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Original Article

Antibacterial study of silver doped zinc oxide nanoparticles against *Staphylococcus aureus* and *Bacillus subtilis*

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ABSTRACT

Objectives: The present study has been undertaken to synthesize silver doped zinc oxide nanoparticles, with pharmaceutical importance. The synthesized particles have been evaluated to study the effect of silver doping on grain size and further on antibacterial activities against the microorganisms *Bacillus subtilis* and *Staphylococcus aureus*.
Methods: Silver doped zinc oxide nanoparticles were prepared by the solution route spin-coating process, using zinc acetate ($Zn(CH_3COO)_2.2H_2O$) and silver nitrate ($AgNO_3$) as host and dopant precursors respectively. The antibacterial activity of the silver doped zinc oxide were studied against *S. auerus* and *B. subtilis* via using agar well diffusion method.
Results & discussion: : The structure of the powder samples was analyzed by X-ray diffraction (XRD). The effect of silver doping on grain size and further on antibacterial activity against the microorganisms *B. subtilis* and *S. auerus* is discussed.
Conclusion: It was clear from X-ray investigations that its structure is wurtzite type and that an increase in Ag-doping resulted in decrease in the grain size of the ZnO nanoparticles. Antimicrobial study against the microorganisms *B. subtilis* and *S. auerus* shows that in case of *S. auerus* the MIC varies with increase in Ag content but in case of *B. subtilis* the MIC remained constant for all concentration of Ag.

1. Introduction

The field of nanotechnology is one of the most active researches in modern material science. Nanotechnology is emerging as a rapid growing field with its applications in science and technology for the purpose of manufacturing new materials at the nanoscale level. Recently, need for designing new materials with improved properties have forced fast development of nanostructured materials. Thus, researches have been focused on investigation of materials at the atomic, molecular and macromolecular level, with the aim to understand and manipulate the features that are substantially different from the processing of materials on micro-scale. Nanoparticles usually ranging in dimensions from 1 to 100 nm have properties unique from their bulk equivalent. With the decrease in dimensions of materials to the atomic level, their properties change. The nanoparticles possess unique physico–chemical, optical and biological

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properties which can be manipulated suitably for desired applications.[1]

Synthesis of nanosized drug particles is of great interest in the development of new pharmaceutical products.[2] Emergence of new resistant bacterial strains to current antibiotics has become a serious health issue. It raised the need to develop new antibacterial materials.[3] However, the phenomenon of improved biological activity and certain material changes resulting from nanoparticles is not yet understood fairly. A number of research studies have shown encouraging results about the activity of different drugs and antimicrobial formulation in the form of nanoparticles.

ZnO nanoparticles have exhibited a strong bacterial growth inhibiting character.[4] ZnO has also gathered significant attention due to its various applications such as UV light emitting diodes, laser diodes and catalysts.[5] ZnO is widely used to treat a variety of skin conditions, in products such as baby powder, barrier creams to treat diaper rashes and in calamine lotion, antidandruff shampoos and antiseptic ointments.[6] Nano zinc is non-toxic, with wide band gap has also been identified as a promising semiconductor material for exhibiting ferromagnetism (RFTM) at room temperature when doped with most of the transition metal elements.[7] Transition metal doped nanostructure is an effective method to adjust the energy level surface states of ZnO, which can further improve by the changes in doping concentrations of doped materials and hence in its physical and optical properties.

Silver and zinc oxide successfully used in chemical and biological sensors, bactericidal agents, electronics and photo electronic devices and have considerable bio-activity as well.[8–10] Among inorganic antibacterial agents, silver nanoparticles have been employed most extensively. Silver nanoparticles liberate silver ions in liquids that show a broad spectrum of the antimicrobial activities.[11–13] Nanosized silver particles are currently used to control the bacterial growth in a variety of applications, including medical devices, dental composite materials and textile materials. Silver has been extensively used in topical preparations and to saturate bandages so as to restrict bacterial growth in injured skin.[14,15] Satumal et al reported that decrease in particle size with the increased presence of doping element can be attributed to increased solubility of doping element in the base material.[16] Thus, addition of silver in zinc oxide may result in varied particle size and hence may affect its antibacterial activity.

In the present study, we intend to synthesize undoped and silver doped ZnO nanoparticles with different silver content. The synthesized undoped and doped nanoparticles have been characterized for the structure and further investigated for the anti-microbial property with respect to their crystallite size and silver content.

2. Materials and methods

2.1. *Synthesis of nanoparticles*

ZnO nanoparticles can be synthesized by several methods such as, vapor deposition, pulsed laser deposition (PLD), sol–gel synthesis and hydrothermal method. The solution route method is a simple, low-cost and most commonly used technique for the synthesis of ZnO nanoparticles. The synthesis of ZnO nanoparticle was carried out by simple solution route. As starting materials, zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$] (98.5% Fisher Scientific) and silver nitrate [$AgNO_3$] powders (98.8% Fisher Scientific) were used. At room temperature solution A: zinc acetate and solution B: different concentrations of silver nitrate were dissolved in the solution containing 80 ml distilled water and 20 ml ethanol respectively. Then ammonia solution was added into the solution C: mixture of zinc acetate, silver nitrate. The initial solution contained milky colored precipitates of zinc hydroxide at a low concentration of ammonia. With continuous dropping of ammonia solution, zinc hydroxide started to form Zn complex. Then solution became clear. A buffer solution was prepared by dissolving appropriate amounts of sodium hydroxide. The buffer solution was then added drop wise to the vigorously stirred solution until the precipitation. Then the solution was stirred at constant temperature for 1 h. Finally the precipitates were filtered and washed with distilled water. The final product was dried at 500 °C for 1 h in muffle furnace and ground to fine powder.

2.2. *Structural analysis*

X-Ray Diffraction (XRD) data was collected after grinding using diffractrometer (Model: D8 Focus). Standard JCPDS data cards were used for indexing of the XRD data collected. Scherrer's formula was used for estimation of crystallite size of undoped and silver doped nanoparticles.

2.3. *Antibacterial testing*

Antibacterial testing was performed against *Staphylococcus aureus* (MTCC 3160) and *Bacillus subtilis* (MTCC 441) by agar well diffusion method. Bacterial strains were obtained from MTCC (Microbial Type Culture Collection) Chandigarh. For bacterial growth nutrient agar medium is used which contains beef extract, peptone, sodium chloride, and yeast, distilled water at pH 7.2 and incubated at 37 °C for overnight. Wells were then bored into the plates with seeded organisms using sterile cork borer of 6 mm in diameter. 50 μl of different samples of concentration 200 mg/ml were placed in the wells in different plates for study. All bacterial plates were incubated at 37 °C for 24 h. Average values were used for calculation of the inhibition zone area, which was the measure of the antibacterial activity of the studied samples. The diameter of the minimum zone was measured in mm.

2.4. *Determination of minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC), is the lowest concentration of material, which inhibits the growth of an organism. It was determined by broth tube dilution method, which is based on cultures containing different concentration of silver doped ZnO nanoparticles ($Zn_{1-x}Ag_xO$, $x = 0$, 0.1, 0.2, 0.3, 0.4) in suspension.