DOI: 10.1478/C1V89S1P080

AAPP | Atti della Accademia Peloritana dei Pericolanti
*Classe di Scienze Fisiche, Matematiche e Naturali*
ISSN 1825-1242

Vol. 89, Suppl. No. 1, C1V89S1P080 (2011)

# LIGHT SCATTERING STUDY OF ZINC OXIDE NANOPARTICLES FOR THE APPLICATION OF ITS ANTI-BACTERIAL PROPERTY

S. ROY,[a*] N. BARUA,[b] A. K. BURAGOHAIN,[b] AND G. A. AHMED [a]

ABSTRACT. Investigations on the anti-bacterial activity of ZnO nanoparticles on *Staphylococcus aureus* were made by using bio-technical method. Light scattering properties of these particles were studied as a function of scattering angle by using a versatile laboratory light scattering setup in order to find the scattering profile of ZnO nanoparticles and also the mode of action of these particles on bacterial property.

## 1. Introduction

ZnO nanoparticles have important applications as antibacterial and antifungal agents when incorporated into materials, such as surface coatings in paints, textiles, and plastics. It also has novel applications in ultra-violet lasers, gas sensors, biomedical sciences etc. Several authors have reported about the anti-bacterial and anti-fungal properties of ZnO nanoparticles [1] using different bacterial and fungal strains. Scattering and absorption of light from small particles with diameter ranging from nanometer to micrometer, distributed in different continuous media or matrix, holds enormous potential in detection and yielding morphological information *in-situ*, and as such is a subject of intensive research at the present time . Interesting results based on light scattering technique by biological particles were presented by researchers [2]. The scattering properties of such samples are determined not only by their own and the medium's refractive indices but also by their shape, size, density, scattering angle and polarization state of the incident light. It is often constructive to discuss elastic light scattering in terms of Stokes vector and Mueller matrices. The elements of the Mueller scattering matrix at an angle represent the effect of scatterer on the intensity and the polarization state of the incident wave [3]. We have investigated the anti-bacterial action of ZnO by using *Staphylococcus aureus* as bacterial strain, and the morphological changes of the particles in the scattering volume were observed with scanning electron microscopy (SEM). Staphylococcus aureus is a Gram- positive cocci, is a member of the Staphylococcaceae family [4].

In this paper we report for the first time the experimental investigations carried out on the effects of ZnO on bacteria using bio-techniques and scattering experiments involving polarization phase functions of these randomly oriented non-spherical particles. The scattering experiments were performed using an indigenously designed and fabricated versatile laser based light scattering system [5].

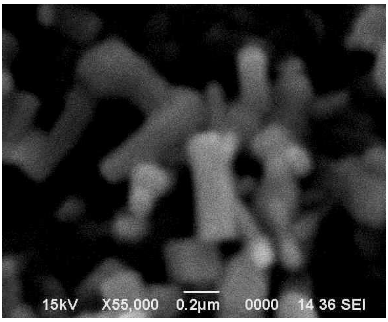


**Figure 1.** Scanning Electron Micrograph image of ZnO nanoparticles.

## 2. Experimental Details

**2.1. Antimicrobial Assay.** The antimicrobial assay was performed against *S. aureus* MTCC 737 using the Agar Well Diffusion Assay [5]. The strain was cultured in Mueller Hinton Broth 2 media at $37^o C$ for 18h. The bacteria were then suspended in a saline solution(0.85% NaCl) to adjust the McFarland standard of the cells to a turbidity of 0.5 corresponding to approximately 108 CFU/ml. The suspension was inoculated in Mueller Hinton Agar media and three wells (6mm) were punched. 50 $\mu$l of the ZnO nanoparticles at the concentration of 50mg/ml, dissolved in 1% (v/v) DMSO (Dimethyl sulphoxide) was added to well 1.1% DMSO was added to well 2 and Streptomycin was used as an antibiotic control in well 3. The plates were incubated at $37^o$ for 18h.

**2.2. Light scattering:** The setup was used to study the light scattering characteristics[5] of ZnO nanoparticles (Fig. 1) having average diameter of $0.15\mu$m,and the anti-bacterial action of action of ZnO on S. aureus. The instrument uses He-Ne laser which can be operated at three different incident wavelengths of 543.5 nm, 594.5 nm and 632.8 nm alternately. The scattered signals were sensed by a photomultiplier tube (H 5784-20) detector and the measurements were taken from $10^o$ to $170^o$ as a function of the scattering angle $\theta$. The analytical part was tackled by comparing the experimental results with theoretical predictions based on Mie theory [6].

## 3. Results and Discussions:

Growth of the bacterial cells was not affected by 1% DMSO as shown by our control experiments in well 2 (Fig.2 (a)). The ZnO nanoparticles exhibited an antimicrobial activity as shown by the zone of inhibition. Well 1 contains ZnO (50mg/ml), Well 2 contains 1% DMSO and Well 3 contains Streptomycin. The ZnO nanoparticles exhibited a potent antimicrobial activity as observed by the MIC value of 0.0390 mg/ml against *S. aureus* MTCC 737 (Fig. 2(b)). The light scattering behaviour of both ZnO and anti-bacterial effect of ZnO on *S. aureus* has been studied as a function of scattering angle. The Time Kill Assay was performed to study the antibacterial effect of the ZnO nanoparticles with respect to time. The ZnO particles exhibited a potent antibacterial activity at the Minimum Inhibitory Concentration (MIC value of 0.0390 mg/ml against *S. aureus* MTCC 737.

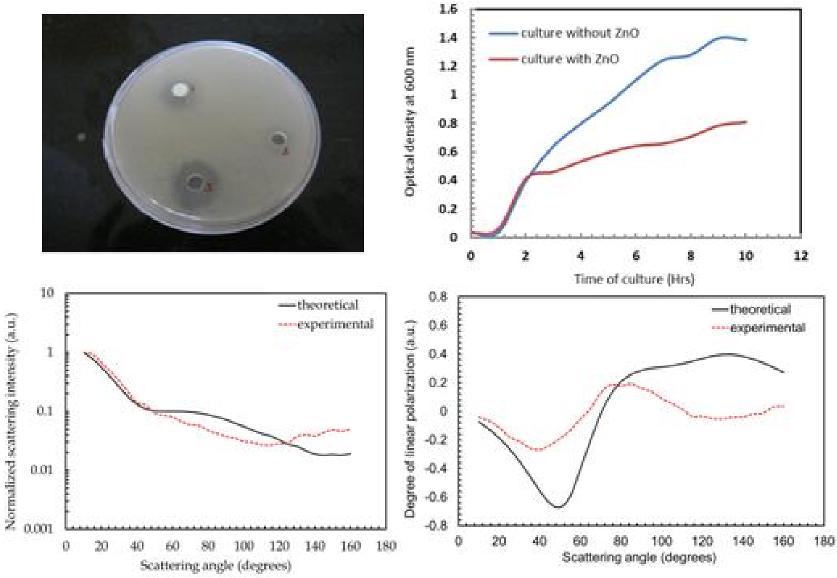


**Figure 2.** (a): The Antimicrobial Assay; (b): Plot showing the bactericidal effect of ZnO on Staphylococcus aureus MTCC 737;(c) : Scattering phase function of ZnO particles at $\lambda$= 632.8nm;(d): Linear polarization phase function of ZnO at $\lambda$= 632.8nm nanoparticles.

We have studied the light scattering from ZnO nanoparticles and ZnO effected *S. aureus* bacteria at 543 nm, 594 nm and 632 nm laser wavelengths respectively.

Overall the $\theta$-dependent light scattering signals (intensities and polarization) from particles is described in the context of Stokes vectors and Mueller matrices [3]. Each of the elements of the matrix contains all the information about intensities and polarization of the scattered light as a function of the scattering angle. Thus, light scattered from a biological particle is rich in information and can be extracted by making detailed measurements of properties of light after scattering. Angle-resolved light scattering measurements [2] by different biological particles including *S. aureus* have been reported earlier, where light scattering was used as a powerful technique to differentiate various bioparticles according to species,strains, growth media etc. In our present work, an attempt was made to study the angle-resolved light scattering of ZnO, and its effect on *S. aureus*, and also purely *S. aureus* bioparticles in order to see the signatures of these particles with changing wavelength of incident light. The scattering results reveal the differences, definiteness and nature of polarized light scattered from such systems. Light scattering techniques are very important tool when a small alteration is introduced in a system. The aim of our experiment was to study subtle differences, especially for bioparticles by detecting significant light scattering signals. Some small morphological changes in the bioparticles under the influence of any external factors like strain, chemical treatment, metabolic change, killing techniques can be monitored *in-situ* by light scattering. In simple words, one might be able to detect precisely what changes occur in bioparticles like bacteria as a result of some environmental or external alterations. The scattering results for scattering and linear polarization phase function of ZnO particles at a wavelength of 632.8nm is shown in Figs. 2(c) and 2(d) respectively. The experimental plot in figs. 2(c) and 2(d) seems to be deviating from the theoretical plot. The reason may be attributed to the presence of a broad size distribution of the particles in the scattering volume. The measured degree of linear polarization, $-S_{12}/S_{11}$ shows (Fig. 2(d)) oscillating behaviour of polarization phase curve indicating higher non-sphericity in case of these particles. From figure 2(d), it was observed that the shape of the polarization phase curves for experimental plot deviates more from the theoretical plot as compared to fig. 2(c). The source of this observed feature might be the strong dependence of polarization on the model size parameter, x=$\pi$s/$\lambda$, where s is the average diameter of the particles and $\lambda$ is the wavelength, as a result of which we obtain actual signature for experimental light scattered signals which again is different from theoretical results. It was also observed that the light scattering behaviour of ZnO effected bacteria significantly vary for the three different laser wavelengths. The points and results will be described in more detail during presentation. The instrument has proved itself to be quite efficient for performing light scattering experiments on small particulate matter which includes aerosols and hydrosols.

## Acknowledgments

The corresponding author wishes to thank Department of Science and Technology, Ministry of Science and Technology, Government of India for the project grant (sanction no. SR/WOS-A/PS-20/2007 dated 04/08/2008) under the Women Scientist-A Scheme.

## References

[1] Y. Liu et al. Antibacterial activities of zinc oxide nanoparticles against Escherichia coli 0157:H7. *Journal of Applied Microbiology*. 107 1193-1201(2009).

[2] W S Bickel, J F Davidson, D R Huffman and R Kilkson .Applications of polarization effects in light scattering: a new biophysical tool. Proc Natl Acad SciUSA1976;73(2):486-90

[3] M. I. Mishchenko, J. W. Hovenier, and L. D. Travis. Light Scattering by Nonspherical Particles: Theory, Measurements, and Applications. San Diego, CA : Academic (2000).

[4] Sibbald et al., Mapping the Pathways to Staphylococcal Pathogenesis by Comparative Secretomics, *Microbiology and Molecular Biology reviews*, Sept. 755-788 (2006)

[5] S. Roy et al. Monitoring of pathogen carrying air-borne tea dust particles by light scattering. *J. Quant. Spectros. and Radiat. Transfer*, 112 1784-1791 (2011).

[6] S. Roy and G. A. Ahmed, "Computation of Mueller scattering matrices of spherical particles with a Gamma size distribution", *J. Opt* 39 (2) 76-81 (2010).

[a] Optoelectronics and Photonics Laboratory
Department of Physics, School of Science and Technology
Tezpur University
Tezpur-784028, Assam, India

[b] Department of Molecular Biology and Biotechnology, School of Science and Technology
Tezpur University
Tezpur-784028, Assam, India

* To whom correspondence should be addressed | Email: rsanchita1@gmail.com

Paper presented at the ELS XIII Conference (Taormina, Italy, 2011), held under the APP patronage; published online 15 September 2011.