Application of semi-solvo thermally synthesized zinc oxide (ZnO) nanoparticles in food technology and their characterization

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Abstract

The problems posed by the foodborne pathogens in food industries associated with contamination of food stuffs can be solved by ZnO nanoparticles. In this trend, ZnO nanoparticles are very significant because they are safe, biocompatible economic and can be synthesized in large amount by proposed method. Present study reports on the antibacterial applications of ZnO nanoparticles synthesized by semi-solvo thermal method and their characterization. Characterization of zinc oxide powder was carried out by using UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The absorption spectrum of ZnO was found to be at 338 nm, SEM results showed accumulated nugget like morphology and which was characterized by TEM images. The antibacterial activity against gram negative model bacteria Pseudomonas aeruginosa reveals their effectiveness to be used in food technology for prevention of food spoilage and pathogenicity.

Keywords: ZnO nanoparticles, antibacterial activity, semi-solvo thermal, food technology, Pseudomonas aeruginosa
1. INTRODUCTION
The contamination of food stuffs by different microorganisms is one of the serious concerns for consumers and food industries, since the growth and metabolism of microorganisms can cause serious foodborne diseases and a rapid spoilage of the food products. The exceptional properties of nanoparticles like quantum size effect, surface to volume ratio, morphological variations etc., and give assorted ideas to do research on nanostructure edifice for applications in different areas [1]. Though there are several types of nanostructures offered now a days, metal oxides constantly attract great attention in the field of nanotechnology because metal element forms a stable compound with oxygen and manage to develop several geometrical forms with typical band gap and binding energy of particular structures [2]. Metal oxide nanoparticles attain greater electronic properties due to change in energy level and have high density on surface because of its nano size [3] compare to their bulk counterparts. Amongst all other metal oxides, zinc oxide nanoparticle attracts a greater attention because of its physical and chemical properties and its biocompatibility. Therefore, ZnO nanoparticles can be used as an alternative antimicrobial agent for protection of food from different food pathogenic bacteria.

Although most of the potential food pathogens in food stuffs are heat sensitive and can be eradicated by pasteurization process, the food industries face potential economic losses due to damage of nutrients and freshness caused by few heat resistant microorganisms which can survive current pasteurization process [4, 5]. At present, as consumers are demanding fresh, nominally processed, preservative free food products to eat, it is required to protect food to preserve its freshness and nutritive value [5]. To do so, variety of substances like organic acids, phenolic compounds, essential oils, bacteriocins etc. have been investigated to substitute chemical preservatives from food products [6-8] but these substances have certain limitations, they either alter the sensory attributes of food or they are costly. Thus, food processors need alternative antimicrobial substances which are functionally effective without altering physico-chemical and organoleptic properties of the food, and which also cost effective. Pseudomonas aeruginosa is an abundant gram negative bacterium and was isolated from tomatoes, radishes, celery, carrots, endive, cabbage, cucumbers, onions and lettuce [9, 10]. Pseudomonas aeruginosa was known to show consistently high resistance to available antimicrobials hence, need to find out alternative [11]. Therefore, fabrication of cost effective antibacterial nanomaterials which should be inert to the nutrients of food stuffs has become supreme mandate.

Here we present synthesis of zinc oxide (ZnO) nanoparticles by semi-solvo thermal method and their study to inhibit the growth of leading food borne pathogenic bacteria, Pseudomonas aeruginosa.

2. MATERIALS AND METHODS
2.1 Synthesis and characterization of ZnO nanoparticles
Synthesis of ZnO nanoparticles were carried out by semi-solvo thermal route [12]. 100 mM zinc acetate was dissolved in 50 mL Distilled water. 600 mM urea solution
was prepared in 50 mL 40% ethylene glycol. Homogenous reaction mixture was obtained by mixing two solutions under constant stirring which was then transferred to Teflon-lined stainless steel autoclave. Reaction mixture was incubated at 160°C for 18 hours in muffle furnace and then allowed to cool down at room temperature. The precipitate was washed with distilled water by centrifugation at 5000 rpm for 15 minutes. The resultant powder of ZnO underwent calcination process at 400°C for 8 hours to remove volatile impurities. The optical and morphological features of resultant ZnO were characterized by UV-Vis spectroscopy, TEM and SEM for identifying optical, morphological and structural information. Absorbance of synthesized material was measured by UV-Vis spectroscopy (Thermo Scientific UV-10).

2.2 Enumeration of antibacterial activity of ZnO
The antibacterial activity of ZnO nanoparticles were tested on *P. aeruginosa* responsible for food contamination. The proposed assays were performed in both qualitative and quantitative manner. The qualitative enumeration of antibacterial activity of ZnO was carried out by agar well diffusion assay (AWDA) [13]. The Muller-Hilton (MH) agar plates were spread inoculated with overnight grown cultures of *P. aeruginosa*. The synthesized ZnO nanoparticles were dispersed in sterile deionized water by ultra-sonication to make colloidal solution of nanomaterials. On the surface of agar plates wells of 5 mm in diameter and of 18 µL in capacity were formed by using sterile gel borer. The 15 µL of ZnO solution were placed in each well and all plates were incubated at 37°C for 24 hours.

The quantitative enumeration of antibacterial activity of ZnO was performed by plate count method on MH agar plates (in the concentration range of 15 µg - 1000 µg/mL). The test cultures of final cell density of $1\times10^5$ CFU/mL were used for spread inoculation. The plates were incubated at 37°C for 24 hours and subsequent growth inhibition of bacterial cultures was determined. All experiments were performed in triplicates.

3. RESULTS AND DISCUSSION
3.1 characterization of ZnO nanomaterials
UV-visible absorbance is extensively used to study optical properties of nanomaterials [1]. The absorption spectrum of ZnO powder shown in figure 1. The maximum absorption was found at 338 nm. The FESEM image of the synthesized ZnO nanoparticles shows accumulated nugget like morphology with size variation in the range of 80 – 260 nm (figure 2(a)). TEM image of ZnO nanoparticle shows that each nuggets made up of smaller nanoparticulate structures (figure 2(b)). One such structures in figure 2 (b) shows that these nanoparticles structures have size of 20-80 nm.
3.2 Antibacterial activity of ZnO nanoparticles

The antimicrobial activity of chemically synthesized ZnO suspension of 1000 µg/mL concentration were tested on model microorganisms and were found to have antibacterial activity (Figure 3(a)). As it was shown in the study of Gunalan et al., it has been found in this study that by increasing the concentration of ZnO in wells, the growth inhibition has also been increased consistently because of proper diffusion of nanoparticles in the agar medium. In our study, ZnO nanoparticles showed a greater and significant zone of inhibition against *P. aeruginosa*. 

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**Figure 1:** UV-Vis Spectrophotometry of ZnO nanoparticles

**Figure 2:** (a) SEM (b) TEM images of ZnO nanoparticles
MIC and MBC of ZnO suspension were determined by incubation of test bacteria with different concentration of ZnO in the range of 15 µg - 1000 µg/mL (Figure 3(b)). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ZnO nanomaterial was found to be 15 µg/mL and 30 µg/mL, respectively, for P. aeruginosa. The antibacterial activity of ZnO nanoparticles may be due to the oxygen species released by the nanostructure which react with hydrogen ions to produce H$_2$O$_2$, causing fatal damage to microorganisms by penetrating the cell membrane and killing bacteria [15, 16]. The results of this study may be useful to food packaging materials like polymers that are coated with nanoparticles to stop the growth of food pathogens.

![Figure 3](image)

**Figure 3:** (a) Antibacterial activity of ZnO nanoparticles against P. aeruginosa, (b) Determination of MIC & MBC of ZnO nanoparticles against P. aeruginosa In the given figure (b); are given from left to right plates of control, 15 µg/mL, 30 µg/mL, 60 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL & 1000 µg/mL

4. CONCLUSION

Semi-solvo thermally synthesized ZnO nanoparticles have nugget like morphology with average size of 80 nm. ZnO nanoparticles were found to be effective antibacterial agent against gram negative food pathogen, P. aeruginosa. The MIC and MBC of ZnO nanoparticles were found to be 15 µg/mL and 30 µg/mL, respectively, for P. aeruginosa. In summary, ZnO nanoparticles can be used in packaging systems for protection of food stuffs from food pathogens.

REFERENCES


