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Optical and Structural Properties of Protein Capped ZnO Nanoparticles and Its Antimicrobial Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS and TK managed the analyses of the study. Author AKB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Protein coated ZnO nanoparticles (NPs) are prepared by green synthesis method in egg albumin medium. HRTEM analysis and absorption at~360 nm along with ~ 280 nm indicate the formation of protein coated ZnO NPs. Fluorescence study reveals the tryptophan emission~ 340 nm as well as green and blue emission from protein coated ZnO NPs. FTIR bands of ZnO along with amide bands confirm the formation of protein capped ZnO NPs. XRD spectrum showed hexagonal phase of the NPs. The DLS and Zeta potential measurements showed 34.2 nm hydrodynamic radious of the NPs with negative surface charge having value -13.4 mV. Such nanoparticles showed potential antimicrobial activity against gram negative bacteria.

Keywords: Nanoparticles; green synthesis; absorbance; fluorescence; FTIR; antimicrobial activity.

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1. INTRODUCTION

The advancement of nanotechnology has step up the progress of nanoparticles (NPs) research in many areas from information technology to biomedical engineering [1]. Different nanomaterials have been studied widely by the researchers.

It is well reported in the existing literature that bare NPs gets almost coated with proteins, after entering into a biological fluid [2] as well as unfolding of plasma and blood proteins are expected due to surface active property of NPs [3].

The concept of bio-safety and bio-compatibility of NPs is a key issue in favour of application in human body especially the bio-molecules having different functional groups can be used for capping bare NPs. Thus the post surface modification of bare NPs by capping them with biomolecules is a technique that facilitates the biomedical research. Therefore, it is very challenging to fabricate safe bio-conjugated NPs for prospective application in drug delivery, bio-labelling as well as bio-imaging.

Zinc Oxide (ZnO) NPs have wide application in preparation of cosmetics, sunscreen lotion, etc. Earlier we have presented the interaction and unfolding of protein with bare ZnO NPs [4]. In this study we have focussed our aim to prepare protein coated ZnO NPs for bio-safe use of ZnO NPs in mankind and study of their antimicrobial activity.

2. EXPERIMENTAL SECTION

2.1 Synthesis of Albumin Coated ZnO Nanoparticles

Fresh eggs were purchased from the local market (Midnapore town). Zinc Acetate (analytical grade) from Merck (India) was used as received without any further purification. Triple distilled water, deionized with a Milli-Q water purification system from Millipore, U.S.A was used for sample preparation. The pH and the resistivity of freshly prepared water were 6.8 and 18.2 MΩ cm, respectively. Protein capped ZnO NPs were synthesized using a simple green synthesis method. Briefly, filtered egg white (albumen, 90 ml) was slowly added into a magnetically stirred solution of Zn (II) Acetate (20 mM) in Millipore water (10 mL), at room temperature. After vigorous stirring for 3 hours, the white precipitate was filtered and carefully washed repeatedly with distilled water and dried in open environment for further characterization. Schematic of the ZnO nanoparticles synthesis process is shown in Fig. 1.

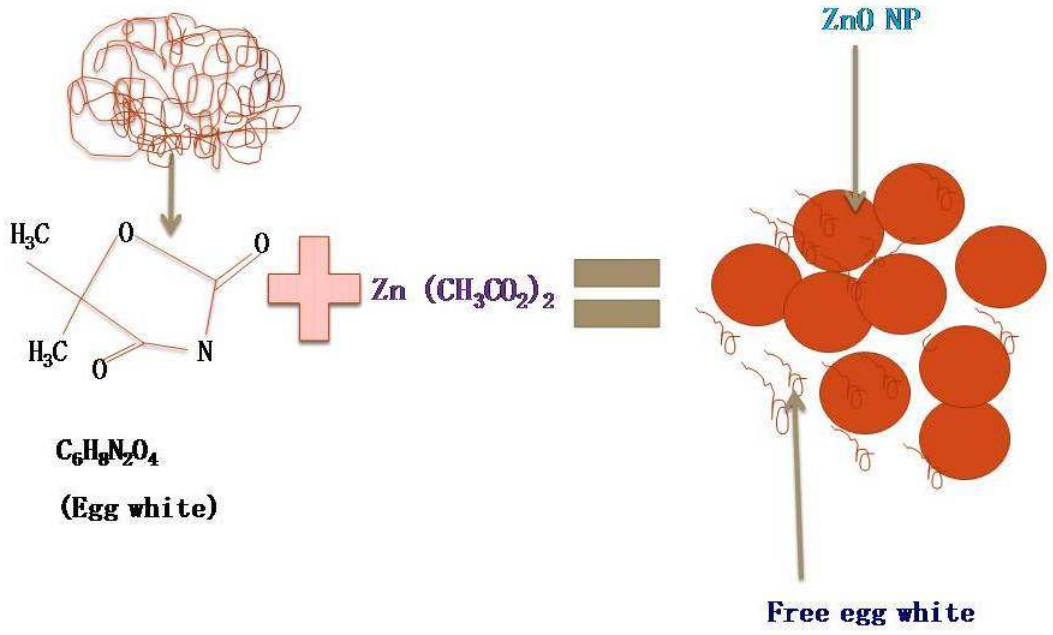

Fig. 1. Schematic of the ZnO nanoparticles synthesis process

2.2 Experimental Details and Characterization Methods

The so prepared ZnO NPs were dispersed in Millipore water using ultrasonification for 120 min. The optical absorption spectra of the prepared samples were recorded in a Shimadzu-Pharmaspec-1700 UV-VIS spectrophotometer. The fluorescence spectra of the as prepared samples were obtained by using Hitachi-F7000-FL spectrophotometer. X-Ray diffraction (XRD) data of the powder sample were collected in a Rigaku X-ray diffractometer using Cu-Kα radiation of wavelength 1.54 Å over the angular range $20° < 2\theta < 60°$. For microstructural study, a small drop of water dispersed samples was placed on a thin carbon film supported on copper grid and kept for some time for drying. The high resolution transmission electron micrograph (HRTEM) of the prepared ZnO NPs was acquired using JEOL-JEM-200 operating at 200 kV. The Fourier Transform Infrared (FTIR) spectra of the samples were obtained by Perkin Elmer LS-55 in transmission mode. Dynamic Light Scattering (DLS) and Zeta potential measurements were made with a Malvern Zeta Sizer Nano ZS instrument operating with a wavelength of 532 nm and a fixed scattering angle of 173°.

E. coli, K. Pnemoniae and *S. aureus* were procure from Persian Type Culture Collection (WDCM124). All these strains were developing aerobically in nutrient broth for 25 h before using as target entity.

2.3 Antibacterial Activity Assay and Determination of Zone of Inhibition

In order to survey the antibacterial activity of the protein coated ZnO nanoparticles on these entity (microorganisms), ZnO NPs were suspended in sterile normal saline and continually stirring until a constant colloidal suspension was found to produce a powder concentration of 100 mg/mL. To assess toxicity range of ZnO NPs against *E. coli, K. Pnemoniae and S. aureus,* an appropriate volume of test bacteria were vaccinate in nutrient broth medium boost with serially diluted ZnO NPs suspensions, from 50 to 0.5 mg/mL. Colony forming units were quantified after an overnight incubation. 0.025 mL and 0.15 mL was added of various amounts of ZnO NPs in discs. The zone of inhibition (ZOI) was measured after 25 h incubation. The antibacterial activity against three different bacteria of ZnO NPs were compared.

3. RESULTS AND DISCUSSION

3.1 Absorption Spectroscopy

Typical absorption spectra of protein coated ZnO NPs is shown in Fig. 2. The sidelong absorption observed at 360 nm might be related to the quantum confinment effect [5] as related to in the bulk absorption edge appearing at 380nm [6] at room temperature. The tryptophan (TRY) of egg protein associated with ZnO NPs shows an absorption peak at 280 nm owing to the π- π* transition of aromatic amino acid residues. The bandgap of the sample is found to be ~ 3.55 eV.

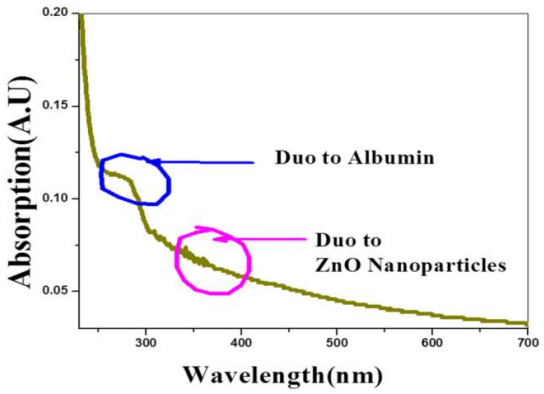

Fig. 2. Absorption spectra of the albumin coated ZnO nanoparticles

3.2 Fluorescence Spectroscopy

TRY fluorescence of protein coated ZnO NPs in Figs. 3(a), 3(b) shows a maximum at ~340 nm when the exciting wavelength is 280 nm (Fig. 3(a)). This value correleates with literature reference [7]. No red shifting of TRY is observed in this system. Therefore, the proteins attached with ZnO NPs are not unfolded [8]. Fig. 3(b) presents the typical PL of the ZnO from protein coated ZnO NPs. The as-prepared protein coated ZnO NPs exhibit a blue emission with peak at ~442 nm [9] and PL peak at~ 393 nm, which is accorded with the typically free excitonic transition [10-11]. However, a very strong broad green band at ~ 511 nm has been observed. Emission spectrum shows that few new states (surface states) must lie in the fundamental gap close to the Fermi level of ZnO nanoparticles [12-13]. The surface states situated near to the Fermi level are due to the supposed dangling bond in elemental semiconductors [14]. A dangling bond on a semiconductor crystal surface likely gives rise to a localized state within the fundamental

band gap [15-16]. The unsaturated covalent bands can easily form states especially in the center of the gap. Although, it is suggested that no gap surface states exist on perfect ZnO surfaces, as no broken sp^3 bulk bonds are present [17]. In the case of ZnO nanoparticles, however, one of the key characteristic is the large surface-to-volume ratio (S/V). That is, the large S/V greatly increases the probabilities of dangling bonds or broken bonds in ZnO nanoparticles, which is confirmed by our experimental results [18-19]. The room temperature Photoluminescence spectrum of the ZnO nanoparticles shows UV and visible emission. The visible emission is possible due to the existence of various defect states of lower energy. We observe PL emission peaks at 393 nm, 442 nm, 462 nm, 511 nm and 594 nm. The average particle size as calculated from the HRTEM pattern was found to be ~18 nm. The size of our ZnO NPs is larger than the ZnO Bohr radius of 1.5 nm. Hence we assumed the weak confinement mechanism of the charge carriers within the ZnO nanocrystals [20]. The transition energy corresponding to $n \rightarrow 1$ is [20]:

$$\Delta E_{n\rightarrow 1} = \frac{h^2}{8MR^2}(n^2-1) = \frac{\alpha}{2}(n^2-1)$$

The calculated value of α was found to be 0.000846 eV [20]. Using this value of α, (taking R = 18 nm), the 393 nm peak in the emission spectrum corresponds to the transition from n = 86 to n = 1 level. The other transition levels are also calculated by similar way and shown in Table 1. The different values of n for different photo illumination wavelengths are shown in Table 1. The various transitions observed in our ZnO nanoparticles are also shown by the arrow lines in a diagram (Fig. 4). The variation of different values of n for different PL emission is shown in Fig. 5.

Table 1. Various transition levels corresponding to various PL emission peaks

Wavelength(nm)	Energy(eV)	α (eV)	n
393	3.155		86
442	2.805	0.000846	81
462	2.684		79
511	2.427		76
594	2.088		69

3.3 FTIR Spectroscopy

The room temperature FTIR spectrum of the albumin coated ZnO NPs is shown in Fig. 6. The strong amide-I peak at 1638 cm^{-1} in the FTIR spectra of egg protein coated ZnO NPs shows the presence of protein in the protein coated ZnO NPs. A strong band is observed at 525 cm^{-1}. This is assigned to the Zn–O stretching bonds. The peak at 1,384 cm^{-1} appeared due to symmetric stretching mode of N = O coming from some unreacted zinc nitrate. The vibration mode of OH group was also observed at 3,467 cm^{-1}. It revealed that some water was absorbed by the sample from the atmosphere.

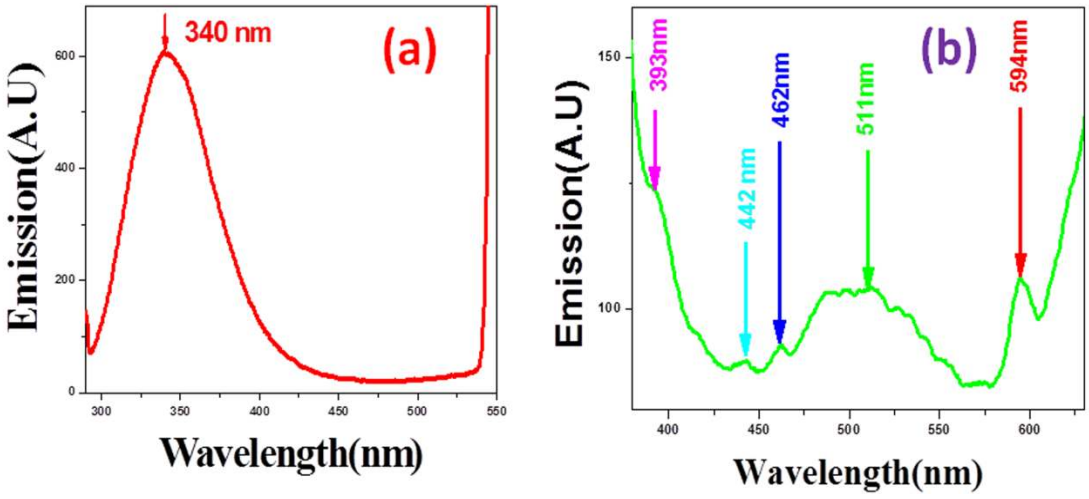

Fig. 3. (a) Emission spectra of the albumin coated ZnO nanoparticles for the excitation wavelength 280 nm. (b) Emission spectra of the albumin coated ZnO nanoparticles for the excitation wavelength 330 nm

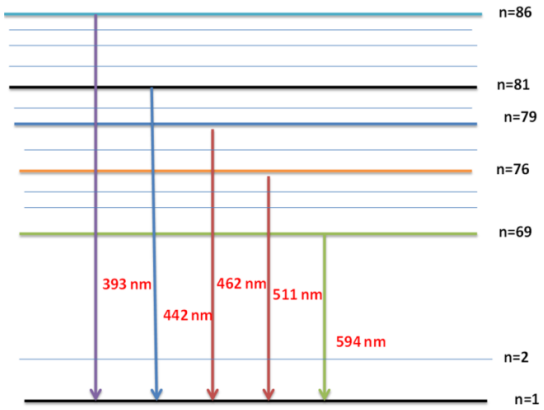

Fig. 4. Various transition levels responsible for PL emission from ZnO nanoparticles

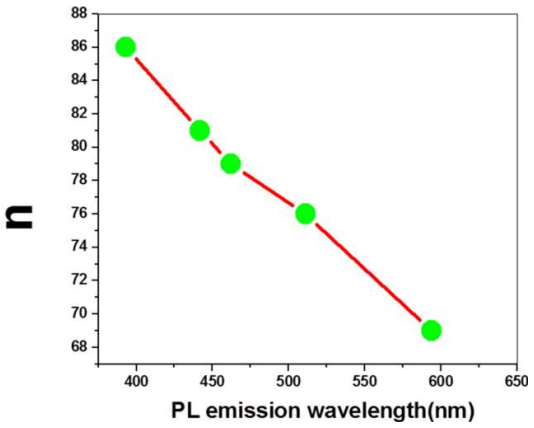

Fig. 5. The variation on n for different emission wavelength

3.4 HRTEM

The morphology and the shape of protein coated ZnO NPs was investigated using HRTEM. Typical HRTEM image of the synthesized ZnO nanocrystals are also shown in Figs. 7(a) & 7(b). Distinct well grown spherical ZnO nanocrystals are formed with aggeregated extra egg white Albumin surrounding the nanocrystals are found. The nanocrystals have an average diameter of ~ 18 nm as revealed from the TEM image. The size distribution of the nanoparticles as observed from the TEM indicates that the basic growth unit is spherical.

3.5 X-ray Diffraction of ZnO Nanoparticles

The XRD pattern of the synthesised ZnO nanoparticles is shown in Fig. 8(a). The diffraction spectrum shows the presence of the (100), (002), (101), (102), (110) peaks and can be indexed to the hexagonal crystal structure [21]. The sharp diffraction peaks arises due to crystalline nature of the ZnO nanoparticles. We have also calculated the crystal size (dimension of the coherent diffracting domains) using Scherrer formula [22-23].

$$R_{hkl} = \frac{0.89\lambda}{\beta \cos\theta}$$

In this calculation, the (101) diffraction plane has been used and assumed the peak to be Gaussian. Fig. 8(b) is the Gaussian fitting of the (101) peak of ZnO nanoparticles. The calculated particle size is ~21.32 nm. The particle size from XRD pattern is slide greater than the average size obtained from TEM measurement this is due to anisotropic nature of the nano sample.

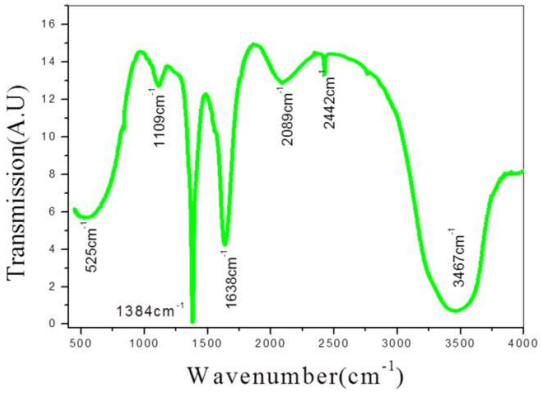

Fig. 6. FTIR spectra of the albumin coated ZnO nanoparticles

3.6 Dynamic Light Scattering and Zeta Potential Study

The ZnO nanoparticle size distribution was measured by dynamic light scattering (DLS). DLS measurements of nanoparticles show that ZnO NP have near mono dispersed PSD with mean hydrodynamic diameter of 34.2 nm (Fig. 9(a)). The hydrodynamic size of the nanoparticle is large then size measurement from TEM is due to albumin coating around the surface of the ZnO nanoparticle. The corresponding correlation Function vs time and size residuals vs time plot are shown in Figs. 9(b) and 9(c) respectively.

In present experiment, it is found that the value of Z-potentials of ZnO NRs is -13.4 mV (Fig. 10). The negative value of the measured zeta potential indicates the full coverage of ZnO NPs by albumin coating [23].

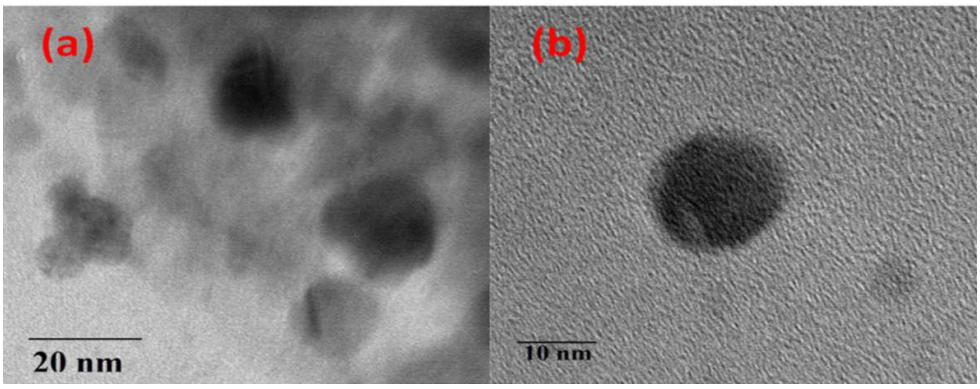

Fig. 7. (a) & (b) HRTEM image of ZnO nanoparticles

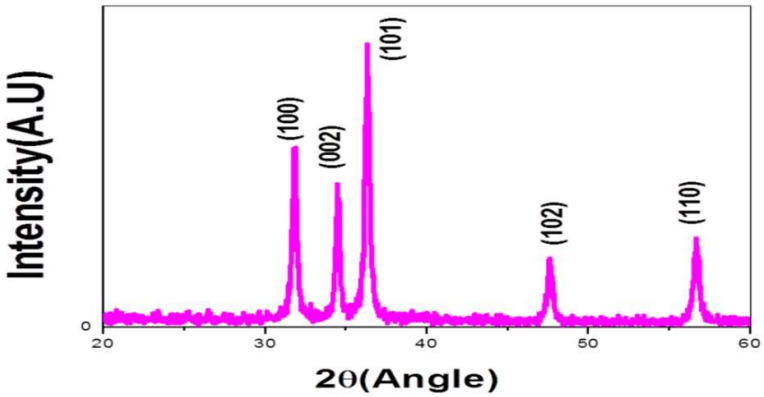

Fig. 8 (a). XRD spectra of ZnO nanoparticles

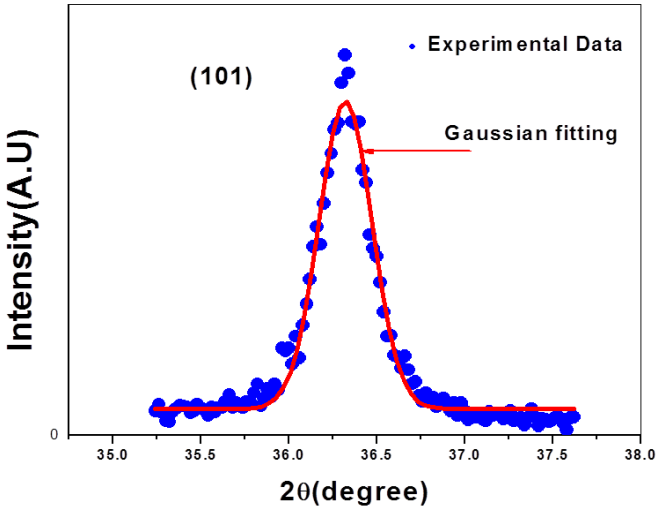

Fig. 8 (b). Gaussian fitting of the (101) peak of ZnO nanoparticles

3.7 Antimicrobial Activity

The white lawn on the Petridis (Fig. 11) is the different bacterium that has been mentioned above in each picture. Among these bacteria *E. coli*, *K. pnemoniae* are the gram negative bacteria but *S. aureus* is the gram positive bacteria. The clear zone on the plate indicates

that protein coated ZnO nano particle has the ability to destroy the bacteria and it is especially effective against gram negative bacteria [24-25]. The different diameter of the clear zone observed in a plate are due to application of different concentration of ZnO sample. Higher clear zone represented higher antimicrobial activity.

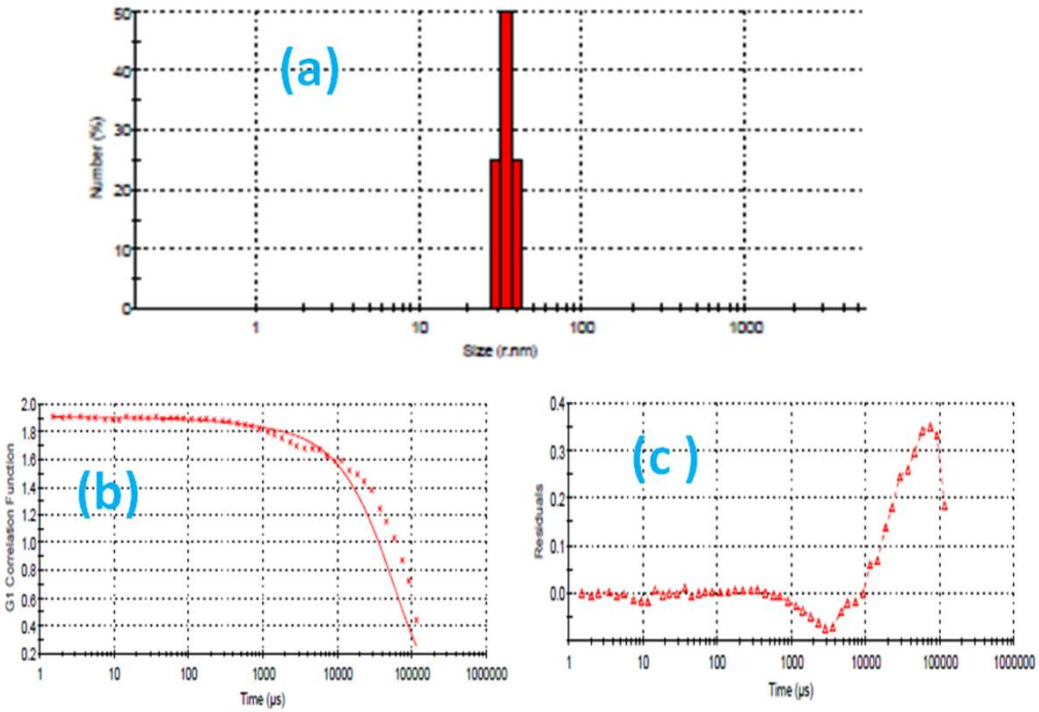

Fig. 9. (a) Dynamic light scattering spectra of albumin coated ZnO NPs, (b) correlation function vs time plot, (c) The corresponding size residuals vs time plot

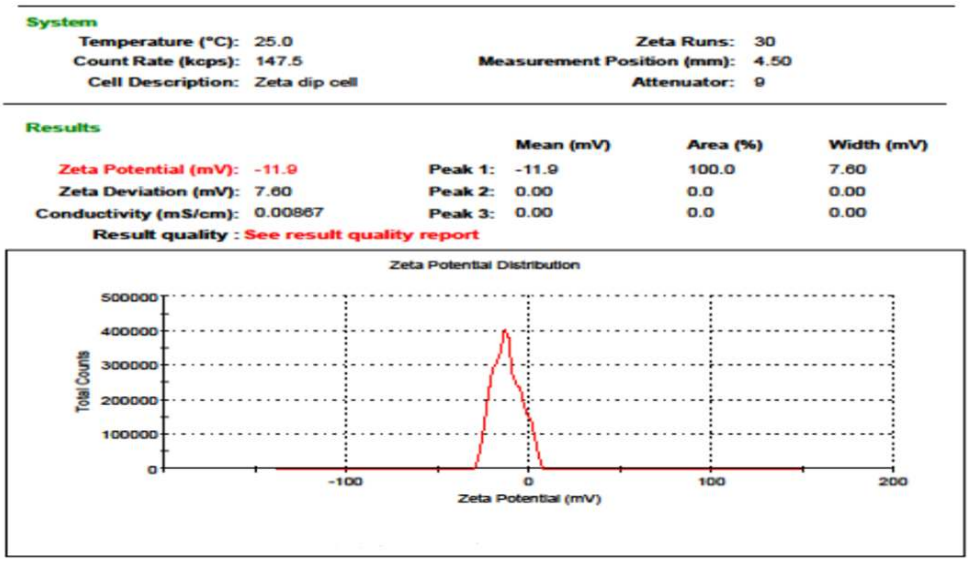

Fig. 10. Zetapotential spectra of albumin coated ZnO NPs

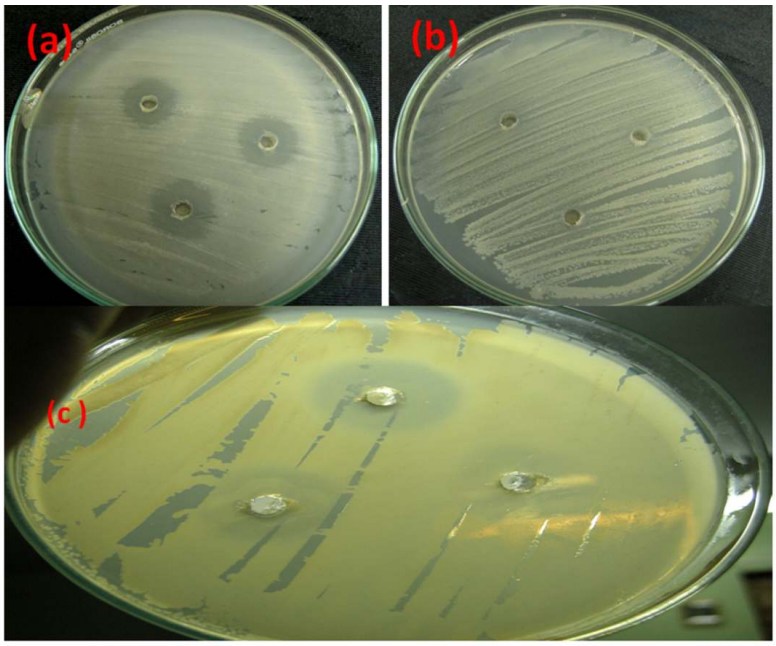

Fig. 11. Antimicrobial activity (image) of the ZnO nanoparticles against (a) *Escherichia coli*, (b) *Staphylococcus aureus*, (c) *Klebsiella pneumoniae*

4. CONCLUSION

We successfully synthesized albumin coated Zinc Oxide nanoparticles of average diameter ~18 nm using a simple wet chemical method. The XRD pattern of the synthesized samples shows a hexagonal crystal structure with dimension of the coherent diffracting domains ≈ 21.32 nm. The emission spectrum of the ZnO nanoparticles shows tryptophan emission~ 340 nm as well as green and blue emission due to formation of surface states from protein coated ZnO NPs. The peak at ~ 1638 cm^{-1} in the FTIR spectra of protein coated ZnO NPs is the signature of strong amide-I bond due to albumin. The string band at 525 cm^{-1} is assigned to the Zn–O stretching bonds. The DLS and Zeta potential studies showed 34.2 nm hydrodynamic radious of the NPs with negative surface charge having value -13.4 mV. The ZnO NPs exhibited inhibitory effect against the gram negative bacteria *E. coli* and *K. pnemoniae* but it had no activity againt the gram positive *S. aureus*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mazzola L. Commercializing nano-technology. Nature Biotechnology. 2003;21:1137.
2. Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, Dwason KA, Linse S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc. Natl. Acad. Sci. U.S.A. 2007;104:2050.
3. Lacerda SHDP, Park JJ, Meuse C, Pristinski D, Becker ML, Karim A, Doglus JF. Interaction of gold nanoparticles with common human blood proteins. ACS Nano. 2010;4:365.
4. Bhunia AK, Samanta PK, Saha S, Kamilya T. ZnO nanoparticle-protein interaction: Corona formation with associated unfolding. Applied Phys. Lett. 2013;103: 143701.
5. Saha S, Kamilya T, Bhattacharya R, Bhunia AK. Unfolding of blood plasma

albumin protein in interaction with CdS nanoparticles. Sci. Adv. Mater. 2014;6:1.
6. Virendra P, Charlnene D, Deepti Y, Shaikh AJ, Nadanathangam V. Spectroscopic characterization of zinc oxide nanorods synthesized by solid-state reaction. Spectrochimica Acta Part A. 2006;65:173.
7. Loh KKP, Lim CT. Selective concentration-dependent manipulation of intrinsic fluorescence of plasma proteins by graphene oxide nanosheets. RSC Adv. 2016;6:46558.
8. Kamilya T, Pal P, Talapatra GB. Interaction of ovalbumin with phospholipids langmuir−blodgett film. J. Phys. Chem. B. 2007;111:1199.
9. Wu JJ, Liu SC. Low-temperature growth of well-aligned ZnO nanorods by chemical vapor deposition. Adv. Mater. 2002;14: 215.
10. Zu P, Tang ZK, Wong GKL, Kawasaki M, Ohtomo A, Koinuma H, Segawa Y. Ultraviolet spontaneous and stimulated emissions from ZnO microcrystallite thin films at room temperature. Solid State Commun. 1997;103:459.
11. Flores-Carrasco G, Carrillo-López J, Martínez-Martínez R, Espinosa-Torres ND, Muñoz L, Milosevic O, Rabanal ME. Optical and morpho-structural properties of ZnO nanostructured particles synthesized at low temperature via air-assisted USP method. Applied Physics A. 2016;122:173.
12. Schmeits M, Mazur A, Pollmann J. Scattering-theoretical method for relaxed and reconstructed surfaces with applications to GaAs(110) and Si(100)-(2×1). Phys. Rev. B. 1983;27:5012.
13. Dorn R, Luth H, Buchel M. Electronic surface and bulk transitions on clean ZnO surfaces studied by electron energy-loss spectroscopy. Phys. Rev. B. 1977;16: 4675.
14. Woolfson MM, Ziman JM. Cambridge University Press London. 1976;186.
15. Wu XL, Xiong SJ, Siu GG, Huang GS, Mei YF, Zhang ZY, Deng SS, Tan C. Optical emission from excess Si defect centers in Si nanostructures. Phys. Rev. Lett. 2003; 91:157402.
16. Puzder A, Williamson AJ, Reboredo FA, Galli G. Structural stability and optical properties of nanomaterials with reconstructed surfaces. Phys. Rev. lett. 2003;91:157405.
17. Ivanov I, Pollmann J. Electronic structure of ideal and relaxed surfaces of ZnO: A prototype ionic wurtzite semiconductor and its surface properties. Phys. Rev. B. 1981; 24:7275.
18. Zhao FL, Gong Z, Liang SD, Xu NS, Chen J, Wang HZ. Ultrafast optical emission of nanodiamond induced by laser excitation. Appl. Phys. Lett. 2004;85:914.
19. ang X, Zhao F, Xie P, Deng S, Xu N, Wang H. Surface emission characteristics of ZnO nanoparticles. Chem. Phys. Lett. 2006;423:361.
20. Samanta PK. Weak quantum confinement in ZnO Nanorods: A one dimensional potential well approach. Optics and Photonics Letters. 2011;4:35.
21. Bhunia AK, Samanta PK, Kamilya T, Saha S. Chemical growth of spherical zinc oxide nanoparticles and their structural, optical properties. J. Physical Sci. 2015;20:205.
22. Bhunia AK, Kamilya T, Saha S. Temperature dependent and kinetic study of the adsorption of bovine serum albumin to ZnO nanoparticle surfaces. Chemistry Select. 2016;1:2872.
23. Bhunia AK, Kamilya T, Saha S. Synthesis, characterization of ZnO nanorods and its interaction with albumin protein. Materials Today: Proceedings. 2016;3:592.
24. Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). Journal of Nanoparticle Research. 2007;9:479.
25. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: A comparative study. International Journal of Nanomedicine. 2012;7:6003.

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