



Synthesis of zinc oxide nanoparticles by homogeneous precipitation method and its application in antifungal activity against *Candida albicans*

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) were prepared by a simple homogeneous precipitation method without using any surfactant, chelating or gelating agents. The influence of calcination temperature on the morphology, specific surface area and pore volume of the nanoparticles has also been investigated. The synthesized samples were characterized by powder X-ray diffraction, FT-IR spectroscopy, thermal gravimetric analysis, UV–visible diffuse reflectance spectroscopy, surface area measurements, field emission scanning electron microscopy coupled with energy dispersive X-ray analysis and transmission electron microscopy. The antifungal activity of the ZnO-NPs against *Candida albicans* was assessed using the disc-diffusion susceptibility tests. It was observed that with the increase in concentration of ZnO-NPs the antifungal activity is enhanced toward pathogenic *C. albicans*.

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1. Introduction

Zinc oxide nanoparticles (ZnO-NPs) possess several interesting properties such as optical transparency, electric conductivity, piezoelectricity, non-toxicity, wide availability, low cost and stability [1,2]. They are n-type semiconductor with a wide band gap (3.3–3.6 eV) and large excitation binding energy (60 meV) at room temperature [1]. They are used for many applications in various fields including catalysis, gas sensors, solar cells, paints, varnishes, plastics, pharmaceuticals, laser and optoelectronic devices [3–6]. It is usually employed as protective agent in cosmetics and sunscreen products because of its ability to filter UV radiations [7]. ZnO-NPs are well known for its antimicrobial and antifungal activity [8,9]. ZnO powder is an active ingredient for dermatological values in creams, lotions and ointments for its antibacterial properties [10]. They have also been used as photocatalysts in degradation of methylene blue due its high photosensitivity

and stability [11]. ZnO-NPs have been synthesized using different methods in various morphologies such as fibers [12], wires [13], flowers [14], rods [15], mallets [16], tetrapods [17] and rings [18] with diverse properties.

ZnO-NPs powder has been synthesized by several methods such as thermal decomposition [1], sol–gel [3,4], laser ablation [19], microemulsion [20], sonochemical [21], solid state [22], combustion [23], polymerization [24], vapor deposition [25], spray pyrolysis [26], solvothermal [27] and hydrothermal method [28]. Homogeneous precipitation method has also been used by some workers to synthesize ZnO-NPs [29–34]. Ming et al. have reported [29] the synthesis using a Teflon-lined autoclave which required high calcination temperature (450 °C) while Liang et al. [30] employed a pulse alternative field with a frequency of 50 HZ. Samaele et al. [31], Tang et al. [32] and Behnajady et al. [33] have reported the preparation using sodium dodecyl sulfate (SDS) and oxalic acid as the surfactant and chelating agent, respectively. In the present work, ZnO-NPs powder has also been synthesized by homogeneous precipitation method but at low calcination temperature (300 °C) without using any surfactant, chelating agent, Teflon-lined autoclave or

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pulse alternative field. The homogeneous precipitation method offers easy control of uniform particle size, environmental-friendly and preparing samples at low temperature in short time in large scale production, not employing any expensive raw materials and complicated equipments. The pathogenic *Candida albicans* is the most common microorganism which is responsible for fungal infection [9]. Its overgrowth causes a variety of infections in the mouth, skin, blood stream and genital regions of both men and women [35,36]. Recently, fungal infections especially *Candidiasis* has significantly increased and in its treatment only a few antifungal drugs are available. They have developed drug resistance and side effects. Hence, developing of the novel antifungal agents is in urgent demand. This prompted the authors to synthesize ZnO-NPs and to test these for antifungal activity against pathogenic *C. albicans*.

2. Experimental

2.1. Materials

Zinc acetate (MERCK[®]), ammonia solution (25%, RAN-KEM[®]), potato dextrose broth (PDB) medium for fungus cultures (SRL[®]) were used as received without further purification. For testing antifungal activity *C. albicans* strain (MTCC 221) was purchased from the Culture Collection, Chandigarh, India. The solutions were prepared using Millipore[®] water.

2.2. Synthesis of ZnO-NPs

In the present study, ZnO-NPs powder was prepared using suitable precursor by homogeneous precipitation method. The details of procedure are as follows.

In 250 mL beaker zinc acetate (8 mmol) was dissolved in 90 mL of Millipore water. To this 10 mL ammonium hydroxide (25% ammonia solution) was added and the contents were heated to ~85 °C with continuous stirring for 2 h. During the reaction, a milky white precipitate was obtained. It was filtered off, washed with water several times to remove any impurities present and then dried at 70 °C in an oven for 6 h. The as-prepared powder was calcined in air at 300 °C and 400 °C at a heating rate of 2 °C/min for 2 h inside a muffle furnace. The color of the ZnO-NPs powder before and after calcination was white.

2.3. Characterization methods

Powder X-ray diffraction (XRD) patterns were recorded using a Bruker AXS-D8 diffractometer operating with Cu-K α radiation ($\lambda=0.15406$ nm) in the 2θ range 5°–80° with a scanning speed of 1°/min. Thermal gravimetric analysis (TGA) was carried out in air using a Perkin Elmer (Pyris Diamond) instrument at a heating rate of 5 °C/min in the temperature range, 25–600 °C. Diffuse reflectance spectra were recorded with the help of a Shimadzu UV-2450 UV–visible spectrophotometer attached with a diffuse reflectance accessory in the wavelength range 200–800 nm using BaSO₄ as the reference. A Thermo Nicolet Nexus Fourier FT-IR spectrometer in the range 4000–400 cm⁻¹ was used for recording

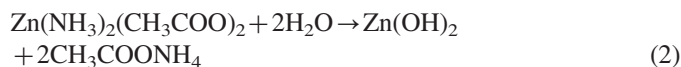
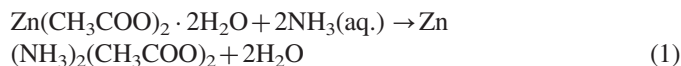
IR spectra of the zinc oxide powder using KBr disk method. The specific surface area of the zinc oxide powder was measured using Brunauer–Emmett–Teller (BET) method by Micromeritics Chemisorb 2720 instrument using N₂ physisorption. Morphology of ZnO-NPs along with elemental analysis (EDXA) data were obtained using a field emission scanning electron microscope (FE-SEM, FEI Quanta 200F) operating at an accelerating voltage of 20 kV and TEM images of the zinc oxide powder were recorded using a FEI TECNAI G2 electron microscope operating at an accelerating voltage of 200 kV.

2.4. Testing of antifungal activity

The antifungal activity of the ZnO-NPs was investigated against *C. albicans* using the disc-diffusion susceptibility method. 50 mL Potato dextrose broth (PDB) medium was prepared by dissolving appropriate amount of potato dextrose powder (39 g/L) in Millipore water and mixed it properly. The final volume of 50 mL was made by adding adequate amount of Millipore water to it. Medium was then sterilized at 121 °C for 15 min. This medium was poured into five different sterilized petri dishes. After solidification of these media, 50 μ L suspension cultures of *C. albicans* was evenly spread on the surface of solidified culture media with sterilized glass spreader. Then sterilized filter paper discs (6 mm diameter) were placed at the center of each petri dish. The ZnO-NPs were taken at different concentrations of 5, 10, 15 and 20 mg/mL and mixed with sterilized Millipore water using a low power sonicator. Further, each concentration of ZnO-NPs was impregnated onto the discs kept in four petri dishes. In the fifth petri dish the growth of *C. albicans* was observed for the disc impregnated with only sterile Millipore water in the absence of ZnO-NPs and it was taken as the fungal control. Then the dishes were incubated in a thermostatic chamber at 28 °C for 72 h. All petri dishes were kept in regular observation to examine the zone of inhibition around each disc. The diameters of the clear zone of inhibition were measured in millimeters (mm). To minimize experimental error the experiment was repeated several times.

3. Results and discussion

Proper amount of aqueous ammonia solution was added to an aqueous zinc acetate solution with continuous stirring for 2 h at ~85 °C yielding Zn(OH)₂ and ZnO (as confirmed by the XRD results). ZnO-NPs were formed by the following reactions:



The XRD data confirm the formation of pure ZnO-NPs after calcination.

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