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Analytical characterization and antimicrobial activity of nano zirconia particles

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ABSTRACT

Metal oxide nanoparticles have been of substantial intrigue with its wide biological applications in the fields of Nano biotechnology. The sizeable surface area to volume proportion, very low thermal conductivity, high strength, heat insulating properties and pliability has made zirconium oxide nanoparticles extremely useful in medical field, especially as dental filling composites and orthopedic implant materials. They may also have notable antimicrobial properties that might be due to this increase in their surface area and also because their distinctive crystal planes. In the present study, FTIR and UV-VIS Spectra were recorded to characterize the commercial yttrium stabilized zirconium oxide nanoparticles. Their particle size, zeta potential and electrophoretic mobility were determined. Their EDX analysis was also carried out. The nanoparticles were dispersed in water and their activity against several MTCC bacterial cultures and isolated skin flora was assessed.

KEYWORDS: Zirconium oxide nanoparticles, Nanoparticle characterization, antimicrobial activity

1. INTRODUCTION

Nanotechnology in the last decade has shown tremendous advance in several areas of research with numerous promising applications (Jangra, 2012). Nanomaterials are used extensively in various fields due their unique physiochemical properties that are not normally found in isolated or bulk solids Rao, 2007. In this context, metal oxide nanoparticles are of great importance due to their interesting properties like high melting points, resistance to acids and alkalis and also importantly, their anti-microbial properties.

Zirconium oxide is one such promising metal oxide nanoparticle, which adds value to several structural and functional materials. Zirconium oxide is often called ceramic steel, as it is used as an abrasive. It is incorporated into many cutting tools and is used to make engine components and furnaces (Rao, 2007). In the medical field, it is emerging as a significant catalyst. It is also used for dental fillings, for making dental crowns and for therapeutic purposes Kumar, 2012. They are currently being employed as an alternative to titanium based materials as the latter leads to severe localized reactions and also pain. Zirconium oxide powders are also used in separation chromatography as they act as supporting surfaces for separation of proteins and enzymes. This gives valuable information about the absorbent behavior of various functional proteins present in living entities and material dyes Cazan, 2014.

ZrO_2 is found naturally in its impure form and is usually extracted chemically from two minerals: zircon and babbelyite (Cazan, 2014). Nanoparticles can be synthesized by different physio-chemical methods like sol-gel synthesis, aqueous precipitation method, thermal decomposition and hydrothermal methods that sometimes require extreme temperatures (Gowri, 2014; Balantrapu, 2014; Rodriguez-Sanchez, 2000; Taleb, 1997). The extraction of zirconia from aloe vera is a widely used technique, as it is less toxic and beneficial to the environment.

Recent years have shown the use of nanoparticles in the field of human health for tumor imaging, cytotoxic activity and drug delivery. Chemotherapeutic drugs are usually found to be toxic to the body because they are unstable in the blood and are highly insoluble. Since they hardly stay in the body they are flushed out faster, and the drugs are not released in the affected area. Therefore high doses are usually manifested so that there is a therapeutic effect. This may lead to toxicity. To avoid such complications, metal oxide nanoparticles are coated with these drugs, thus enhancing the efficiency of the drugs (Rao, 2013). Zirconium oxide is widely used for dental implants and used for imitation of bone tissue and hair. It is highly resistant to bacterial plaque and its high rigidity makes it fracture resistant. It is usually ivory colored but can be dyed to various colors to make better imitations. Recently it has been used for the production of bone-anchored hearing aids (Cazan, 2014). Additionally, it has been reported that these particles show anti-microbial effects against specific microbes and this knowledge is of considerable importance in the health care industry as large numbers of continuously evolving antibiotic resistant strains of microorganisms represent a significant health concern. Hence an unconventional antimicrobial agent, like ZrO_2nanoparticles, that can inhibit a wide range of microorganisms can be exploited to address this issue. However, some studies conclude that certain metal nanoparticles have detrimental effects on both the ecosystem and mankind in general Banerjee, 2015. The results are diverse and range from these particles being reported as toxic (Amato, 2012), partially poisonous (Sengupta, 2014), or completely harmless (Tong, 2007). Hence these particles maybe employed, albeit carefully for its versatile applications.

In the present study, commercial yttrium stabilized ZrO_2 nanoparticles have been characterized by UV, SEM, FTIR and particle size analysis. Additionally, its concentration dependent activities against several standard MTCC cultures and normal skin flora have been assessed to determine its suitability not only for human handling but also

to explore its use as an antimicrobial agent. The results of the study also establish its hazard potential towards the ecosystem during its life cycle.

2. MATERIALS AND METHODS

Requirements: Standard isolates of *Escherichia coli* MTCC 443, *Proteus mirabilis* MTCC 9493, *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 737, *Serratia marcescens* MTCC 7298, and *Vibrio parahaemolyticus* MTCC 451 were obtained from Microbial Type Collection Centre (MTCC), Chandigarh, India.

Chemicals: All media components were procured from Hi media. Yttrium stabilized ZrO_2 nanoparticles were kindly donated by CSIR-NAL, Bangalore, India.

Characterization of the nanoparticles:

Particle size analysis: The mean particle size, zeta potential and electrophoretic mobility of the nanoparticles were measured using Horiba Scientific, SZ 100 instrument. The scattering angle was taken to be 90º, the dispersion medium viscosity was 1996 mPa.s and the temperature for the study was 25ºC respectively. The count rate of the nanoparticles was 1154 kCPS.

FT-IR spectroscopic analysis: The nanoparticles were characterized for functional group analysis using FTIR spectrophotometer (Bruker Optics) at room temperature using attenuated total reflection procedure with diamond crystal. Spectral range of 4000-500 cm^{-1} was chosen for the analysis (Sangappa, 2013).

UV-Vis spectroscopic analysis: UV-Vis spectrophotometer (Perkin Elmer Lambda 35) was used to measure the absorption spectra of the nanoparticles at 25^0C, in the range of 200-800 nm (Sangappa, 2013). The readings were taken at intervals of 10nm between 200-300nm and 50nm between 300-800 nm.

Antimicrobial Activity: Isolation and identification of skin flora: To estimate the toxicity of ZrO_2 towards skin commensals, skin flora was collected and spread on plates containing nutrient agar, using sterile cotton swabs. The plates were incubated for 24 hours at 37ºC.The isolated colonies were subjected to gram staining and their colony characteristics were recorded. Distinct agar slants were then used to subculture these colonies.

Activity against skin flora: Kirby Bauer well diffusion method was employed to determine the antimicrobial activity of the nanoparticles against isolated microorganisms from the skin (Jangra, 2012; Azam, 2012). Nutrient broth was used to inoculate the isolated colonies and it was incubated at 35ºC ± 2ºC for 24 hours and swabbed on Muller Hinton agar plates. Wells of about 5mm were punched into the plate with a sterile well borer into which 50µg/ml, 100µg/ml, 200µg/ml of the nanoparticle suspension was added. Streptomycin, penicillin, ciprofloxacin antibiotic discs served as positive controls. The plates were incubated overnight at 37^0C and then checked for zones of inhibition.

Activity against MTCC Cultures: The antimicrobial activity of the nanoparticles were examined against six MTCC bacterial cultures and analyzed by the well diffusion method as reported earlier (Jangra, 2012; Azam, 2012). The test cultures were inoculated into nutrient broth and incubated at 37^0C for 24 hours and then swabbed onto Muller Hinton agar plates using sterile swabs. The concentration of the nanoparticles prepared was same as above. 50µg/ml, 100µg/ml, 200µg/ml of samples were added to the wells. Antibiotic discs of bacitracin, ciprofloxacin, streptomycin, chloramphenicol and rifampin were used as positive controls. The plates were incubated at 37^0C and observed for zones for inhibition.

3. RESULTS AND DISCUSSION

The nanoparticles were characterized by particle size analysis, SEM, FTIR and UV-Vis Spectroscopy

Particle Size analysis: From figure 1, the mean size distribution of the nanoparticles was found to be 282.3nm with a Polydispersity Index (PI) of 2.0.

Zeta potential and electrophoretic mobility analysis: The Zeta Potential measures the electric charge on a nanoparticle surface and gives information on the stability of the particle Qasim, 2015. Zeta potential here was found to be 0.1mV as seen from figure 2. A low value suggests that the repulsive forces are greater than the attractive forces between the particles and hence it would lead to rapid coagulation or flocculation in water. The electrophoretic mobility, that depicts the movement of the particles, was found to be 0.000001cm^2/Vs.

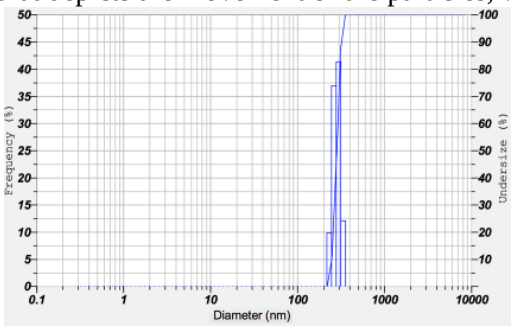


Figure.1.Particle size analysis of ZrO_2 nanoparticles

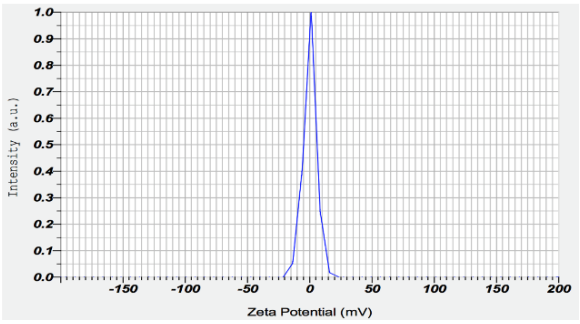


Figure.2.Zeta potential of ZrO_2 nanoparticles

FT-IR analysis: FTIR analysis was performed to identify the functional groups, if any,that were present in the compounds usually added to the nanoparticles for their stabilization. Figure 3 shows the FTIR spectrum. The peaks found in the range of 500 to 700 $cm^{-1}$are characteristic of tetragonal Zr-O-Zr vibrations (Gowri, 2014). The frequency of the resultant peaks was compared with standard FTIR chart.

UV-Vis Spectroscopy: The nanoparticle was analyzed by UV-Vis spectroscopy. The UV spectrum showed a maximum absorbance at 270nm as seen from figure 4. The peak at 270nm depicts the transition of the electrons of the inner shells (Mahmoud, 2013). The UV-Vis absorption studies of metal and metal oxide nanoparticles studied by other authors have shown similar results (Singh, 2010).

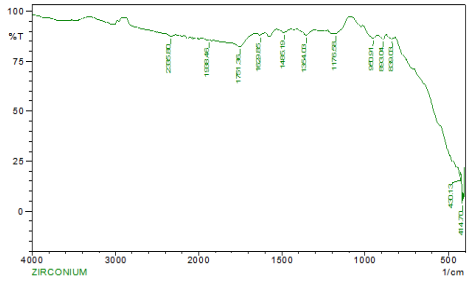


Figure.3.FTIR analysis of ZrO_2 nanoparticles

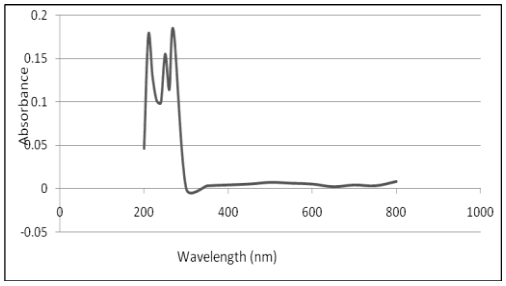
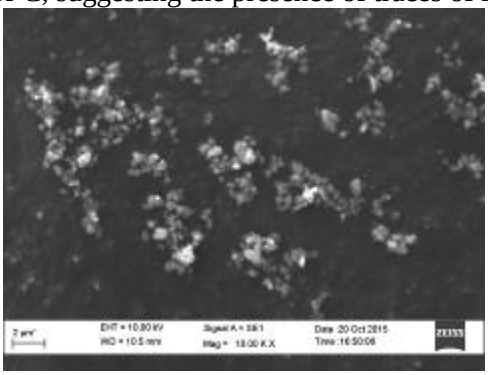
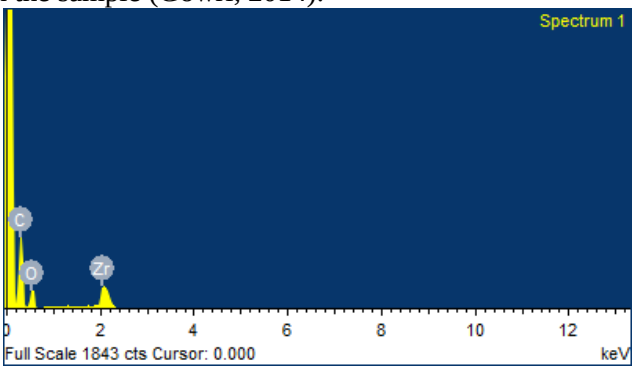


Figure.4.UV-Vis spectrum of 1% ZrO_2 nanoparticles in water

SEM analysis: SEM analysis is done to gauge the morphology of the nanoparticles. Prior studies suggest that zirconium oxide is observed as perfect polydispersed spherical balls under SEM analysis (Mahmoud, 2013). The grain size of the nanoparticle is around 2µm. The structure was spherical with irregular morphology. The corky appearance may be due to particle agglomeration. Crystallization can lead to increase in grain size. Energy-dispersive X-ray spectroscopy analysis confirmed signal characteristics of Zr and O. It also showed the presence of traces of C, suggesting the presence of traces of impurity in the sample (Gowri, 2014).



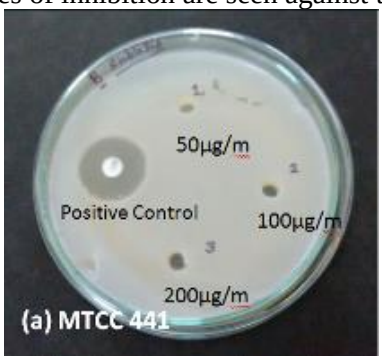
(a)



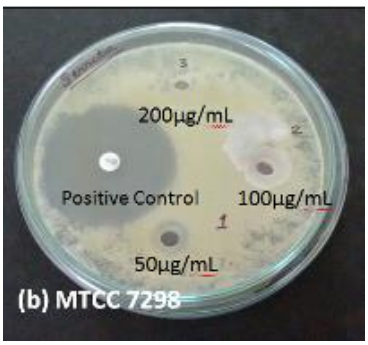
(b)

Figure.5.(a) SEM image of the nanoparticles, (b) EDX analysis of the nanoparticles

Antimicrobial Activity: Antimicrobial activity was assessed using Kirby Bauer well diffusion method. The dilution of nanoparticles for activity studies were 50µg/ml, 100µg/ml and 200µg/ml. A total volume of 100µl of the nanoparticle dispersion was added into the wells. Activity was checked against six MTCC standard cultures and normal skin flora as seen in figures 6 and 7. No zones of inhibition were observed against any of the cultures. Thus, it is inferred that these particles are suitable for handling by humans as they do not harm the skin flora. Also do not disturb the ecosystem, with respect to the experimented organisms, during the nanoparticle life cycle. However, these particles cannot be used as antibacterial agents against pathogenic strains of the studied organisms as no significant zones of inhibition are seen against them.

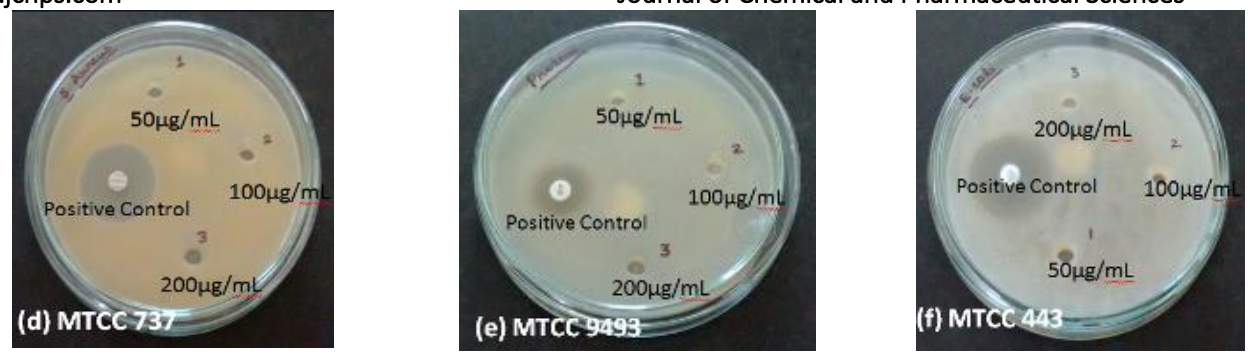


(A)



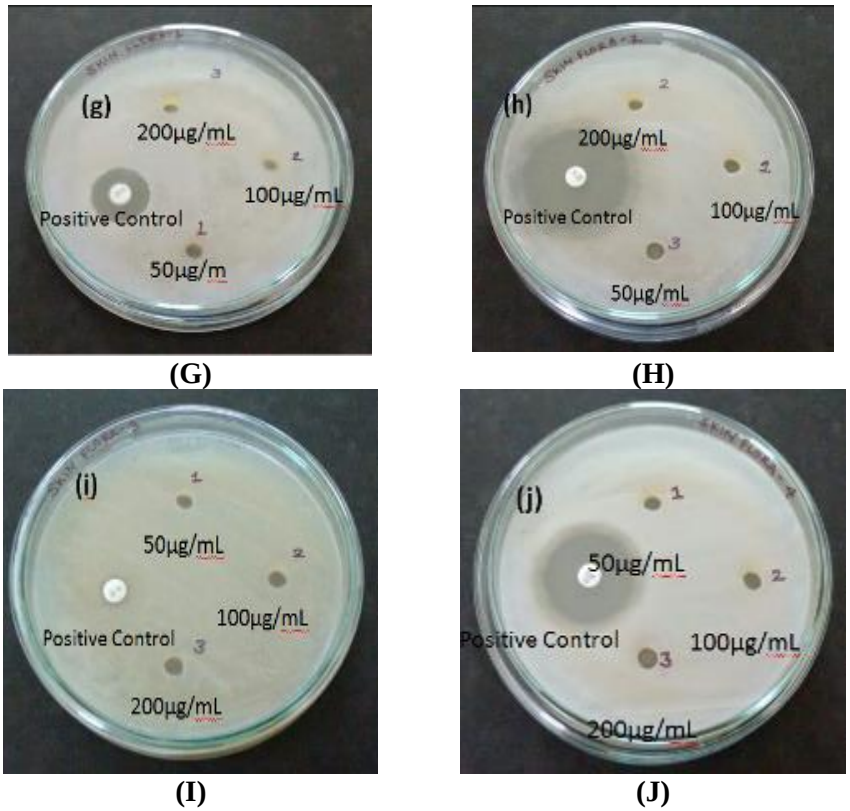
(B)

(C)



(D) **(E)** **(F)**

Figure.6.(a,b,c,d,e,f) Antimicrobial activity against MTCC cultures 441 (*Bacillus subtilis*), 7298 (*Serratiamarcescens)*, 451*(Vibrio parahaemolyticus)*, 737 *(Staphylococcus aureus)*, 9493 *(Proteus mirabilis)*, 443 *(E.coli)* respectively.



(G) **(H)**

(I) **(J)**

Figure.7.(g,h,i,j) Antimicrobial activity against skin microflora *Proteus spp., Bacillus spp., Pseudomonas spp., Staphylococcus spp.* respectively.

4. CONCLUSION

Commercially acquired yttrium stabilized Zirconium oxide particles were characterized using UV-VIS Spectrophotometry, FTIR, Particle size analyzer and SEM. They showed a maximum absorbance at 270nm and a mean particle size of 282.3nm. The dispersed particles in water showed no antimicrobial activity against specific MTCC cultures and the isolated skin flora. Hence the use of these particles in industry may be safe for human handling at the concentrations studied. Also they may not harm the ecosystem during their product life cycle at these concentrations in solution form.

5. ACKNOWLEDGEMENT

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