ANTIBACTERIAL STUDIES WITH METAL OXIDE (ZnO and Fe_3O_4) NANOPARTICLES

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ABSTRACT

In the present paper we report the antibacterial activity of different metal Oxide Nanoparticles which are prepared by combustion synthesis where Zinc nitrate and Ferric nitrate solution are precursors and Urea. Antimicrobial activity of metal Oxide Nanoparticles against sixbacteria *E.coli, P.vulgaris, B.subtilis, S.aureus, Xanthomonas*and*Serratia*have been tested by disc diffusion technique. Metal Oxide nanoparticles exhibited a very strong antibacterial activity against bacterial species. The microbial property of metal Oxide nanoparticles was analyzed by measuring the inhibition zone. Metal Oxide NPs were characterized in order to study different properties of the acquired particles the crystalline size and shape analysis by X-ray diffraction, Thermogravimetric (TG) analysis for weight loss and particle size by Particle Analyzer.

1. Introduction:

With new advances in nanotechnology different metal and metal oxide nanoparticles with antimicrobial activity have been synthesized [1-9]. Metal nanoparticles containing magnesium oxide [6], copper [7, 8], silver [1-5], iron [10], zinc oxide [11- 13], and nickel oxide [14, 15] are exhibit antimicrobial properties. The antimicrobial activity has been observed to vary as a function of surface area in contact with the microbe; therefore nanoparticles with large surface area ensure a broad range of reactions with the bacterial surface [16]. When compared with other methods, the reverse micelle method is one of the most promising wet chemistry synthesis approaches [17] of synthesis of metal nanoparticles. This method provides a favourable microenvironment for controlling the chemical reaction. As such the reaction rate can be easily controlled, and it is possible to obtain a narrow nanoparticle size distribution [18]. The size of the core of the reverse micelle can also be controlled by changing water to surfactant ratio [19].

In order to safeguard successfully the public life antibacterial materials are widely made use in our daily life. Materials of wide-ranging like antibiotics [20] metal ions [21] and quaternary ammonium compounds

[22] are used in check out the growth of microbes on surfaces of the materials. On the other hand worries about these materials are also related such as resistance of antibiotic, pollution of the environment and costly as well [23, 24]. In the recent past nanomaterials are being discovered in the field of antibacterial properties to face the above, mentioned difficulties such as silver nanoparticles [25], titanium oxide nanoparticles [26] and carbon nanotubes (CNTs). We have tried to study the effects of high concentration of metal oxide nanoparticles in the antibacterial properties.

2. Experimental Details:

2.1 Synthesis of Metal oxide Nanoparticles

Freshly prepared aqueous solutions of zinc nitrate and urea were used for the synthesis of nanoparticles. At room temperature the chemicals are added one by one with the 0.1 M solution of zinc nitrate and 0.15 M solution of urea. The mixture of chemicals was then heated on a hot plate in separate beakers which led the chemical mixture to self-combustion. After combustion the final precipitate is calcinated for 1 hr at 400^0C. Thus we successfully obtained a pure ZnOnano powders in this synthesis. Similarly we have synthesized $Fe_3O_4$with ferric nitrate.

2.2 Disc diffusion method

Antibacterial activity of ZnOand Fe_3O_4 nanoparticles were tested against *E.coli, P.vulgaris, B.subtilis, S.aureus, Xanthomonas*and*Serratia* using the agar well diffusion assay method [27]. Approximately, 25.0 ml of molten and cooled nutrient agarmedia were poured in the sterilized petri dishes. The plates were left over night at room temperature to check for any contamination to appear. The bacterial test organism *E.coli, P.vulgaris, B.subtilis, S.aureus, Xanthomonas*and*Serratia* were grown in nutrient broth for 24 hours at 37 °C. A 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells were prepared with the help of a sterilized stainless steel cork borer. Agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 100 µl of different concentrations i.e.0.20, 0.30, 0.40 and 0.50 µg/ml of ZnO and Fe_3O_4 nanoparticles.

3. Results and Discussion

3.1 XRD Analysis

The crystallite size of ZnOis calculated from full width at half maximum (FWHM) of the peaks (1 0 0) (0 0 2) (1 0 1) (1 0 2) (1 1 0) (1 0 3) (1 1 2) and (2 0 1). Simairally crystallite size of Fe_3O_4 is calculated from full width at half maximum (FWHM) of the peaks (2 2 0) (3 1 1) (4 0 0) (4 2 2) (3 3 3) and (4 4 0) using the Debye–Scherrer's [28] equation.

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

Where D is the average crystallite size perpendicular to the reflecting planes, λ is the -ray wavelength, β is the full width at half maximum FWHM, and θ is the diffraction angle. All the diffraction peaks of ZnO and Fe_3O_4 can be assigned toJCPDS No.36-1415 andJCPDS No.89-4319 respectively shown in Fig. 1 and Fig. 2. The average crystalline size of ZnO and Fe_3O_4 is 36 nm and 40 nm respectively.

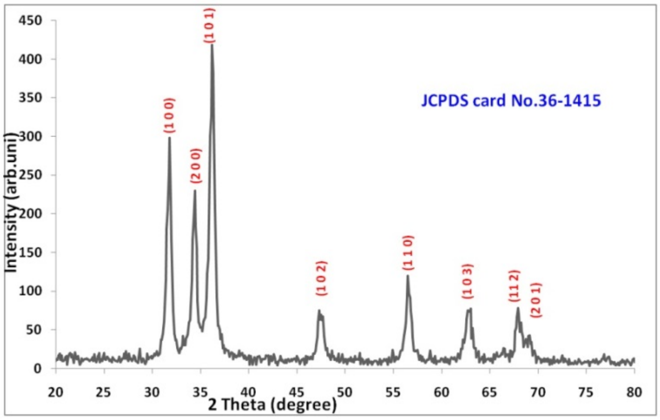

Fig.1XRD patterns ofZnO

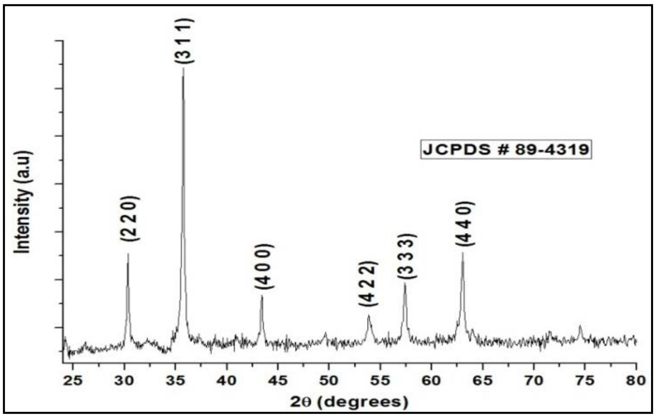

Fig. 2XRD patterns of Fe_3O_4

3.2 Thermal Properties

Thermogravimetric (TG) analysis is performed for ZnO and Fe_3O_4 between 30°C to 800°C at the rate of 20°C / min for the samples and the weight loss is observed Fig.3 and Fig 4. Between the temperatures 30°C to 150°C was about 1.09% and 0.281% respectively due to removal of water molecules on the surface between 150°C to 550°C was about 2.05% and 0.577% respectively due to decomposition of carbon and nitric compounds and between 550°C to 800°C the weight loss was about 1.99% and 0.935% respectivelydue to loss of oxygen. The total weight loss for ZnO and Fe_3O_4 is 5.14% and 1.79% respectively.

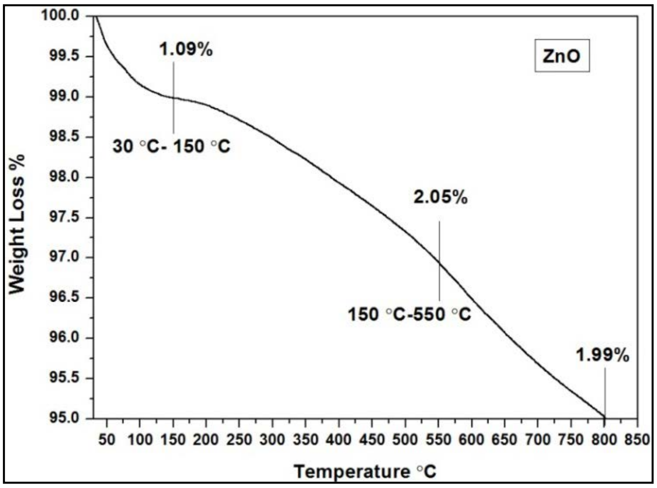

Fig.3Thermogravimetric (TG) analysis of ZnO

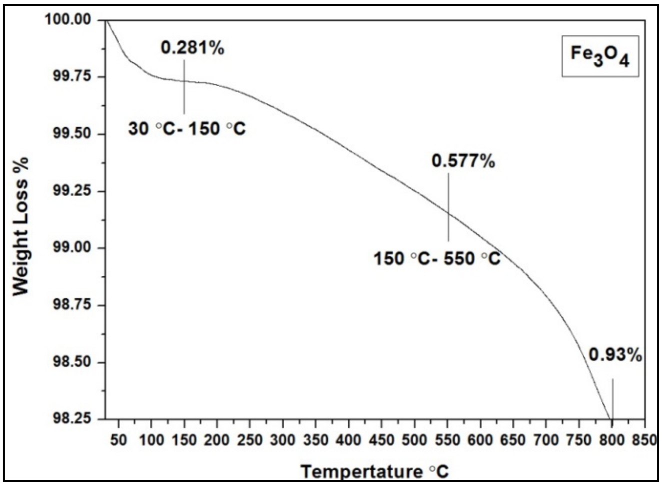

Fig. 4Thermogravimetric (TG) analysis of Fe_3O_4

3.3 Particle Analyzer

The size of the nano powders is measured using Nano Particle Analyzer (SZ100). The average particle size for sample is shown with histogram in Fig. 5 and Fig.6 for ZnO and Fe_3O_4 respectively. The result from particle analyzer is in good agreement with the XRD result of crystallite size.

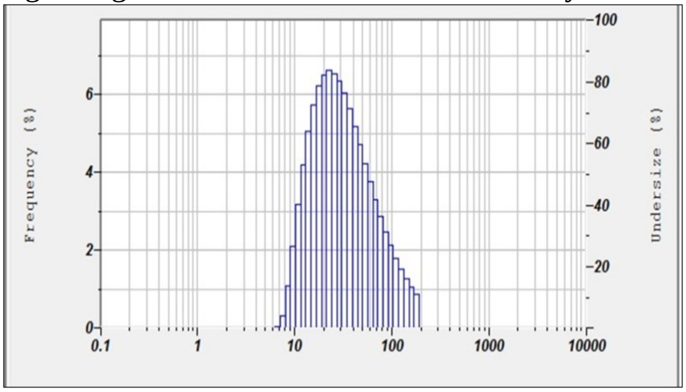

Fig.5Particle Analyzerof ZnO

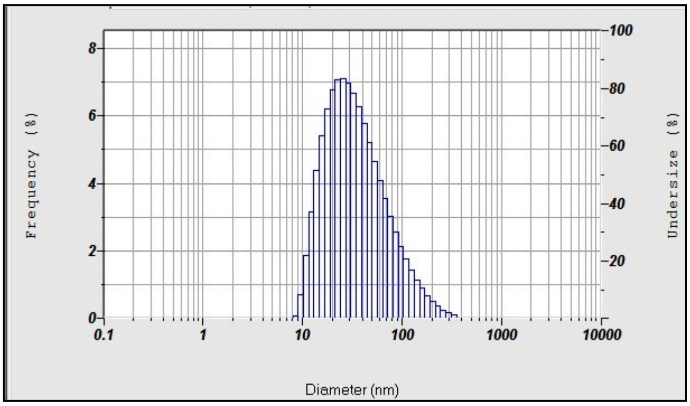

Fig.6 Particle Analyzerof Fe_3O_4

3.4 Antibacterial Activity

The effect of different concentrationsuch as 0.20, 0.30, 0.40 and 0.50 µg/ml of ZnO and Fe_3O_4 nanoparticles respectively are shown in Fig. 7 and Fig.8which show the increase of the inhibition zone measurements with increase the concentration of ZnO and Fe_3O_4 nanoparticles.

ZnO shows the highest antibacterial activity *Pseudo Vulgaris* followed by *Xanthomonas, E.coli, B.subtilis, Serratia*and *S. aureus*. The growth of microorganisms was repressed by the synthesized ZnO nanoparticles showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free Zinc ions. Thus gram positive bacteria may allow less ZnO to reach the cytoplasmic membrane than the gram negative bacteria [29].The Fe_3O_4 Nanoparticle showed a good antibacterial activity on *Pseudo Vulgaris* followed by*Xanthomonas*, *E.coli*, *B.subtilis*,*Serratia*and *S. aureus*. Gram-negative bacteria are more sensitive when compared to Gram- positive bacteria. Earlier studies also indicate that gram-negative bacteria are less sensitive than gram-positive bacteria. A strong bactericidal activity was observed against Proteus vulgaris.

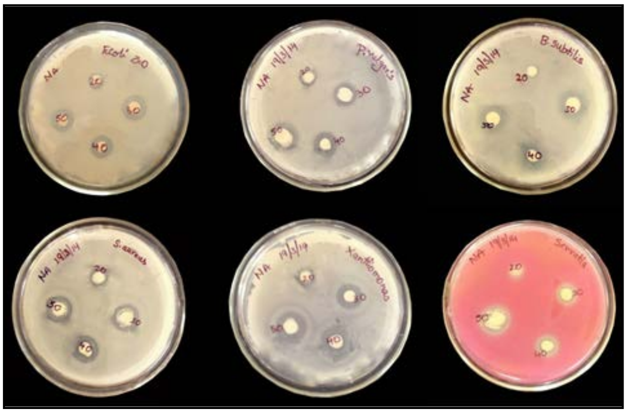

Fig.7Antibacterial Activityof ZnO

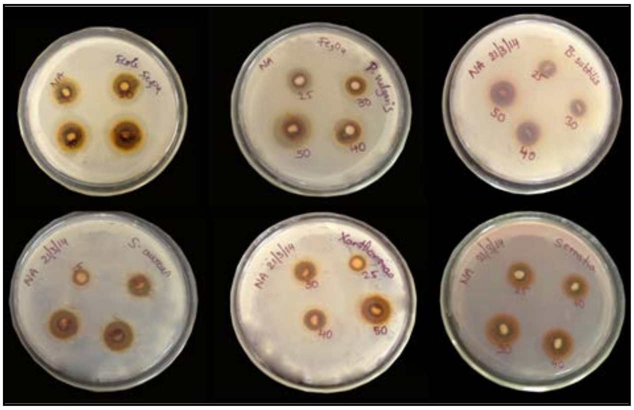

Fig.8Antibacterial Activityof Fe_3O_4

4. Conclusions

ZnO and Fe_3O_4powder was successfully synthesized by chemical combustion method. The crystallite size of ZnO and Fe_3O_4 calculated from the XRD is 36 nm and 40 nm respectively and they are in good agreement with Particle Analyser results. Thermal analysis indicated that the samples obtained were of extreme pure in nature, since it was observed that the total weight loss of the samples ZnO and Fe_3O_4 were only 5.14% and 1.79% respectively. The antibacterial activity of ZnO and Fe_3O_4 nanoparticles were confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that ZnO and Fe_3O_4isalso a very effective antibacterial agent. ZnO and Fe_3O_4 nanoparticles are effective against both the bacteria which gives a conclusion that it is effective against gram +ve and gram –ve bacteria. Thus gram positive bacteria may allow less metal to reach the cytoplasmic membrane than the gram negative bacteria. Therefore we can conclude that ZnO and Fe_3O_4 nanoparticles are a very effective antibacterial agent.

References:

1. Alt V., Bechert T., Steinrcke P., Wagener M., Seidel P., Dingeldein E., Domann U., Schnettler R., 2004, Biomat. Biomat., 25, pp. 4383-4389.
2. Furno F., Morley K.S., Wong B., Sharp B.L., Arnold P. L., Howdle S. M., Bayston R., Brown P.D., Winship P.D., H. Reid, 2004, J. Antimicrob. Chem., 54, 1019.
3. Jeong S.H., Yeo S.Y., Yi S.C., 2005, J. Mater. Sci., 40, 5407.
4. Chou W. L., Yu D. G., Yang M. C., 2005, Poly. Adv. Technol., 16, 600.
5. Sambhy V., MacBride M. M., Peterson B. R. Sen, A., 2006, J. Amer. Chem. Soc., 128, 9798.
6. Stoimenov P. K., Klinger R.L., Marchin G.L., Klabunde K., 2002, Lang., 18, 6679.
7. Hsiao M. T., Chen S. F., Shieh D.B., Yeh C.S., 2006, J. Phys. Chem. B, 110, 205.
8. Theivasanthi T., Alagar M., 2011, Ann. Bio. Res., 2, 368.
9. Morones J. R., Elechiguerra J. L., Camacho A., Holt K., Kouri J. B., Ramírez J. T., Yacaman M. J., 2006, Nanotechnol., 16, 2346.
10. Lee C., Kim Y., Lee W.I., Nelson K.L., Yoon J., Sedlak D.L., 2008, Environ. Sci. Technol., 42, 4927.
11. Zvekic D., Srdic V.V., Karaman M.A., Matavulj M. N., 2011, Proc. App. Cer., 5, 41.
12. Rajendran R., BalaKumar C., Ahammed H.A.M , Jayakumar S., Vaideki K., Rajesh E.M., 2010, Int. J. Engg. Sci. Technol., 2, 202.

13. Gondal M.A., Dastageer M.A., Khalil A., Hayat K., Yamani Z.H., 2001, J. Nanopart. Res., 133, 423

14. Wangsaprom K., Maensiri S., 2010, Proceed in 3rd Int. Nanoelec. Conf., 1044.

15. Kavitha T., Yuvaraj H., 2011, J. Mater. Chem., 21, 15686.

16. Holister P., Weener J. W., Vas C. R., Harper T., 2003, Nanoparticles-Technology white papers 3, Cientific Ltd., London.

17. Arriagada F. J., Osseo-Asare K., 1999, J. Coll. Inter. Sci., 211, 210.

18. Bae D. S., Jungkim E., Bang J. H.,Kim S.W., Han K. S., Kyulee J., Kim B. I., Adair J. H., 2005, J. Met. Mater.-Int., 11, 291.

19. Ganguli A. K., Vaidya S., Ahmad T., 2008, J. Bull. Mater. Sci., 3, 415.

20. Moran Aviv, Israela Berdicevsky, Meital Zilberman. Gentamicin-Loaded Bioresorbable Films for Prevention of Bacterial Infections Associated with Orthopedic Implants. J. Biomed. Mater. Res., Part A 83A: 10–19 (2007).

21. Madeleine Ramstedt, Nan Cheng, Omar Azzaroni ,Dimitris Mossialos, Hans Jörg Mathieu,Wilhelm T. S. Huck. Synthesis and Characterization of Poly (3-Sulfopropylmethacrylate) Brushes for Potential Antibacterial Applications. Langmuir 23: 3314–3321 (2007).

22. Bradley C. Allison, Bruce M. Applegate, Jeffrey P. Youngblood. Hemocompatibility of Hydrophilic Antimicrobial Copolymers of Alkylated 4-Vinylpyridine. Biomacromolecules 8: 2995–2999 (2007).

23. Philip S Stewart, J William Costerton. Antibiotic Resistance of Bacteria in Biofilms. Lancet 358: 135–138 (2001).

24. Margaretha Adolfsson-Erici, Maria Pettersson, Jari Parkkonen, Joachim Sturve. Triclosan a Commonly Used Bactericide Found in Human Milk and in the Aquatic Environment in Sweden. Chemosphere 46: 1485–1489 (2002).

25. Ashavani Kumar, Praveen Kumar Vemula, Pulickel M. Ajayan1, George John. Silver-Nanoparticle-Embedded Antimicrobial Paints Based on Vegetable Oil. Nat. Mater. 7: 236–241 (2008).

26. Chang. Wei, Wen Yuan. Lin, Zulkarnain. Zainal, Nathan E. Williams, Kai. Zhu, Andrew P. Kruzic, Russell L. Smith, Krishnan. Rajeshwar. Bactericidal Activity of TiO2 Photocatalyst in Aqueous Media: Toward

a Solar-Assisted Water Disinfection System. Environ. Sci. Technol. 28: 934–938 (2002).

27. Perez C, Paul M, Bazerque P., 1990, Act. Bio. Med. Exp., 15, 133.
28. B D Cullity. *Elements of X-ray diffraction*, Addison-Wesely Publishing Company, Inc., chap-6, Philippines (1978).
29. N Ahmad, S Sharma, V N Singh, S F Shamsi, A Fatma, B R Mehta. *Biotech. Res. Int*. 1-8 (2011) doi.10.4061/2011/454090