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Antimicrobial activity of wet chemically engineered spherical shaped ZnO nanoparticles on food borne pathogen

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Abstract

Nanotechnology is being envisioned as a hurriedly developing field, it has potential to revolutionize food systems and improve the conditions of the food quality. The present study reports the synthesis of ZnO nanoparticles using wet chemical method and used as antimicrobial agent against Food borne Pathogens. The antibacterial activity of ZnO nanoparticles was examined against *E. coli*, and *Pseudomonas aueroginosa*, the maximum inhibition was occurred at 100 µl. The concentration of ZnO in 100 µl is 100 µg. The antifungal activity of ZnO nanoparticles was also analyzed against *Aspergillus niger* and the maximum inhibition was found at 400 µl. This wet chemical mediated synthesis of ZnO nanoparticles is a rapid and it could not produce any toxic chemical along with the nanoparticles. Moreover, the antimicrobial effect of ZnO nanoparticles against food borne pathogen may leads to the proficient application in food packaging and preservation process.

Introduction

Zinc oxide is non-toxic, II-VI semiconductor with wide band gap (3.37eV) and natural n-type electrical conductivity (Wellings et al., 2007). ZnO becomes one of the most important functional materials due to its unique optical, electronic properties such as near-UV emission, optical transparency, electric conductivity, piezoelectricity (Ma *et al*., 2008). ZnO have superior durability, greater selectivity and heat resistance than organic and inorganic materials (Padmavathy and Vijayaraghavan, 2008; He *et al*., 2011b).

Due to noble properties such as high refractive index, high thermal conductivity, binding energy, antibacterial and UV-protection of ZnO it could be used in various materials and products, including medicine, cosmetics, varistors, solar cells, rubber and concrete, foods (Klingshirn, 2007). ZnO has high biocompatibility and fast electron transfer kinetics, such features advocate the use of this material as a biomimic membrane to immobilize and modify the biomolecules (Kumar *et al*., 2008). Among a variety of semiconducting materials, zinc oxide is rich in nanostructures and it has the capability to produce into a variety of morphologies (Wahab *et al*., 2008) such as nanowires (He *et al*., 2011a), nanorods (Huang e*t al*., 2011), nanocombs (Fan *et al*., 2010), nanoflowers and nanosheet (Kou *et al*., 2011). ZnO nanoparticles has been prepared by various methods such as thermal decomposition (Yang *et al*., 2003), solvothermal reaction (Tonto *et al*., 2008) reactive electron beam evaporation technique (Asmar *et al*., 2005), chemical vapor deposition (Wu *et al*., 2002), hydrothermal method (Baruah *et al*., 2009). The metal oxides such as titanium oxide, magnesium oxide, zinc oxide and copper oxide are liked better than nano silver because of cost consideration (Yadav *et al*., 2006; Kathirvelu *et al*., 2009). Zinc oxide is non toxic to human beings and noxious to microorganisms. Moreover, zinc is a mineral element necessary to human health and ZnO is a form in the daily supplement for zinc. ZnO nanoparticles also have good biocompatibility to human cells (He *et al*., 2011b; Padmavathy and Vijayaraghavan, 2008). Currently ZnO is listed as generally documented as safe material by FDA (Food and Drug Administration, USA) (Emamifar *et al*., 2010). Early works have been reported that the antimicrobial textiles can be prepared using ZnO coating on cotton fabrics (Rajendran *et al*., 2010). Another investigation has shown the antibacterial activity against food borne pathogen using ZnO powder coated PVC films (Li *et al*., 2009).

Electron resonance measurements show that aqueous suspension of ZnO nanoparticles generate

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augmented level of reactive oxygen species, i.e. hydroxyl radicals. Increased oxidative stress is detected after the antibacterial treatment, beyond the level yielded by the ZnO itself and the bacteria coming to contact with little amount of ZnO nanoparticles effects in an increased cellular internalization of the nanoparticles and bacterial cell damage (Applerot *et al*., 2009; Thati *et al*., 2010). A few studies suggested that the dissolution of Zn ions from ZnO nanoparticles responsible for toxicity of ZnO nanoparticles and also the dissolution of ZnO nanoparticles into Zn ions were found to be size dependent. Thus, engineered ZnO nanostructures may change their toxicity by influencing their dissolution rate (Franklin *et al*., 2007; Heinlann *et al*., 2008; Meulenkamp *et al*., 1998; Aruoja *et al*., 2009; Miller et al., 2010; Peng *et al*., 2011).

Nowadays, numerous methods have been employed to control or prevent the growth of pathogens in food by the involvement of synthetic and natural antimicrobial agents (Bajpai *et al*., 2007). In recent years, nanoparticles play an important role in food preservation and packaging and it has a larger work of art and greater potential in food nanotechnology (Sastry *et al*., 2011). In the present investigation, the ZnO nanoparticles were synthesized using wet chemical method. The structural characterization of ZnO nanoparticles were carried out using X-ray diffractometer, scanning electron microscope, Fourier transform infrared spectrophotometer. Further, the antimicrobial activity of ZnO nanoparticles were carried out against *Escherichia coli* and *Pseudomonas aeruginosa* which was isolated from mint leaf extract and freezed ice cream, respectively and a fungus, *Aspergillus niger* was isolated from bread.

Materials and Methods

Materials

All the chemicals, culture medium and broth used in this experiment were purchased from Himedia Laboratories Mumbai, India.

Preparation of ZnO nanoparticles

Wet chemical method was used for preparation of ZnO nanoparticles. In a typical experiment 1% of Glucose was added to 500 ml of double distilled water followed by Zinc acetate (0.1M) and kept in magnetic stirrer for continuous stirring to dissolve Zinc acetate completely. Then 0.2 M of sodium hydroxide was added dropwise to the vessel and mixed it using magnetic stirrer and withstand the mixture in stirrer for 2 hours. After 2 hours, the solution was incubated for 24 hours for settlement and the settled white precipitate was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellets were washed several times with distilled water to remove the byproducts. After washing the nanoparticles were dried at 80º C in hot air oven. During drying process, Zinc hydroxide is completely converted into Zinc Oxide.

Characterization studies

The formation of ZnO nanoparticles was initially confirmed by using UV spectrophotometer (Perkin-Elmer) and the particles were scanned at the wavelength ranges from 300-700 nm. The crystalline nature of prepared ZnO Nanoparticles were observed using powder X- ray diffractometer (X'per PRO model) using CuKα radiation, at 40 keV in the ranges of 10-80. The bulk ZnO and ZnO nanoparticles XRD image was used for compare the results. Further, the morphology of the prepared ZnO nanoparticles was characterized by using scanning electron microscopy (HITACHI Model S-3000H). The Fourier transform Infra red spectroscopy analysis (MAKE – BRUKER Optik GmbH MODEL No - TENSOR 27 SOFTWARE - OPUS version 6.5.) was performed to identify the possible functional groups of glucose involved in the synthesis of ZnO nanoparticles and bulk ZnO FTIR also taken. The sample was mixed with KBr and then pressed into thin pellet. Infrared spectra were measured at the wavelength in the range of 400-4000 cm^{-1}.

Determination of antibacterial activity of ZnO nanoparticles

The antibacterial activity of the ZnO nanoparticles were examined against *E. coli* and *Pseudomonas aeruginosa* (which was isolated from mint extract and freezed ice cream and the microorganism could be identified by biochemical tests followed by Bergeys manual) in Luria Bertani broth (LB). The 24 hrs old bacterial cultures were inoculated into LB Broth supplemented with various concentrations (20 µl, 50 µl, and 100 µl) of ZnO nanoparticles. ZnO free LB broth was used as control. The broth containing conical flasks were incubated at room temperature under stirring for 24 hrs and the vulnerability of the tested organisms was observed by taking optical density at 600nm for various time intervals

Antifungal activity of ZnO nanoparticles

To determine the antifungal activity of ZnO nanoparticles, the *Aspergillus niger* (isolated from bread) was grown in Sabouraud dextrose agar. The ZnO nanoparticles were taken at different concentrations like 100 µl, 200 µl, 300 µl and 400 µl. The culture was inoculated and then well was formed

in the medium. Further, the plates were incubated at room temperature and after 48 hours of incubation the growth inhibition was examined by the formation of zone.

Results and Discussions

ZnO nanoparticles have some outstanding properties such as optical, electrical, catalytic, electronic, and antibacterial, UV Absorption, as well as low cost and wide application in various fields (Tang *et al*., 2006). The ZnO nanoparticles were obtained by using wet chemical method. The formation of ZnO nanoparticles was explained in the equation 1 and the reactions as follows,

$$ZnCH_3(COO)_2.6H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2NaCH_3(COO)_2$$
$$Zn(OH)_2 \xrightarrow{80\,^{\circ}C} ZnO + H_2O \quad (1)$$

The UV absorption spectrum was taken for ZnO nanoparticles. Figure 1 shows the absorption spectrum of ZnO nanoparticles, the UV spectrum reveal a characteristic absorption peak located at 370 nm.

Figure 2, shows the X-ray diffractometer pattern of the Bulk ZnO (2A) and prepared ZnO nanoparticles (2B). The diffraction pattern and spacing closely matched to the diffraction pattern of the bulk ZnO (Yadav *et al*., 2006). The peaks allotted to diffractions from various planes correspond to hexagonal close packed structure of ZnO. The peaks at 2θ = 31.73º, 33.02 º, 36.19 º, 47.73 º, 56.57 º, 59.05 º, 67.85 º are assigned to (100), (002), (101), (102), (110), (103), (112). The crystalline size of the particles was determined using Debye-Scherrer equation (2).

$$D = \frac{K\lambda}{\beta_{1/2}COS\theta} \quad (2)$$

Here k is scherrer constant, λ the wavelength of X- ray, β the peak width at half of maximum, θ is the Bragg diffraction angle. The size of the wet chemically synthesized ZnO was calculated using three main diffraction peaks by scherrer formula and the average crystalline size of the Zinc Oxide nanoparticles was 106 nm.

The functional groups of the bulk ZnO and prepared ZnO nanoparticles were analyzed using FT-IR spectrum. Figure 3A, shows the FTIR spectra of bulk ZnO and Figure 3B, shows the FTIR spectra of prepared ZnO nanoparticles occurred in the range of 4000-400 cm^{-1} at room temperature. In Figure 3A the peak at 3640.63 cm^{-1} and in Figure 3B the peak at 3423 cm^{-1} represents the presence of hydrogen-bonded O-H stretch. In Figure 3A the peaks at 2922.59 cm^{-1}, 2855.05 cm^{-1} shows the C-H Stretch,

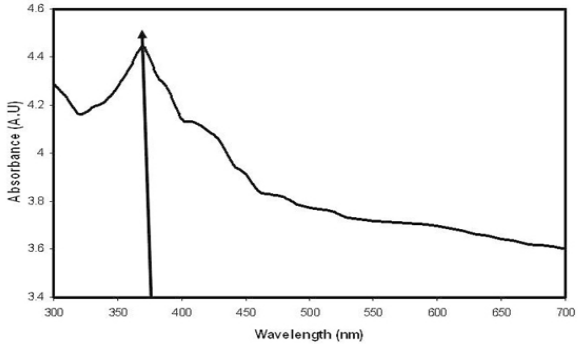

Figure 1. UV visible spectrum of wet chemically synthesized ZnO nano colloidal solution.

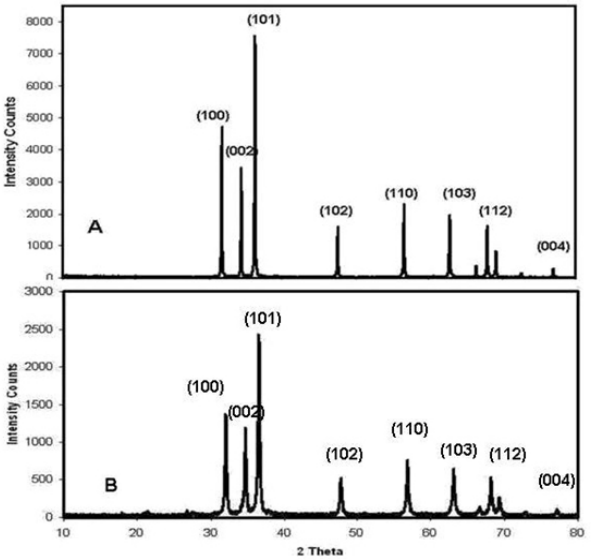

Figure 2. XRD pattern of the bulk ZnO and air dried ZnO nanoparticles using CuKα radiation

The peaks at 2362.37 cm^{-1} represents the CO_2. In Figure 3B the absorption peak at 1583cm^{-1} indicates the presence of nitro group N=O stretch. In Figure 3A and 3B the peak at the range of 1401 cm^{-1}, 1635.34 cm^{-1}, 1404 cm^{-1}, 1336 cm^{-1} corresponds to the carbonate groups (Music *et al*., 2007; Wahab *et al*., 2008). In Figure 3A and 3B the bands incidence at 1119 cm^{-1}, 1019.19 cm^{-1}, 759.81 cm^{-1}, 676.96 cm^{-1}, 614.21 cm^{-1}, and 1026 cm^{-1}, 925 cm^{-1}, 675 cm^{-1}, and 615 cm^{-1} illustrates the chemical bonding, crystal structure and relative intensities of the IR bands of the carbonate (Goldsmith *et al*., 1966; Wahab *et al*., 2008). In Figure 3A and 3B, the band around 542.86 cm^{-1}, 424.26 cm^{-1}, and 471.36 cm^{-1} was associated to the stretching vibration of ZnO (Wahab *et al*., 2008).

The wet chemically synthesized Zinc Oxide nanoparticles morphology was characterized using HITACHI Model S-3000H. The SEM image was taken at X 40,000 magnification and the wet chemically synthesized and dried (80ºC) Zinc Oxide nanoparticles is seen clearly in Figure 4. Shows the SEM Image of ZnO nanoparticles. The image shows

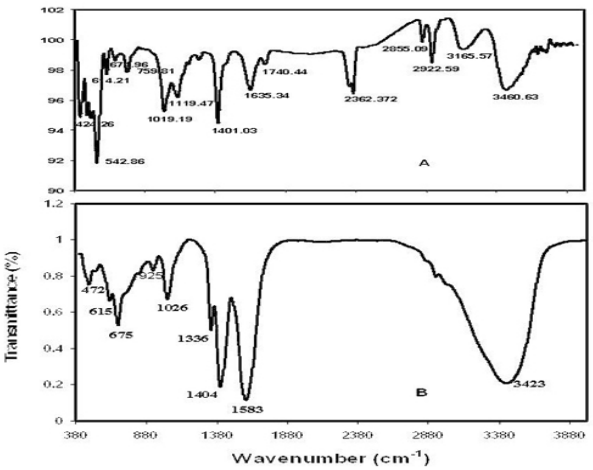

Figure 3. FTIR spectrum of bulk ZnO and ZnO nanoparticles obtained by wet chemical method

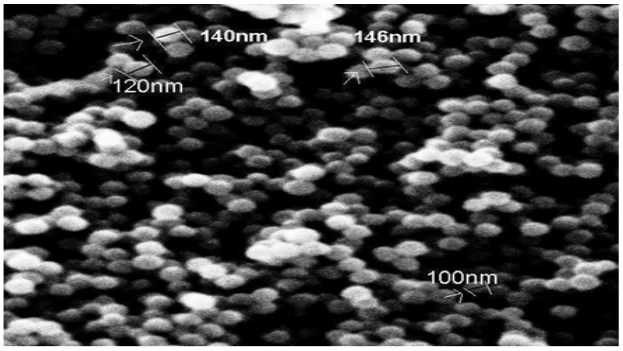

Figure 4. Scanning Electron Microscopic image of ZnO nanoparticles at x40,000 magnifications

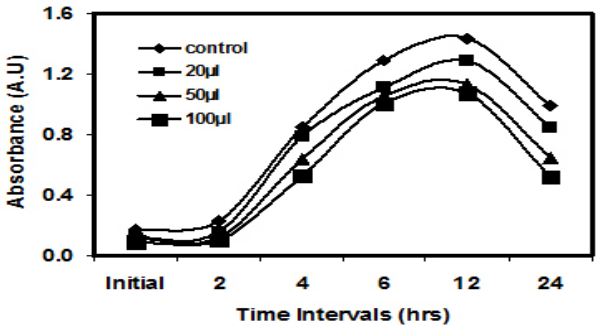

Figure 5. Growth curves of *E. coli*, LB broth containing various concentrations of ZnO nanoparticles and negative control without ZnO nanoparticles

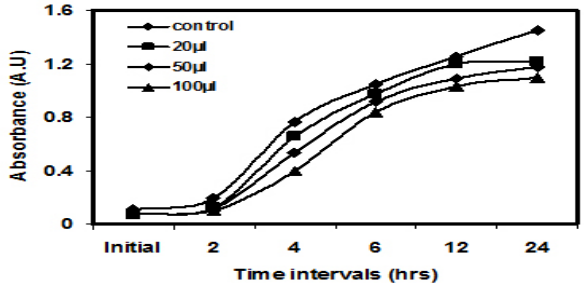

Figure 6. Growth curves of *Pseudomonas auerogenosa*, LB broth containing various concentrations of ZnO Nanoparticles and negative control without ZnO nanoparticles

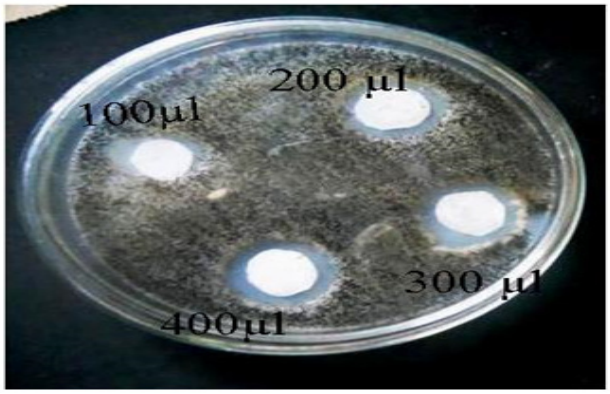

Figure 7. Antifungal activity of ZnO nanoparticles against *Aspergillus niger*, shows the inhibition of growth after 48 hours of incubation

ZnO nanoparticles are spherical in shape with smooth surface and the size of the nanoparticles around 100-146 nm.

Figure 5 and 6, shows the antibacterial effect of silver nanoparticles against *E. coli* and *Pseudomonas aeruginosa* in LB broth. The inhibitory effect of various concentrations of ZnO nanoparticles (20 µl, 50 µl, and 100 µl) was examined through UV-vis spectrophotometer by taking optical density values (OD). The growth rate of biomass was compared with and with addition of ZnO nanoparticles with biomass. The growth rate of *E. coli* and *Pseudomonas aeruginosa* was decreased at the increased concentration of ZnO nanoparticles and the maximum inhibition of growth was obtained at 100 µl. Similarly, Li *et al*. (2009) reported the antibacterial effect of ZnO powder coated PVC film against Gram-positive and Gram-negative bacteria. The larger surface area and higher concentration are responsible for the antibacterial activity of ZnO nanoparticles (Zhang *et al*., 2007; Peng *et al*., 2011). The previously reports demonstrated that the generation of H_2O_2 from ZnO leads to the penetration of particles into the cell membrane of bacteria leads to the formation of injuries and finally the death of bacterium was occurred (Sawai *et al*., 1996; Li *et al*., 2009). Conversely, the electrostatic interaction between bacterial cell surface and nanoparticles may be one of the reasons for the inhibition of growth (Stoimenov *et al*., 2002; Li *et al*., 2009). Based upon the above possible phenomena, our present study reports the growth inhibition of *E. coli* and *Pseudomonas aeruginosa* was obtained by damage the cell membrane through penetration of ZnO nanoparticles. This proves that the ZnO nanoparticles synthesized by wet chemical method can be used in food preservation and packaging.

As per the literature survey there is limited studies carried out for antifungal activity of ZnO nanoparticles. Figure 7, shows the inhibitory

effect of ZnO nanoparticles against the fungus Aspergillus niger. Herein, the maximum inhibition of fungal growth was achieved at 400 µl and the figure 7 exhibits the increased concentration of ZnO nanoparticles resulting in the decreased growth rate of *Aspergillus niger*. Previous works have been reported the antifungal activity of ZnO nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. They suggested that the cell functions of *Botrytis cinerea* was affected by ZnO nanoparticles due to increased production of nucleic acids through the stress response in fungal hyphae leads to the death of cells (He *et al*., 2011b). Whereas in *Penicillium expansum* (ZnO nanoparticles treated cells) the release of protein, carbohydrates and lipids through the damaged cell membrane results in the decreased amount of proteins, carbohydrates and lipids in fungal cells leads to death of the cells (Peral *et al*., 2002; He *et al*., 2011b).

Conclusion

A simple, rapid inexpensive method has been developed to prepare ZnO nanoparticles. The present developments in nanotechnology were evaluating for their possible to enhance food safety. The results proved that the ZnO nanoparticles have potential to be used as a antimicrobial agent. The maximum inhibition was occurred at 50 µl for both two bacteria. The maximum inhibition of growth for Aspergillus niger was achieved at 400 µl and in both the bacterial and fungal growth was decreased at the increased concentration of ZnO nanoparticles. Due to the intense antimicrobial, non toxic nature and other noble properties the ZnO nanoparticles can be used in food nanotechnology as a food preservation material.

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