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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 growt completely inhibited at concentrations 60 µg/ml.

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

I. INTRODUCTION

In the rapidly emerging field of nanopiotechnology, 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 microgram (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 FeSO4 .7H2O was dissolved in 100 ml of an aqueous solution of ammonium persulphate ((NH4) 2S2O8). 1.85 g of sodium borohydrate (NaBH4) 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 × 106 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 ofZNVI nanoparticles (10µg, 20µg……100µg) were added and the plates were kept for incubation at 37ºC 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.

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

III- FIGURES AND TABLES

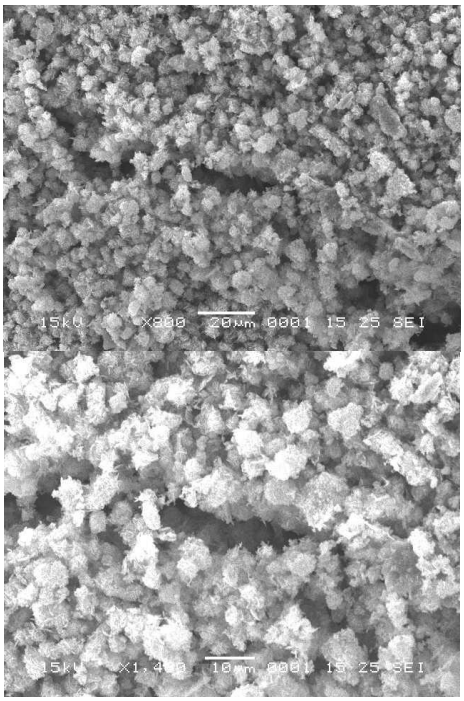

Fig.1 SEM images of NZVa: at 20 µm / 200 X. b: at 10 µm / 1000 X

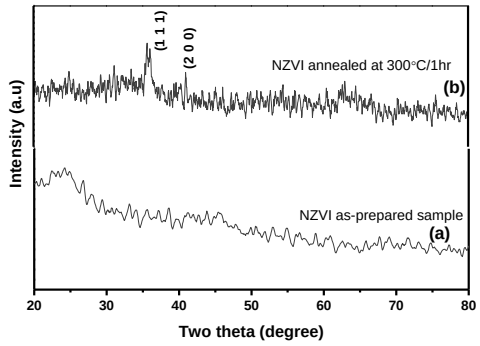

Fig.2 X-Ray Diffraction analysis of NZVI samples.

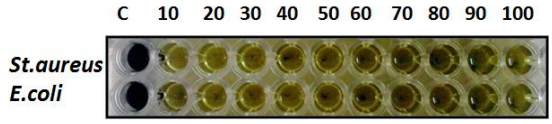

Fig.3 The effect of ZVIN on bacterial growth in MTT plate

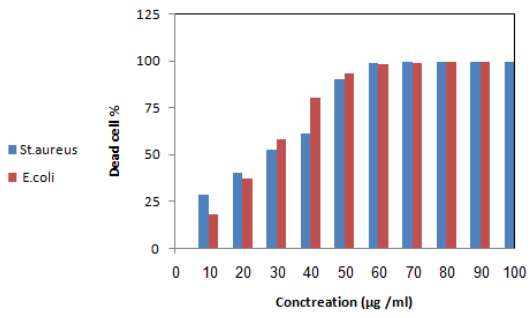

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Fig.4 Antimicrobial Activity

IV. RESULTS AND DISCUSSION

NZVI SYNTHESIS AND CHARACTERIZATION

The scanning electron microscopy (SEM) image of synthesized NZVI particles is shown in Fig 2. Results indicate that the synthesized NZVI particles are almost spherical. Fig.2 (a) shows evenly distributed spherical particles approximately 2µm in size, and Fig.2 (b), under higher magnification, confirms the spherical shape and the size range of each particle. On the spherical particles there were threads-like or tube-like structures clearly visible in Fig.2 (b). These structures increased the available surface area of reaction. Fig.3 (a) shows the X-ray diffraction pattern of the as-prepared NZVI sample. The as-prepared samples are amorphous, as no diffraction peaks appear. Fig.3 (b) shows the X-ray diffraction pattern of NZVI annealed at 300°C in air for 1 h. The spectrum shows two major diffraction intensity peaks at 2θ = 36.08° and 41.01°. The peaks were identified to originate from the (1 1 1) and (2 0 0) planes of FeO respectively (JCPDS no: 772355). The X – ray could be indexed to the $Fm^{-}3m$ (225) face group (Face – centered) cubic structure, with cell parameter a = 4.309 Å.

The information of the particle size was obtained from the full width at half maximum (FWHM) of the diffracted beam using the sherre: The crystalline size is calculated using the Debye-Scherrer formula :

$$D = \frac{0.9\,\lambda}{\beta \cos 6}$$

The sample annealed at 300°C/1h has an average crystalline size of 31.1nm ± 0.5. As the annealing time or temperature increases, the crystalline size increases.

The antimicrobial activity of FeO nanoparticles on bacterial growth are shown in figure 3 was determined by MTT assay. In this experiment E.coli and St.aureus test strains were inoculated in Muller Hinton medium supplemented with different concentrations of FeO nanoparticles. The MIC values for the FeO nanoparticles were 30µg/ml, for both. Increasing concentration of FeO nanoparticles substantially inhibited the growth of E.coli and St.aureus test strains completely inhibited in 60 µg/ml shown in figure 4.

There are several factors that caused the presently studied FeO nanoparticles to be bactericidal. The main mechanism by which antibacterial drugs and antibiotics work is via oxidative stress generated by ROS.[20]ROS, including superoxide radicals(O2-), hydroxyl radicals (-OH), hydrogen peroxide (H2O2), and singlet oxygen (1O2), can cause damage to proteins and DNA in bacteria.[21] In this case, metal oxide FeO could be the source that created ROS leading to the inhibition of St. aureus. A similar process was described by Keenan et al in which Fe2+ reacted with oxygen to create hydrogen peroxide. This H2O2 consequently reacted with ferrous irons via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macromolecules.[22] Other research has demonstrated

that the small size of nanoparticles can also contribute to bactericidal effects. For example, Lee et al reported that the inactivation of Escherichia coli by zero-valent iron nanoparticles [23] could be because of the penetration of the small particles (sizes ranging from 10–80 nm) into E. coli membranes. Nano-Fe0 could then react with intracellular oxygen, leading to oxidative stress and eventually causing disruption of the cell membrane. Several other studies on ZnO and MgO nanoparticles also concluded that antibacterial activity increased with decreasing particle size. [24,25] In this study, the concentration of nanoparticles was a major contribution to St. aureus activity inhibition. A similar concentration-dependent behavior was observed by Kim et al when they investigated the antimicrobial effects of Ag and ZnO nanoparticles on St. aureus and E. coli. [24,25] In a study of bactericidal effects of IO nanoparticles on St. epidermidis, Taylor et al also reported concentration dependent bacteria inhibition.[26] Briefly, St. epidermidis density progressively decreased at time points of 12, 24, and 48 hours when incubated with 100 μg/mL, 1 mg/mL, and 2 mg/mL IO. It is also important to note that IO nanoparticles do not negatively influence all cells. Specifically, osteoblast (boneforming cell) proliferation was enhanced in the presence of Fe2O3 nanoparticles (at 4.25 mg/mL. [27] Such results showed that FeO nanoparticles could have a dual therapeutic function which can enhance bone growth and inhibit bacterial infection. Lastly, this present study provided evidence that with an appropriate external magnetic field, FeO nanoparticles may be directed to kill bacteria as needed throughout the body.

V. CONCLUSION

Stable FeO nanoparticles were successfully synthesized. The particles were characterized with SEM, dynamic light scattering, XRD. A live/dead assay showed that at the highest dose of iron oxide (30μg/mL), the growth of E. coli and S. aureus was inhibited significantly compared with the control samples. Indicates that ZVIN have potential for use as antimicrobials. ZVIN has several advantages such as low cost, easy preparation, and high reactivity compared to other metal nanoparticles.

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