of ZnO/Ag nanoparticles

The surface morphology of the synthesized nanoparticles was carried out using FE-SEM and shown in Fig. 2(a) and 2(b). From surface morphology analysis, it has been observed that the size of particles is clearly of the order of few nanometers. The synthesized particles exhibit spherical symmetry, agglomerated in cluster form which are randomly scattered and have a uniform size distribution. The size of nanoparticles estimated by inception method for both the samples is found in the range of 40-50 nm.

EDX analysis of ZnO/Ag nanoparticles

EDX technique utilizes the interaction of the excitation source with the sample for the elemental analysis. Fig. 3(a) and 3(b) show the EDX spectra for Ag doped ZnO nanoparticles. It is clear from the spectra that different peaks belong to zinc (Zn), oxygen (O) and silver (Ag) are identified in both the samples.

Antibacterial activity of ZnO/Ag nanoparticles

To investigate the antibacterial activity of the synthesized ZnO/Ag nanoparticles against gram-negative bacteria (*E. coli*), three petri-dics/ plates with different compositions have been prepared (Table 1). Inoculation of *E. coli* has been done with 1.5 ml.

These prepared plates have been kept for the incubation at 37 ^{o}C for 24 h. The growth of *E. coli* strain has been observed in plate 1 as shown in fig. 4(a), whereas no growth of *E. coli* strain is obtained in plates 2 and 3 as shown in Fig. 4(b) and 4(c) respectively. It means that the ZnO/Ag samples have been prevented the growth of *E. coli*. Hence, it can be concluded that the synthesized nanoparticles show excellent antibacterial response against gram-negative bacteria *E. coli* in the present study.

A strong antimicrobial activity of ZnO/ Ag nanoparticles has been found against the *E. coli* bacterial strain. It may be due to the interaction between nanoparticles and CTAB surfactant which leads to nano-size particle formation. The small size of the ZnO/Ag nanoparticles and CTAB surfactant may promote the antibacterial activity. The small size nanoparticles can easily penetrate the small thickness of the bacterial cell wall. These ZnO/Ag nanoparticles discharge ions that interact with the protein cell membrane and able to disrupt their functions which finally lead to the death of microorganism[21, 24].

CONCLUSION

ZnO/Ag nanoparticles have been synthesized by the surfactant mediated route through the single step and double step procedure. The study of the structure, morphology and composition of the synthesized nanoparticles has been performed by XRD, FE-SEM and EDX. The average particle size is 13.4 nm and 6.9 nm for both ZnO/Ag nanoparticles synthesized via single step and double step procedure respectively. EDX analysis confirms the presence of Zn, O and Ag elements in the nanoparticles. The antibacterial activity of spherical shaped ZnO/Ag nanoparticles has been tested against oral pathogen *E. coli* and is found to have a good antimicrobial response. The results of the present work clearly indicate that ZnO/Ag nanoparticles synthesized by above described procedures can be utilized as an antimicrobial agent against the antibacterial and antifungal infections and also in other industrial and agricultural practices.

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Conflicts of Interests

Declared none

Funding Source

Nil

Authors Contributions

All authors have contributed equally in this work.

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