

Antimicrobial Activity of zero-valent Iron Nanoparticles

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ABSTRACT

This work reports on the toxicity of ZVIN nanoparticles on gram-negative and gram-positive bacterial systems, *Escherichia coli* and *Staphylococcus aureus*. Detailed characterization of the nanoparticles using x-ray diffraction (XRD), scan electron microscopy (SEM) confirmed the presence of 31.1nm sized ZVIN particles. Further, *St. aureus*, *E.coli* were grown in the presence of different ZVIN nanoparticles concentrations for 24 hours. MTT assays were performed and the results provide evidence that ZVIN nanoparticles. FeO nanoparticles MIC of *E. coli* and *St. aureus* at concentrations 30 µg/ml, where as growth completely inhibited at concentrations 60 µg/ml.

Keywords- Nanotechnology, ZVIN, bactericide effect, *Staphylococcus aureus*, *Escherichia coli*.

I. INTRODUCTION

In the rapidly emerging field of nanotechnology, metal nanoparticles are extensively used in drug delivery [1], biosensors [2], bio imaging [3], antimicrobial activities [4], food preservation [5] etc. by exploiting their unique physical chemical and biological properties. There has been a great interest in using microorganisms as a tool for synthesis of new functional inorganic nanomaterials [6, 7] which are free from any kind of toxic chemicals and byproducts. Iron oxide (IO) has been widely used in biomedical research because of its biocompatibility and magnetic properties. [8] IO nanoparticles, with sizes less than 100 nm, have been developed as contrast agents for magnetic resonance imaging (MRI), [9, 10] as hyperthermia agents, [11, 12] and as carriers for targeted drug delivery to treat several types of cancer. [13,14] It is further believed that through the use of magnetic nanoparticles, an optimal drug delivery system can be developed by using an external magnetic field to direct such nanoparticles to desirable sites (such as implant infection) for immediate treatment.

Several recent studies have reported on the antimicrobial activity of nanoparticulate zero-valent iron (ZVIN) [15, 16]. We previously found that ZVIN exhibited a stronger antimicrobial activity than other iron-based nanoparticles, and that the inactivation of *E. coli*, *Staphylococcus aureus* by ZVIN was greater under deaerated than air-saturated conditions [17].

The scope of the present study is the synthesis of NZVI particles from ferrous sulfate, and were characterized using scanning electron micrograph (SEM) and X-ray diffraction (XRD).and study its antimicrobial activity against *St. aureus* and *E. coli* in MTT assay.

II. MATERIAL AND METHODS PREPARATION OF NZVI

The NZVI particles were synthesized by the well-known liquid phase reduction method [18-19]. 10 mmol (2.78 gm) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in 100 ml of an aqueous solution of ammonium persulphate ($(\text{NH}_4)_2\text{S}_2\text{O}_8$). 1.85 g of sodium borohydride (NaBH_4) was dissolved in 50 ml of distilled water. This solution was added drop wise into the above solution. After addition, this reaction continued for 5 h with constant stirring. The solution was centrifuged for 10 min at 6000 rpm and the supernatant was discarded. The pellet was washed with ethanol three and then dried in vacuum.

MICROORGANISMS AND CULTURE MEDIA

Pure cultures of *E. coli* (MTCC 118) and *St. aureus* (MTCC 96) were obtained from Microbial Type Culture Collection (India), were inoculated in 50 mL of Mullar Hinton Broth (Difco Co., Detroit, Mich.) medium and grown at 37°C for 18 h. According to the standard curve correlating bacteria number with optical density, this value was equivalent to 5×10^6 cells/mL

Antimicrobial activity of ZVIN nanoparticles by MTT assay

To measure the activity of living cell by assessing the activity of the bacterial dehydrogenase enzymes. 95µl of the freshly prepared Muller Hinton Broth and the different concentration of ZNVI nanoparticles (10µg, 20µg.....100µg) were added and the plates were kept for incubation at 37°C for 24 hours. 5mg of MTT(3-(4, 5 -Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was weighed and dissolved with 1 ml of milli Q water and 10 µl of this preparation is added to each well and kept for 4 hours incubation. The contents were collected and centrifuged at 8000 rpm for 15 minutes, and the pellets were dissolved with 100µl of Dimethyl sulphoxide. Then the contents were transferred to the appropriate well and read at 570 nm in the ELISA reader. The percentage of viable cells was calculated using the following formula.

$$\% \text{ Dead cells} = \frac{\text{Control O.D} - \text{Test O.D}}{\text{Control O.D}} \times 100$$