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Synthesis, characterization and antibacterial activity of simple ZnO and metal doped ZnO nanoparticles

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Abstract: The objective of this study was to synthesize pure ZnO and metal doped ZnO nano-particles, determine its physical, chemical characteristics and antibacterial activity against selected bacterial strains. Pure ZnO was synthesized and metals including Manganese (Mn), Magnesium (Mg), Calcium (Ca), Copper (Cu) and Silver (Ag) were doped with ZnO to produce nanoparticles through co-precipitation method. X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy were used for detection of synthesized nanoparticles, their crystalline structures, size and other chemical characteristics. An altered version of Kirby Bauer method of disk diffusion was used for determining the antibacterial activity of these nanoparticles. The XRD results showed that the average size of pure ZnO nanoparticles was 55.3 nm. While the size of metal doped ZnO particles were affected by dopant elements. Results of SEM indicated that these nanoparticles were roughly spherical, rod shape and fiber like rod shape with certain degree of aggregation. Antibacterial studies showed that all samples had the potential to inhibit the growth of selected bacterial strains; *E. coli*, *S. choleraesuis*, *S. typhimurium, S. marcescens*, *B. subtilus* and *S. aureus.* With 90µg/ml concentration ZnO nanostructures inhibited *B.subtilus* and silver doped Zinc nanoparticles suppressed growth of *S. marcescens.* Characterization and antibacterial study indicated the importance of these nanoparticles at industrial and pharmaceutical level.

Keywords: Metal doped ZnO nanoparticles, X-ray diffraction, scanning electron microscopy, fourier transform infrared, antibacterial activity.

INTRODUCTION

Nanotechnology is one of the emerging fields because of its uses in various sectors of medicine, technology and in the field of research including electronics, optics, biomedical and material sciences. Nanotechnology is the study of nanoparticles, the size of its atomic and molecular aggregates is less than 100nm (Griffin, Masood *et al*. 2018) (Kato, 2011; Daniel, 2004). The chemical and physical properties of metal NPs such as Zinc Oxide (ZnO), Silver Oxide (SiO_2) and Titanium Oxide (TiO_2) are characterized by their configuration, size, crystal lattice and morphology. ZnO nanoparticles are formed by the procedure of a wet chemical substance. Interestingly, the characterization of the samples can be done by UV-Vis spectroscopy as well along with Fourier transformer-infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and photoluminescence also. (Ravichandrika, Kiranmayi *et al*. 2012). Nanoparticles are reduced to nano-scale size to influence their mechanical, chemical, structural, electrical, morphological, and optical properties (Rasmussen *et al.,* 2010). Nanoparticles have larger structure with respect to their counterparts due to its small size. And this allows the possible applications of NPs in various biological fields such as biosensors, nanomedicine, and bio-nanotechnology (Ashe, 2011). Thus, nano-materials play an important role in the basic and practical sciences as well as in bio-nanotechnology (Zottel, Videtič Paska *et al*. 2019).

Zinc Oxide belongs to inorganic compounds with molecular formula ZnO. It exists in the form of white powders and water insoluble. The powder zinc oxides are used as additive in various products and materials such as rubber, plastic, glass, cement and paints etc (Borysiewicz 2019). Zinc oxide exhibits very attractive chemical properties including semi-conductivity, chemical sensing, piezoelectric properties and electric conductivity (Navale, Navale *et al*. 2017, Nour, Nur *et al*. 2017). At room temperature, it shows wideband gap (3.3 eV) and high excitonic binding energy (60 meV) (Mirzaei and Darroudi 2017, Rahimi, Yazdani *et al*. 2018).The optical absorption and electrical conductivity are affected by wide band gap of zinc oxide(Wisz, Virt *et al*. 2017),(Parihar, Raja *et al*. 2018). Structures of zinc oxide are found in 1D, 2D and 3D in the form of nano needles, rods, springs and helix in 1D; nano-sheets and nano-pellets in 2D; and 3D structures

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are in the form of flower, snowflakes, and dandelion (Choudhary and Gangopadhyay 2018).

Doping in nano Zinc Oxide has been proved to be very effective. By a classic solvothermal pathway, transition metal doped ZnONPs (TM-ZnONPS) are successfully produced with dopant material of 3%. In this work, Manganese, Iron, Cobalt, Nickel and Copper ions have been highlighted as dopant transfer metals. It was confirmed by XPS studies that transition metal particles have been doped in ZnO crystal lattice. (Qi, Xing *et al*. 2020). ZnO has weak optical properties which can be fixed through doping that in turn alters the properties of ZnONPs and improves its eligibility in different fields (Bharat, Mondal *et al*. 2019).

To cover different and unique properties and to make ZnO adaptable, different methodologies are used to synthesize zinc oxide nano-particles. These properties depend on the size and structure of nano-particles and all these result in the novel the application of these particles. To adopt a method for preparation of nano-particles its application, morphology and properties should be kept in considerations. Its parameters such as pH, temperature, type of solvent and other precursors should be also considered (Yahya *et al.,* 2010). Precipitation method is mostly used and important method by researchers for nanoparticles synthesis. Hydrothermal method is non-reproducible and the process is difficult to control. It involves placing the solutions in electric furnace involves the use of expensive machinery. the setup of this machinery takes too much of space and it also consumes electricity. While in case of solid state method, very little volume of nanoparticles is obtained using a lot of energy. The green synthesis method consumes a lot of time and produces very less amount of materials. It is also difficult to maintain the conditions of the culture broth. Also, other methods including physical methods like. Also, the method which involves chemical can be dangerous for the environment and the person who is supervising it due to toxic chemicals. So, the best choice is co-precipitation method in which we can control the size of nanoparticles by changing the parameters of reactants. The reactants are cost effective as well (Agarwal, Kumar *et al*. 2017, Jamkhande, Ghule *et al*. 2019). The objective of this research is to discover antimicrobial activities with pure ZnO and metal doped ZnONPs as this may open the door for the progress of therapies against diseases caused by pathogens.

MATERIALS AND METHODS

Synthesis of Simple ZnO nanostructures and metal doped ZnO nanostructures

Synthesis of Simple ZnO nanostructure

Pure ZnO nanostructures were prepared by dissolving zinc acetate dehydrate [Zn $(CH3COO)_2 \cdot 2H_2O$] in distilled water and then dropwise addition of aqueous sodium hydroxide (NaOH) to it. The solution was stirred for half an hour at room temperature and then at 80°C for 5 hours. The precipitates were washed by de-mineralized water and ethanol and then dried at 120°C for an hour and finally at 300°C for 2 hours to form Nano powder (Gandhi *et al.,* 2014).

Synthesis of Manganese doped ZnO nanostructures

Manganese acetate tetra hydrate solution (Mn $(CH_3COO)_2 \cdot 4H_2O$) was added slowly to zinc acetate dehydrate solution with constant stirring. Then sodium hydroxide (NaOH) was added dropwise to it to form precipitates. The pH was set at 8.5 with 2 hours of stirring at 85°C. Washing with ethanol and distilled water was done. Then it was calcinated at 500°C for 4 hours to obtain nanostructures (Dhanalakshmi *et al.,* 2016).

Synthesis of Magnesium doped ZnO nanostructures

Magnesium nitrate hexahydrate salt solution was used as a doping agent for magnesium and then added to Zn $(NO_3)^2.6H_2O$ solution with constant stirring at 60°C. After that, sodium hydroxide solution was added dropwise. This yielded white precipitates. The final solution was constantly stirred for 4 hours and then refluxed at 37°C for the next 24 hrs. The solution was then washed with distilled water and ethanol several times. The resultant precipitates were first dried at 120°C and then annealed at 500°C in a furnace for about 6 hours to form nanostructures (Ahamed *et al.,* 2016).

Synthesis of Calcium doped ZnO nanostructures

A solution of Calcium nitrate tetra hydrate was added slowly to the solution of zinc nitrate hexahydrate with constant stirring. Sodium hydroxide was then added dropwise to form precipitates. Final solution was stirred first at room temperature for half an hour and then at 60°C for 4 hours. It was refluxed for the next 24 hours afterwards at room temperature to form a clear solution. This clear solution was washed with distilled water and ethanol several times to remove impurities. The precipitates were first dried at 120°C and then annealed at 600°C for 4 hours (Hamed *et al.,* 2013).

Preparation of Cu-doped ZnO nano-particles

Aqueous solution of copper chloride was slowly added to aqueous solution of zinc chloride ($ZnCl_2$) with constant stirring. Aqueous Ammonia was then drop-wise added to it to produce white precipitates. The pH was set to 8 and solution was heated and stirred. After about 10 minutes of stirring, the solution was filtered and washed via ethanol and distilled water. The precipitates formed were dried in the oven and then annealed at 600°C for 3 hours under reflux to obtain nanostructures of Cefotaxime and copper doped zinc oxide (Singhal *et al*., 2012). The reflux in the temperature and other parameters caused the formation of Cu and Cefotaxime-doped ZnO nanowires instead of Cu-doped ZnO nanoparticles (Prescott and Schwartz, 2008).

Synthesis of Silver doped ZnO nanostructures

Silver nitrate salt solution was added slowly to zinc acetate solution to form a homogeneous mixture. Sodium carbonate solution was added dropwise to the homogeneous mixture with vigorous stirring that resulted in the formation of precipitates. The precipitates were washed with distilled water and ethanol multiple times and separated. After that, they were dried in a drying oven at 80°C and then annealed for 2 hours at about 300°C (Shojaei *et al.,* 2015).

Characterization of nanostructures

XRD

X-Ray Diffraction crystallography or XRD analyzed the crystallographic structure of molecules and atoms present in the samples. The results were compiled with the help of Bragg's equation. Diffraction peaks were compared to the normal planes to determine the type of crystal and shape. Microsoft Excel was used to prepare the graphs from the readings of XRD (Klug and Alexander, 1954; Zak *et al.,* 2011).

SEM

The samples were observed with scanning electron microscope and images were saved to visualize the synthesized nanostructures. They were further processed, and the particle size was found by ImageJ software version 1.51u (Abranoff *et al.,* 2004).

During the image processing, the radius square (r^2), radius (r), and mean diameter (d) of the nanostructures were calculated by the formulas given respectively:

Radius square (r^2):

r^2=Area

Radius (r):

r=Area

Mean diameter (d):

d=2(r)

FTIR

The spectroscopy of Fourier-transform infrared (FTIR) was used to collect data from the samples with high resolution spectrum. This is done by measuring the absorbance and transmittance of different wavelengths of light through the samples (Bell, 2012). 'Spectrum Two' FTIR was used to measure the values of the given samples.

Antibacterial Activity

E. coli K88, *E. coli* ATCC 25922, *S. choleraesuis* ATCC 50020, *S. typhimurium* ATCC 50013, and *S. aureus* ATCC 25923 were provided by PCSIR and stored at 4°C.

Sigma Aldrich Prepared Nutrient agar media, 1 liter double distilled and de-ionized water, aluminum foil and cotton rolls were purchased.

For antibacterial activity, an altered version of Kirby Bauer method of disk diffusion was used (Bauer *et al.,* 1996; Azam *et al.,* 2012). The bacterial strains were sub-cultured a day before the experiment into fresh Müller-Hinton broth media. Nutrient agar media was prepared in a flask and sterilized with the help of an autoclave. This media was then poured onto petri plates and left to solidify. After the solidification of media, it was incubated for 24 hours for a sterility check. After 24 hours, about 100 µL fresh bacterial cultures were swabbed onto the plates by using a sterile glass rod. Number of colonies of each bacterial strain on Petri plates was 106 colony-forming units (CFU)/mL. Plates were left to stand for some time to let the bacteria get absorbed in media. After 10 mins, holes of about 8mm were punched in media by the help of a cork borer. A drop of molten agar was added in well to prevent leakage of nanostructures from the well. After that, samples with different concentrations (30µg, 60µg and 90µg) were added to the wells with the help of a micropipette. Each well contained 100µL of sample. Plates were labelled and then incubated at 35°C ± 2°C for 12 hours. For positive control, antibiotics including Tetracycline, Ampicillin were used while negative control was assessed by using blank solvent in the well. Measurement of inhibition zone was done in order to find out antibacterial potential of samples (Azam *et al.,* 2012).

RESULTS

Characterization Results

X-Ray Diffraction study

The XRD study was done to confirm the crystalline nature of ZnO NPs. XRD results confirmed NPs synthesis as shown in the fig 1A, 1B, 1C, 1D, 1E and 1F. They present the\ XRD spectra of pure ZnO NPs, Manganese doped ZnO NPs, Magnesium doped Zinc Oxide NPs, Calcium doped ZnONPs, Copper doped ZnONPs and Silver doped ZnONPs respectively. Moreover, the sizes of these nano-particles were analyzed by using Debye Scherrer's equation. The average size of pure ZnO was 55.4 nm; while doping elements influenced the size of ZnO nanoparticles such as average size of Mn, Mg, Ca, Cu and Ag doped ZnO nano-particles were 17.6 nm, 23.3 nm, 3.1 nm, 1.7 nm and 4.8 nm respectively.

Scanning Electron Microscopy (SEM)

The physical shape and structure of the nano-particles (synthesized through co-precipitation method) were investigated through SEM. The micrographs of SEM of Mn doped ZnO showed that the NPs exhibited spherical shape with definite degree of accumulation.

This is because of magnetic properties of nanoparticles produced by the competitive repulsive (electro steric) and attractive (dipolar and Vander Waals) interactions among the particles (Garcia *et al*., 2007).

Table 1: Results of antibacterial activity of nanostructures against *S. marcescens*, *E. coli* and *P. aeruginosa*

Sample Name	*Serratia marcescens* (B1) inhibition in mm at sample concentration of			*Escherichia coli* (B2) inhibition in mm at sample concentration of			*Pseudomonas aeruginosa* (B3) inhibition in mm at sample concentration of		
	30 µg/ml	60 µg/ml	90 µg/ml	30 µg/ml	60 µg/ml	90 µg/ml	30 µg/ml	60 µg/ml	90 µg/ml
ZnO Simple	0	12	14	0	0	13	0	0	0
ZnO+ Mn	15	16	18	0	10	12	0	0	0
ZnO+Mg	15	18	20	17	18	19	15	16	17
ZnO +Ca	15	22	24	0	14	15	15	18	20
ZnO +Cu	18	20	22	16	17	18	16	17	20
ZnO +Ag	23	25	27	15	18	20	16	18	20

Table 2: Results of antibacterial activity of nanostructures against *S. typhi*, *S. aureus* and *B. subtilis*

Sample Name	*Salmonella typhi* (B4) inhibition in mm at sample concentration of			*Staphylococcus aureus* (B5) inhibition in mm at sample concentration of			*Bacillus subtilis* (B6) inhibition in mm at sample concentration of		
	30 µg/ml	60 µg/ml	90 µg/ml	30 µg/ml	60 µg/ml	90 µg/ml	30 µg/ml	60 µg/ml	90 µg/ml
ZnO	0	16	18	0	0	0	20	23	27
ZnO + Mn	0	0	0	24	26	30	16	20	24
ZnO +Mg	0	0	19	12	14	15	17	18	21
ZnO +Ca	0	0	0	19	21	28	19	21	28
ZnO +Cu	0	0	20	20	23	28	20	23	28
ZnO +Ag	19	22	26	15	18	21	20	24	25

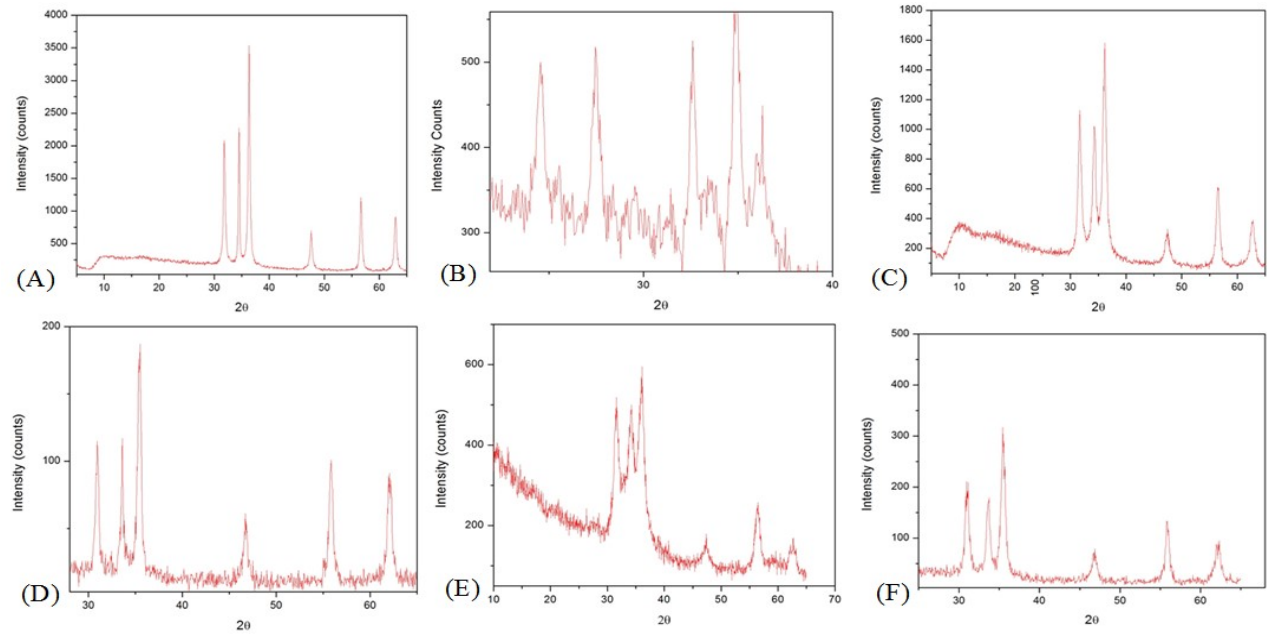

Fig. 1: XRD spectra of pure ZnO (A), Mn-doped ZnO (B), Mg-doped ZnO (C), Ca-doped ZnO (D), Cu-doped ZnO (E) and Ag-doped ZnOnano-particles.

The average size of these NPs was calculated using imageJ and hence the size was reported as 130 ± 97 nm. SEM morphology of simple ZnO NPs showed an average size of 15-30nm with a beautiful spherical shape as shown in Fig 3.1. Mn doped ZnO revealed a clustered morphology with an average size of ±90 nm. The range of sizes was between 50-150 nm. Mg doped ZnO showed clustered spherical shapes with range of 50-100nm. Irregular morphology with average size of 30-60 nm was reported for Ca doped ZnO. Clear rods were formed in case of Cu doped NPs with a 20-90 nm size range. For Silver doped ZnO, the SEM analysis was carried out and hence irregular shape with certain type of agglomeration was found. The size using Image J was calculated as 20-90 nm. Small size of crystals of ZnO NPs and doped ZnO is important to provide it altered physical, chemical and biological properties.

Fourier-transform infrared spectroscopy (FTIR)

This study was done to confirm the presence of vibrational bands present in the samples. Molecular geometry and the functional groups along with the inter-molecular and intra-molecular interactions were also observed. The peak appearance at 654 cm^{-1} may be due to

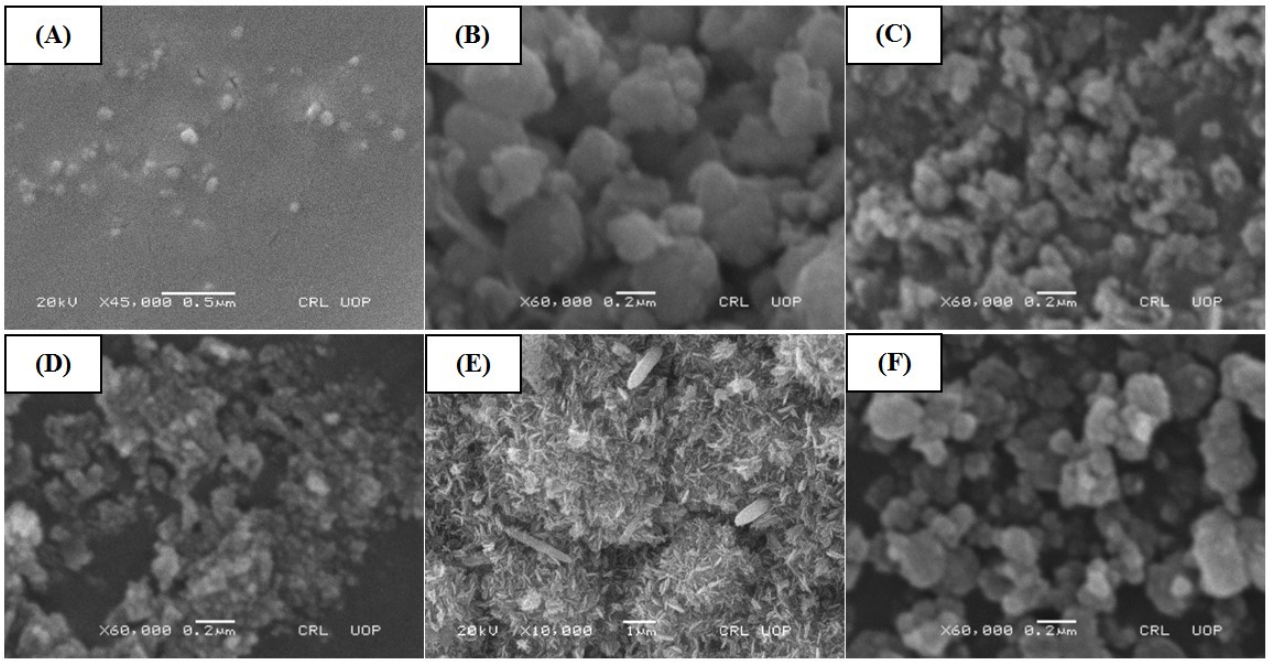

Fig. 2: SEM micrographs of ZnO (A), Mn doped ZnO (B), Mg doped ZnO (C), Ca doped ZnO (D), Cu doped ZnO (E) and Ag doped ZnO nanoparticles (F)

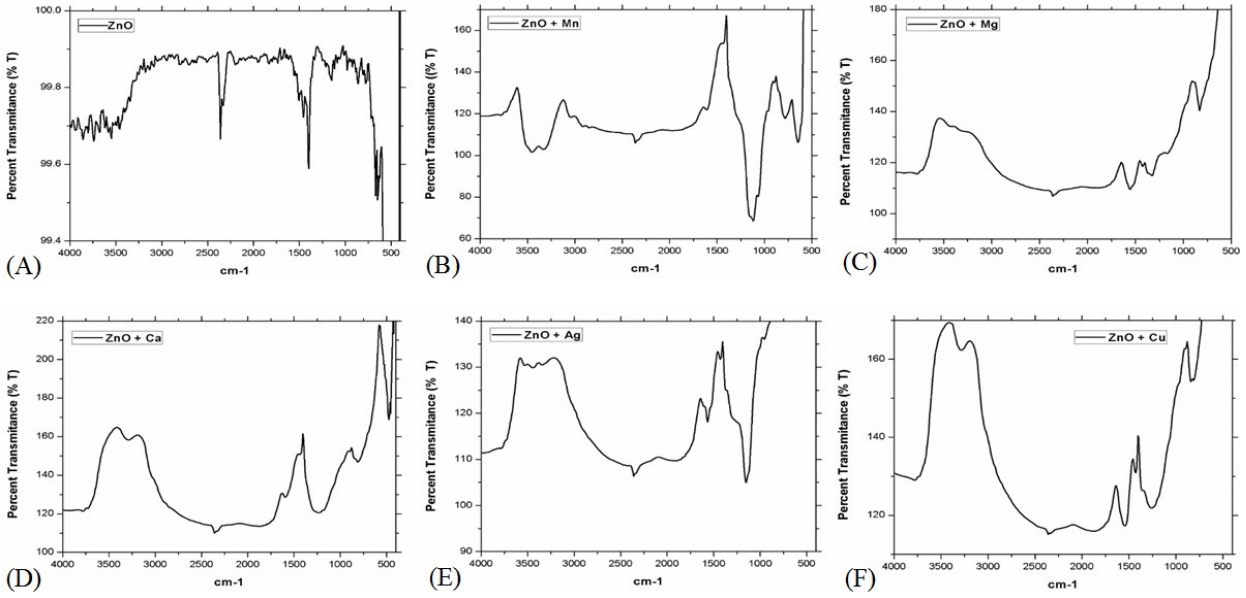

Fig. 3: FT-IR spectrum of ZnO (A), Mn doped ZnO (B), Mg doped ZnO (C), Ca doped ZnO (D), Cu doped ZnO (E) and Ag doped ZnO nanoparticles (F).

the vibration mode of MnO which is not observed for pure ZnO. The characteristic peak of ZnO was observed at 450 cm^{-1}.

Mn doped ZnO nanoparticles showed a symmetric peak between 1400 cm^{-1} and 1600 cm^{-1} range that denoted the presence of carboxyl group stretching at 1589 cm^{-1}. It was due to the loss of acetic acid present before. The absorption peak at 2400 cm^{-1} was found that denoted existence of CO_2 in air. A highest peak of 2350 cm^{-1} was observed for Mg doped ZnO representing a triple bond nitrile group CN. A peak 1529 cm^{-1} was observed which represents the C=C bending. The peak at 1310 cm^{-1} indicates the amine functional group which is probably attached to Mg because Mg is usually present as Mg_3N_2. The peaks at 525 cm^{-1}, 484 cm^{-1},446 cm^{-1},425 cm^{-1} represent the characteristic ZnO peaks.

FTIR spectra of Ca doped ZnO nanoparticles showed several peaks at different wavelengths. For example, the peak 1587 cm^{-1} represents amide N-H bending and the peak at 1417 cm^{-1} shows the medium weak multiple bonded functional group such as C=C. Similarly, the peak at the 1224 represents the C-N stretch medium weak bonded functional group. The peaks 802 cm^{-1} and 476cm^{-1} represent the =C-H functional group containing compounds having strong bonds. Multiple peaks of FT-IR were observed for Cu-doped ZnO nano-particles. The highest peak at 3279 cm^{-1} represents O-H functional group which shows the water molecule. The peak 2351 shows triple bond nitrile group CN. At 1536 cm^{-1} the peak represents the double bond aromatic C=C bending. The peak at 1448 cm^{-1} indicates -C-H functional group with variable bond strength.

Various peaks detected in Ag doped ZnO samples were 417 cm^{-1},452 cm^{-1}, 521 cm^{-1}, 836 cm^{-1}, 960 cm^{-1}, 1152 cm^{-1}, 1433 cm^{-1} and 1379 cm^{-1}. The highest peak observed was 1433 cm^{-1} and 1379 cm^{-1} represent -C-H functional group with variable bond strength. The peak at 1152 cm^{-1}represents the C-N functional group of medium weak bond. At 960 cm^{-1} peak and at 836 cm^{-1} it shows =C-H functional group with a strong bond. Similarly, the peaks 417 cm^{-1},452 cm^{-1} and 521 cm^{-1} are the characteristic peaks representing ZnO nanoparticles.

Antibacterial study

Results of antibacterial activity against given samples were recorded in tables 1 and 2. These results showed that with different potency, all the samples of nanostructures were effective against the microbial growth. The sample that suppressed growth was mostly that of (Zn+Ag) nanoparticles that retarded growth of *Serratia marcescens* at 90µg/ml. and Zinc oxide (ZnO) nanostructures that stopped the growth of *Bacillus subtilus* at 90 µg/ml. Samples that inhibited the growth most effectively against *Serratia marcescens* were silver doped zinc oxide nanoparticles (ZnO+Ag), calcium doped zinc oxide (ZnO+Ca) nanoparticles, magnesium doped zinc oxide (ZnO +Mg) nanoparticles and copper doped zinc oxide (ZnO +Cu) nanoparticles. The suppression of bacterial growth against *E. coli* was done excellently by Silver-doped Zinc oxide (ZnO+Ag) nano-particles, ZnO+Mg nanoparticles and Copper doped Zinc oxide (ZnO+Cu) nanoparticles. Antibacterial activity against *P. aeruginosa* was best covered by Silver-doped zinc oxide (ZnO+Ag) nanoparticles. While in case of *S. typhi*, the samples included that of Silver-doped zinc Oxide (ZnO+Ag) nanoparticles and zinc oxide nanoparticles (ZnO) which inhibited the growth. In case of *S. aureus*, all the samples were good antibacterial agents except ZnO+Mg nanoparticles and simple zinc oxide (ZnO) nanoparticles. Growth of *B. subtilus* was easily inhibited by all samples except manganese doped zinc oxide nanostructures.

DISCUSSION

This study was done under precipitation method which is the most commonly used procedure as other methods such as green synthesis and physical methods consumes so much of power, electricity, time, space and money as well and has less control on nanoparticles size. (Agarwal, Kumar *et al*. 2017). Moreover, the chemicals method can turn out dangerous as it involves toxic chemicals which can harm the surroundings and the person who is handling the process. It also can be hazardous fir the medical field in the process of its application. (Agarwal, Kumar *et al*. 2017). Despite of that, the typical co-precipitation method have some limitations including inadequate diffusion, large and uncontrolled molecule size dispersion, complication in mass formation and nanoparticles phase-shift control. Hence, it is difficult to achieve extreme magnetic response and uniform-sized molecule; particularly, when the size of the molecule should be in nanoscale. (Zarnegar and Safari 2017). Moreover, zinc oxide nano-sized structures have many attractive applications in different fields which make them interesting for researchers. By enhancing the chemical and physical properties of these nano-particles their biological and catalytic activities can be controlled in a positive way. This can be done by doping of pure nano-particles by adding different metals. Dopants influence the characteristics of pure nano-particles and make them more applicable at industrial level. For characterization of chemical properties and morphological study different techniques were used in this study such as XRD, SEM and FT-IR spectroscopy. The result showed that the synthesized nano-particles were spherical, rod and fiber rod like in structure. This present research study showed similarity with previous studies reported for characterization of nano-particles. The average size of ZnO powder was reported 55.4nm. Manganese doped ZnO nano-particles average size was noted 17.6 nm while Ca and Cu doped ZnO average size was measured 3.1 nm and 1.7 nm respectively. Zinc Oxide and metal doped ZnO showed very sound antibacterial activity against bacterial strains; *E. coli*, *S. marcescens*, *B. subtilus* and *S. aureus.* Zinc oxide (ZnO) nanostructures with 90 µg/ml concentration stopped the growth of *Bacillus subtilus*. Samples that inhibited the growth of *Serratia marcescens* and *E.Coli* were Ag-ZnO, Ca-ZnO, Mg-ZnO and Cu-ZnO nano-particles. Furthermore, all the metals showed superior activity against the microbial growth of *Pseudomonas aeruginosa* and *Salmonella typhi* except Simple ZnO NPs and Mn-ZnO nanoparticles. Ag-ZnO nanoparticles were the best candidate among all since it inhibited growth of almost all the bacterial species even at low concentration. While simple ZnO nanoparticles showed low antibacterial activity even at higher concentration except in case of *Bacillus subtilus*. This antibacterial study was in agreement with the previously reported studies. More study is required to know the exact

process of how these nanoparticles damage the bacteria via bioinformatics software. There is also a need to test the cytotoxic effects of these nanoparticles if it is intended to use as a drug.

CONCLUSION

In the present study, nano-sized particles of ZnO and metal doped ZnO were prepared by precipitation method. Metal doping gave totally novel properties to pure ZnO nano-particles, physical and chemical properties were enhanced by doping and made them attractive for industrial and pharmaceutical applications. XRD, SEM and FT-IR were used for characterization of these particles, which confirmed the synthesis, shape, size and morphology of these particles. Different bacterial strains were effectively inhibited by prepared nano-particles. Especially Ag-ZnO NPs excellently suppressed the microbial growth even at a lower concentration. So, it becomes clear that Pure ZnO and doped ZnO nano-particles can play vital role in therapeutics.

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