SYNTHESIS OF Mg DOPED ZnO NANOPARTICLES AND ITS APPLICATION IN MEDICAL PHYSICS

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Received: February 20, 2019 Accepted: March 21, 2019

ABSTRACT: *Mg-doped ZnO nanoparticles (ZnO NPs) were synthesized in the present research with an aim to investigate its biological properties. The structure, chemical composition and antibacterial activity of the synthesized nanoparticles (NPs) were studied with respect to Mg-doped ZnO nanoparticles at three different concentrations (5%, 7.5% and 10%). Among the three different concentrations, 10% Mg2+doped ZnO NPs showed maximum inhibitory zones of 27mm and 28mm against Escherichia coli and Staphylococcus aureus respectively. About 16mm and 21mm of inhibitory zones were recorded for 5%Mg2+doped ZnO NPs. The difference in the inhibitory zones was mainly due to the variation in concentrations and also the chemical composition of the cell wall layer in Gram-Negative and Gram-Positive organisms. As most of the antibiotics are found and reported to be highly specific to attack either Gram-Negative or Gram-Positive organisms. But the synthesized were found significant and interesting that the higher concentrations could play an important role in targeting the cell wall layer of both Gram-Positive and Gram-Negative organisms. SEM analysis of the developed ZnO NPs revealed two different shapes. The spherical shaped particles measured size of <50.5nm. The present research highlighted that the developed ZnO NPs could play a major role in the field of medical industries in the near future.*

Key Words: *Mg-doped ZnO nanoparticles, Antibacterial activity, Escherichia coli, Staphylococcus aureus, Reactive oxygen species*

INTRODUCTION

Nanoparticlesof commercial importance are being synthesizeddirectly from metal or metal salts(Husen and Siddiqi, 2014). Nanostructured zinc oxide has shown a great potential for applications in UV laser devices (Dai *et al.* 2003), quantum wells with superior interface morphologies (Beaur*et al.* 2011), cell imaging (Xiong*et al.* 2008) and solar cells (Kruefu*et al.* 2010). In addition, these particles are also more stableat high temperature and pressure (Sawaj, 2003). Some of them arerecognized as non-toxic and even contain mineral elementswhich are vital for the human body (Roselli et al., 2003). Recently, it has been reported that the doping of ZnO nanostructures with other elements can enhance its various properties. Commonly, different elements as a dopant in ZnO can be categorized into two groups: one group can substitute for zinc (Zn) and the second group for oxygen (O). These different dopants can tune various properties of ZnO nanostructures (Jung *et al.* 2011). Recent literature reported that Mg-doped ZnO nanostructures can exhibit excellent properties for device application (Tripathi et al., 2015). Investigation on doping Group II elements with ZnO showed that the dopants can alter the band gap energy (Eg) with an increase in the UV-Visible luminescence intensity (Ivetica et al., 2014). Doping of Mg into ZnO is expected to modify the absorption, physical, and chemical properties of ZnO. Metal ion-doped ZnO nanostructures are the most promising catalyst for the degradation of various pollutants because of its enhancement in its optical properties (Arshad et al., 2005).Nanosize inorganic compounds have shown remarkable antibacterial activity at very low concentration due to their high surface area to volume ratio and unique chemical and physical features (Rai et al., 2009). Earlier literature reported that ZnO nanoparticles can resist bacterium and they have the ability to shield ultraviolet radiations (Sirelkhatim et al., 2015). Zinc oxide NPs have the ability to disrupt the gram-negative cell membrane structure of *Escherichia coli*. It was reported also that nanoparticles with a positive charge could bind the gram-negative cell membrane using electrostatic attraction (Karthikeya et al., 2016).

ZnO NPs doped with different metal ions were evaluated against E. coli, and Staphylococcus aureus showed the antibacterial activity increasing with crystallite size (Gopinath et al., 2015). Different types of infectious diseases caused by bacteriapose a severe menace towards the public health worldwide.To enhance the antibacterial activity of ZnO, differenttypes of physiochemical properties such as particlesize, crystallinity index, and optical properties should bemodified by doping with metal or non-metal.For thesynthesis of ZnO nanoparticles, mostly reportedmethods are from sol–gel (Omri*et al.* 2014), co-precipitation (Raoufi*et al.* 2013), hydrothermal (Kumar *et al.* 2012), solvothermal (Chen *et al.* 2015), mechanochemical (Radzimska*et al.* 2014), spray pyrolysis (Ghaffarian*et al.* 2011) etc. Among all these methods,co-precipitation method is relatively simple and inexpensive.Furthermore, it can give high yield at roomtemperature for the synthesis of pure and doped ZnONPs (Bagabas et al., 2013).Here, we have investigated the preparation andcharacterization of ZnO nanoparticles with different concentrationsof Mg dopants by using a simple chemicalco-precipitation method. The effects of Mg2+ ion concentrationinside the ZnO lattice have been evaluated interms of structural, morphological, optical, and photocatalyticstudies. Further, the effect of Mg2+ ions on the antibacterialactivity was studied against (Gram-positive)*Staphylococcus aureus* and (Gram-negative) *Escherichia coli*.

MATERIALS AND METHODS

Synthesis of Mg doped ZnO nanoparticles

Mg doped ZnO nanoparticles were synthesized byco-precipitation method. Zinc nitrate hexahydrate (Sigma Aldrich), Sodium hydroxide (Sigma Aldrich) and Magnesium nitrate hexahydrate (Sigma Aldrich), were AR grade and used in the synthesis of Mg-doped Zinc Oxide without further purification. For synthesis of Mg doped ZnO nanoparticles, 0.005 M of aqueous Magnesium nitrate hexahydrate solution was added into 0.095 M of aquous zinc nitrate solution. 0.8 M of aquous NaOH solution was added drop by drop to homogenous mixture to get a white precipitate. The solution with the white precipitate was stirred at room temperature and then temperature of 60 °C for 4 hr.This solution was refluxed at room temperature for 24 hr. Then, a clear solution was obtained, which found to be stabe at a ambient condition. Thereafter, the solution was washed several times with double distilled water and ethanol. Finally, the precipitate was dried at 120 °C. Thus Mg doped ZnOnanopowder was obtained. These samples were annealed at 500 °C for 6 hr.

Antimicrobial activity of ZnO NPs

Antimicrobial activity of Mg doped ZnO nanoparticles were done by agar well diffusion method against two pathogenic bacterial strains *S.aureus* (gram positive) and *E. coli* (gram negative) on Muller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) (Wright *et al.* 2000). The media plates (MHA) were streaked with bacteria 2-3 times by rotating the plate at 60 °C angles for each streak to ensure the homogeneous distribution of the inoculums. Then the agar plates were swabbed with 100 mL each of overnight cultures of *S.aureus* and *E. coli* using a sterile L-shaped glass rod. Using a sterile corkborer, wells (6 mm) were created in each petri plate. Varied concentrations of ZnO NPs (1 mg/ml, 3 mg/ml and 5 mg/ml for both G+ and G- both bacteria) were loaded onto the petri plates followed by incubation for 24 hr at 37 °C, for bacteria. After the incubation period, the diameter of the zone of inhibition (DZI) was recorded. Kanamycin (Hi-Media) was used as the positive control against gram negative and gram positive bacteria respectively to compare the efficacy of the test samples.

Scanning Electron Microscopy (SEM) analysis of synthesized ZnO nanoparticles (Ogunyemi*et al.*, 2019)

The structural morphology of zinc oxide nanoparticles was examined and measured by Scanning Electron Microscopic (SEM) using TM-1000, Hitachi, Japan. An aliquot of each sample was fixed on a carbon-coated copper grid, and the film on the SEM grid was then dried by fixing it under a mercury lamp for 5min. The particle size and particle shape was also confirmed using SEM analysis.

RESULTS AND DISCUSSION

Antibacterial activity

In the present scenario, the NPs are studied extensively to explore their utility as a potential antibacterial agent. Several factors such as less toxicity and heat resistance are accountable forthe use of NPs in the biological applications.In the current study, Mg2+ doped ZnO NPs at three different concentrations were tested against Gram-Negative (*Escherichia coli*) and Gram-Positive bacteria (*Staphylococcus aureus*) using agar well diffusion method to determine their ability as a potential antimicrobial agent. During the analysis, it was observed that all the three concentrations of Mg2+doped zinc oxide nanoparticles inhibited the growth of test bacteria at greater extend. The zone of inhibition was increased with the increase in concentration of nanoparticles (Table-1).

Table-1: Antibacterial activity of Mg2+doped ZnO NPs against bacteria

S. No.	Organisms	Zone of inhibition (mm)		
		5%	7.5%	10%
1	*Escherichia coli*	16	21	27
2	*Staphylococcus aureus*	21	23	28

In Table-1, the variation in inhibitory zones of Mg2+doped ZnO NPs against the test organisms was presented. Among the three different concentrations, 10% Mg2+doped ZnO NPs showed maximum inhibitory zones of 27mm and 28mm against *Escherichia coli* and *Staphylococcus aureus* respectively. And 7.5% of doped nanoparticles exhibited inhibitory zones of 21mm and 23mm against the test organism. About 16mm and 21mm of inhibitory zones were recorded for 5%Mg2+doped ZnO NPs. The recent literature survey also showed the antibacterial potential of ZnO nanoparticles developed from different methods. Manyasree*et al*., (2018) the antibacterial assay clearly expressed that *E. coli* showed a maximum zone of inhibition (32±0.20 mm) followed by *Proteus vulgaris* (30±0.45 nm) at 50 mg/ml concentration of ZnO nanoparticles. Farzana et al., (2018) described Kirby's Disc diffusion assay using different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0) mg/ml of ZnO NPs with and without β lactam antibiotics (Ciprofloxacin and Imipenem). The results of antibacterial activity indicated that ZnO NPs possess strong antimicrobial activity and can enhance the antimicrobial activity of some beta-lactam antibiotics. Sharmila*et al*., (2018) reported that ZnO NPs showed a significant antibacterial activity against Gram-negative bacteria *P. aeruginosa* and *E. coli* than Gram-positive bacteria. *B. tomentosa*leaf extract-derived ZnO NPs showed a significant zone of inhibition for *P. aeruginosa* (20.3 mm) and *E. coli* (19.8 mm), whereas the zone of inhibition was observed less for *B. subtilis* (8.1 mm) and *S. aureus* (10.7 mm).

The difference in the inhibitory zones was mainly due to the variation in concentrations and also the chemical composition of the cell wall layer in Gram-Negative and Gram-Positive organisms. In this study, Gram-Positive *Staphylococcus aureus* was found highly sensitive against the doped nanoparticles. This showed that mode of action of the developed nanoparticles were more potent to attack the cell wall composition of Gram-Positive group of organisms. Most of the antibiotics are found and reported to be highly specific to attack either Gram-Negative or Gram-Positive organisms. But the synthesized were found significant and interesting that the higher concentrations could play an important role in targeting the cell wall layer of both Gram-Positive and Gram-Negative organisms. The reason for the antibacterial mode of action was identified during the literature survey. Sharmila*et al*., (2018) reported that the mechanism of antibacterial activity of ZnO NPs may be attributed to the penetration and disintegration of the membrane by smaller sized NPs which lead to cell lysis. The release of H_2O_2 from the surface of ZnO also reported as the possible mechanism for bactericidal activity. The generation of H_2O_2 is highly depended on the surface area of ZnO and the generated H_2O_2 penetrates the cell membrane and cause damage to kill the bacteria. This could play a major role in the field of medical industries in the near future.

Three different concentrations of Mg2+doped ZnO NPs exhibiting the antibacterial activity in the present study werehighly reactive due to their high surface to volume ratio. From Fig.1 and 2, the difference in the inhibitory zones is proportional with the amount of Mg doping in ZnO NPs. This might be attributed to the reduction in their band gap values.

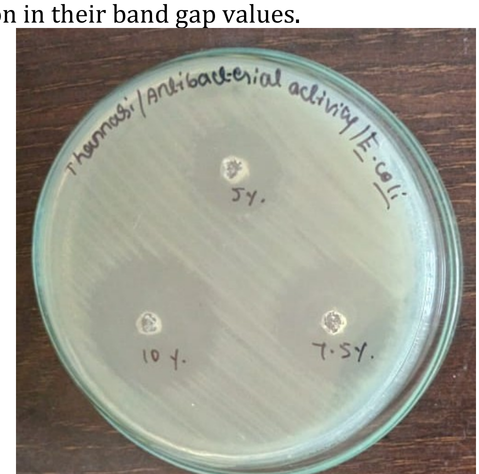

Fig.1: *Escherichia coli*

Fig.2: *Staphylococcus aureus*

The variation in the sensitivity or resistance of different Mg2+doped ZnO concentrations was due to the differences in the cell structure, physiology, metabolism or degree of contact of organisms with nanoparticles. The antibacterial efficiency of ZnO NPs generally depends on the higher ROS which is mainly attributed to the larger surface area, increase in oxygen vacancies, the diffusion ability of the reactant molecules and the release of Zn2+ (Brayner*et al.* 2011). Also differences in the antibacterial activity might be due to different particle dissolution. Basically, the antibacterial efficiency of pure and Mg-doped ZnO NPs is mainly dependent on the increased levels of reactive oxygen species (ROS), mostly hydroxyl radicals (OH) and singlet oxygen (Mahendraet al., 2008). This is mainly due to the enlarged surface area which causes increase in oxygen vacancies as well as the diffusion capacity of the reactant molecules inside the NPs (Vijayakumar et al., 2016). The reactive oxygen group contains superoxide radical and hydrogen peroxide. Both of them can damage the DNA and cellular protein leading to cell death (Jha et al., 2012). Furthermore, due to the various surface-interface characteristics may have different chemical-physical, adsorption-desorption abilities in the direction towards bacteria, make sure in different antibacterial performances (Suresh Kumar et al., 2014).The interaction between the NPs and the cell wall of bacteria was changed due to doping of Mg. Moreover, the presence or addition of the nanostructures on the surface or cytoplasm of the bacteria can cause the disruption of cellular function as well as disorganization of the cell membranes (Storz and Imlayt, 1999). The doping of Mg with ZnO may lead to the variation in grain size, morphology, andsolubility of Zn2+ ions. All these factors combined together have a robust impact on the antibacterial activity of ZnO(Seyedeh et al., 2013).

Scanning Electron Microscopy (SEM) analysis of synthesized ZnO nanoparticles

SEM analysis of the developed ZnO NPs showed size <50.5nm. Two different shapes were also found evident from the SEM anaylsis. The spherical shaped and cube shaped nanoparticles were identified. Interestingly, the spherical shaped particles measured in nanometer and the cube shaped particles measured in micron size (Fig. 3). During the literature survey different sized and shaped ZnO nanoparticles were described. The variation in size and shape were found varied due to the method of synthesis involved. Ogunyemi*et al*., (2019) developed green synthesized ZnO NPs using three different herbal plants. The size and shaped found varied with the type of herbs used in the study. The sizes of the nanoparticles by the SEM ranged from 49.8nm to 191.0nm for ZnO NPs synthesized by *Matricariachamomilla*, 40.5nm to 124.0nm for ZnO NPs synthesized by *Oleaeuropaea*, and a size range of 65.6nm to 133.0nm for ZnO NPs synthesized by *Lycopersiconesculentum.* Manyasree*et al*., (2018) found spherical shaped particles with the average crystallite size of ZnO nanoparticles of 35nm. A facile, eco-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) employing *Bauhinia tomentosa*leaf extract as bioreducing agent was reported by Sharmila*et al*., (2018). The researchers found hexagonal morphology exhibiting nanosizedZnO from the TEM and SEM studies. Upadhyaya et al., (2018) used mehendi extract (Lawsoniainermis) for phytosynthesis of ZnO nanoparticles using 0.1 M $Zn(NO_3)_2$ as a precursor. SEM images showed change in shape and size during their analysis. Interestingly the researchers reported that hexagonal shaped and rod shaped particles were formed in the presence of plant extract and in the absence of plant extract respectively.

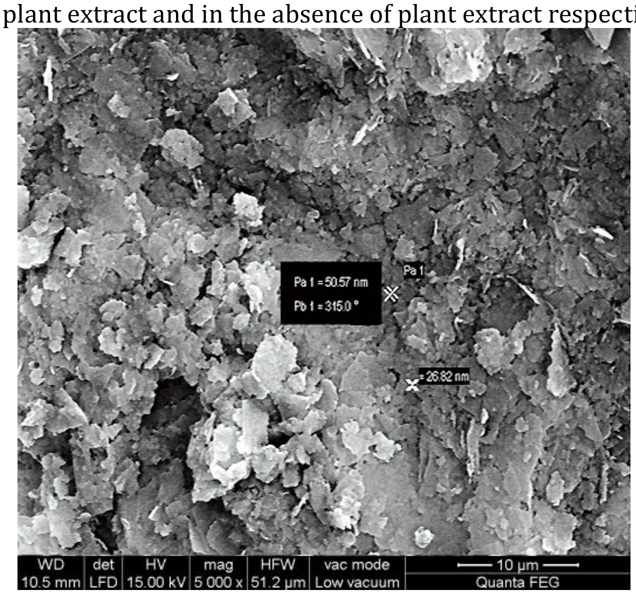

Fig.3: SEM analysis of ZnO nanoparticles

CONCLUSIONS

To conclude, pure and Mg-doped ZnO structures were successfully synthesized by co-precipitation method. The antibacterial activities of the synthesized nanosamples were tested against *E. coli* (Gram-negative), *S. aureus* (Gram-positive bacteria). The zone of inhibition by using Mg-doped ZnO NPs for *E.coli* (Gram-negative), *S. aureus* (Gram-positive bacteria), and Proteus (Gram-negative strains) was significantly observed in the present research. It was carried out using disc diffusion method to observe their ability as a potential antimicrobial agent. SEM analysis of the developed ZnO NPS revealed two different shapes. The spherical shaped particles measured size of <50.5nm. The results have revealed that Mg-doped ZnO nanostructures will be a promising candidate to be used for potential drug delivery systems tocure some significant infections in the near future. The synthesized were found significant and interesting that the higher concentrations could play an important role in targeting the cell wall layer of both Gram-Positive and Gram-Negative organisms. This could play a major role in the field of medical industries in the near future.

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