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Green Synthesis, Characterization and Antagonistic Activity of Chitosan/Zinc Oxide Nanoparticles

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ABSTRACT

The chitosan/ zinc oxide Nano particles are one of the well-known and commonly used metal oxide nanoparticles. It has stable physical as well as chemical properties. These metal oxides Nps have biological applications due to their excellent biocompatibility, economic and low toxicity characters. The present studyis to prepare Chitosan/ zinc oxide nanoparticles using chemical precipitation technique. The precursor is ZnO, Chitosan as the stabilizer and NaoH as the precipitator. The obtained nanoparticles were characterized by UV visible Spectroscopy, FTIR, XRD, SEM and EDAX techniques. The antibacterial activity of Chitosan/ ZnO nanoparticles was evaluated against pathogenic bacteria.

Key words: *Chitosan/ ZnO nanoparticles, Chemical precipitation, Characterization, Antibacterial activity,*

Introduction

Nanotechnology mainly deals with the synthesis and stabilization of various semiconductor nanoparticels (NPs). These NPs have novel optical, electrical and mechanical properties. Nanoparticles can be used as an alternative to antibiotics.

Chitosan is a biodegradable, non-toxic and biocompatible polymer obtained from partly deacetylation procedure of chitin. It is a linear polysaccharide with biotechnological, Bio-Medical and Pharmaceutical applications (Muxika *et al.*, 2017; Rinaudo, 2006; Muzzarelli, 2010; Dash *et al.*, 2011). It also has antibacterial and antifungal properties. It is safe and nontoxic to the environment (Kim and Rajapakse, 2005).

Since chitosan has tremendous ability to form metal complexes with zinc metal (Wang *et al.,* 2017; Predoi *et al.,* 2020) because of its amine groups and hydroxyl groups interact with ZnO metal to form Chitosan ZnO complex (Muzzarelli and Sipos, 1971; Muzzarelli and Tubertini, 1969). Currently, chitosan/ZnO complex attracted great interests for its potential use as UV protector and medicament. Chitosan/ ZnO NPs (CSNPs) are a nanosized, metal oxide nanoparticle. It is an effective antibacterial agent due to its highantibacterial activity, thermal stability and good bio compatibility (Ambika and Sundrarajan, 2015; Svetlichnyi *et al.,* 2016). CSNPs have good bactericidal activity against gram positive as well as gram negative bacteria (Luo *et al.*, 2013; Zhang *et al.*, 2013; Jain *et al.*, 2013; Yousef and Danial, 2012). CSNPs have been prepared by various techniques. The important techniques are sol gel method (Hubbard *et al.*, 2006; Lee *et al.*, 2003), precipitation (Wang and Muhammed, 1999), hydrother-

([1]*Research Scholar, [2]Associate Professor and Head, [3]Assistant Professor) mal synthesis (Xu *et al.*, 2004) and spray pyrolysis (Tani *et al.*, 2002). Recently Nps have also been employed as a carrier to provide therapeutic agents to cure bacterial infection (Husen and Siddiqi, 2014; Siddiqi and Husen, 2017). CSNPs have cytotoxicity in concentration, dependent manner and type of cells exposed due to different sensitivity (Ng *et al.*, 2017; Stankovic´ *et al.*, 2013; Laurent *et al.*, 2008). Further, accumulation of the NPs on the bacterical surface stabilized by electrostatic forces (Zhang *et al.*, 2008) and the intrinsic antibacterial properties of ZnO^{2+} ions released by Zno in aqueous medium leads to cell lysis take place. On the other hand, Chitosan based ZnO nps acts as a stabilizing agent it prevent particle aggregation in aqueous environment. Interestingly, CSNPs not only have less toxic impact compared to the chitosan and metal nanoparticles alone, but also shows an inhibitory effect on the growth of several microorganisms (Ghadi *et al.*, 2014; Yahya *et al.*, 2020). Due to its small size, high permeability, biocompatibility, biodegradability, and cost-effectiveness CSNPs have been applied in many fields such as nano medicine and biomedical engineering.

The antimicrobial activity of the chitosan polymers and nanoparticles may be mainly attributed to their electric charge and high ability of adsorption as well as chemical reactions that allow them to interact efficiently with the bacterial cell membrane (Wang *et al.*, 2018), while the inhibitory effect on the bacterial growth depends on their sizes and shapes as well as biological and structural properties (Li *et al.*, 2014). Compared to the antibacterial activity of chitosan and metal nanoparticles alone, significant inhibition could be expected by the combination of chitosan with metal nanoparticles. Indeed, chitosan has also been studied as the main structural unit of nanomaterials due to its low toxicity, non-immunogenicity, and biodegradability.

The present study mainly focused on (i) preparation and characterization of chitosan/zinc oxide nanoparticles using precipitation method and (ii) application of chitosan/ZnO nanoparticles to impart antibacterial properties.

Materials and Methods

Material and sample preparation

Chitosan/ ZnO nanoparticles were synthesized by biogenic method from the Crab shell waste.The fresh raw sea flower Crabs (*Portunus pelagicus*) were procured from the Colachel fish market, Kanyakumari, Tamil Nadu, India (Fig. 1). Crabs' shells were cleaned thoroughly to remove sand, dirt and other impurities using distilled water and air dried for a couple of week. The dried Crab shells were kept in a mortar and pestle and powdered. The particles were sieved to fine sizes using a sieve of 2.0 mm mesh size for easy extraction. The sieved shell sample was stored in an opaque glass bottle for further analysis (Sumaila *et al.*, 2020).

Fig. 1. Preparation of crab shell for the synthesis of Chitosan/ ZnO nanoparticles

Chitosan extraction from crab shell

The chitosan extraction from the crab shell (Fig. 2) followed Demineralization, Deproteinization and Deacetylation procedures in Sumaila *et al.* (2020).

Green synthesis of Chitosan/ ZnO nanoparticles

One gram of extracted Chitosan is dissolved in 100 ml of 0.1 M Acetic acid and 1gram of Zinc oxide powder is added to this mixture. The whole solution

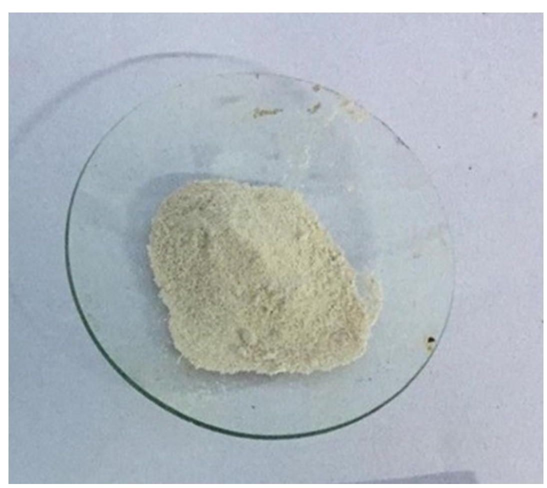


Fig. 2. Extracted Chitosan from Crab shells for the production of Chitosan/ ZnO nanoparticles

is agitated vigorously using magnetic stirrer for about 4 hours. The complex was stabilized. After stabilization 1ml of 1% NaOH solution was added drop wise. The solution was heated for about 8-12 hours in a water bath. The homogenized solution was then washed with water as well as double distilled water. The solution was kept inside the oven for 24hours at 60 ºC. After which it was centrifuged at 6000 rpm for 30 min. The obtained precipitate was washed several times with bi-distilled water to get pH 7 dried at 90 °C for 8 h (Fig. 3).

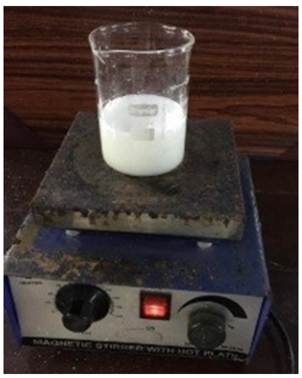


Fig. 3. Green synthesis of Chitosan/ ZnO nanoparticles (Magnetic stirrer)

Fig. 4. Green synthesis of Chitosan/ ZnO nanoparticles (Water bath)

Characterization of Chitosan/ ZnO nanoparticles

The green synthesised chitosan/ *ZnO nanoparticles* was characterized for its functional groups; structure and morphology by the Ultra-violet spectroscopy (UV), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDAX).

Fig. 5. Green synthesized Chitosan/ ZnO nanoparticles

Antogonistic activity of Chitosan/ ZnO nanoparticles

The green Synthesized *Chitosan/ ZnO nanoparticles* were evaluated for their antimicrobial activity against the gram positive microorgaisms viz., *Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis* and *Proteus mirabilis*, and gram negative microorgaisms viz., *Enterococcus faecalis, Proteus vulgaris, Klebsiella pneumonia* and *Escherichia coli* byagar well diffusion method (Klink *et al*., 2022).

Results and Discussion

UV spectrum of Chitosan/ ZnO nanoparticles

The UV spectrum of Chitosan/ Zinc oxide nanoparticles are shown in Figure 6. A peak obtained at 250 nm to 350 nm confirms the existence of

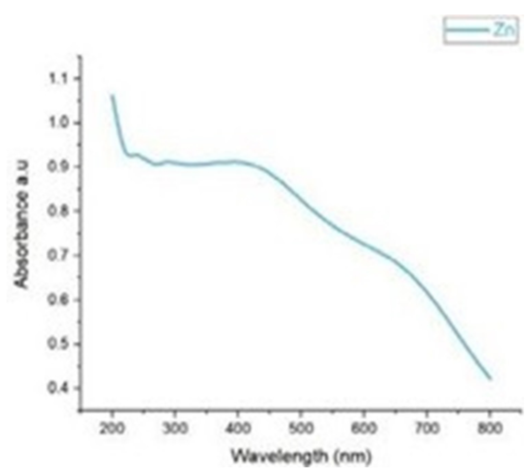
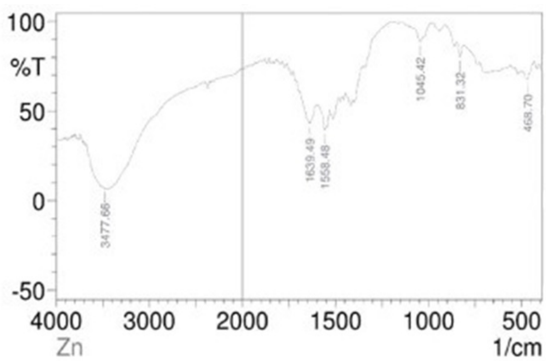


Fig. 6. UV Spectrum of Chitosan/ZnO nanoparticles

zinc metal in the Chitosan/ ZnO nanoparticles sample. The same peak was obtained for the presence of zinc in various studies (Satyanarayana *et al.,* 2012; Jin *et al.,*2000).

FTIR spectrum of Chitosan/ ZnO nanoparticles

The FTIR spectrum of Chitosan/Zinc oxide nanoparticles are shown in the Figure 7. The functional groups and the mode of attachment of metal oxygen bond are interpreted by FTIR spectra. The recorded spectrum for the sample is in the range of 4000cm^{-1}- 500cm^{-1}. The sample produced six characteristic peaks at 3477 cm^{-1},1639cm^{-1}, 1568cm^{-1},1045cm^{-1}, 831cm^{-1}and 468cm^{-1}. The peak obtained at 3477 cm^{-1} - 3160 cm^{-1}represents the presence of O-H and N-Hgroups. Two peaks formed at 1639 cm^{-1} and 1568 cm^{-1} indicates the bending vibrations. A new absorption peak at 400 nm - 468 nm confirms the existence of zinc in the Chitosan/Zinc oxide nanoparticles (Narayanan *et al.,* 2012).



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	468.7	67.636	7.233	495.71	433.98	8.993	1.333
2	831.32	80.127	7.565	848.68	812.03	2.718	0.616
3	1045.42	88.409	4.143	1138	1028.06	2.536	0.208
4	1558.48	39.445	3.554	1598.99	1550.77	15.72	-0.136
5	1639.49	43.126	1.762	1697.36	1635.64	17.878	0.354
6	3477.66	6.842	0.078	3496.87	3473.8	28.583	0.06

Fig. 7. FTIR spectrum of Chitosan/ ZnO nanoparticles

XRD spectrum of Chitosan/Zinc oxide nanoparticles

Figure 8 represents the X-ray diffraction pattern of chitosan/ ZnO nanoparticle. An enlarged line of the XRD peak showed that the formulated material comprises the particles in nanoscale range. The experiment helps to conclude the position and width, peak intensity, full-width at half maximum (FWHM) data, using the XRD pattern analysis. The diffraction peaks located at 31.84°, 34.52°, 36.33°, 47.63°, 56.71°, 62.96°, 68.13°, and 69.18° have been keenly indexed as hexagonal wurtzite phase of ZnO (Zhou *et al.,* 2007; Khoshhesab *et al.,* 2011) with lattice constants a=b= 0.324 nm and c=0.521 nm (JCPDS, 1977). In addition, it also proves that the synthesized nano powder does not have any impurities due to its non XRD characteristics peaks other than ZnO peaks. The synthesized ZnO nanoparticle diameter has been computed using Debye-Scherrer formula (Scherrer, 1918) d=0.89$\lambda\beta cos\theta$, (1) where 0.89 is Scherrer's constant, θ is the wavelength of X-rays, λ is the Bragg diffraction angle, and β is the full width at half-maximum (FWHM) of the diffraction peak corresponding to plane 101. The average particle size of the sample was found to be 16.2 nm which is derived from the FWHM of more intense peak corresponding to 101 planes located at 36.33° using Scherrer'sformula. The spectrum shows a broadening of lines and this confirmes that the molecules are in nanoscale. Using Debye Scherrer's formula and comparing the value with JPCDS it is proved that Zinc nanoparticles are hexagonalwurzite crystalline structure (Srivastava *et al.,* 2013).

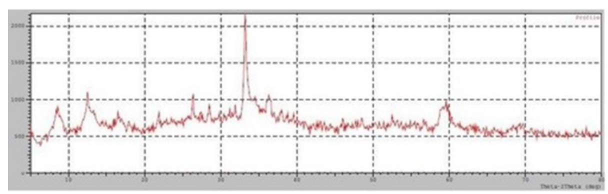


Fig. 8. XRD spectrum of Chitosan/ ZnO nanoparticles

***SEM and EDAX of* Chitosan/ ZnO Nanoparticles**

Figure 9 shows the SEM image of Chitosan/ Zinc oxide nanoparticles. SEM is recorded with two different magnifications such as X10000 and X30000. It is confirmed that the molecule is purely crystalline. Due to agglomeration, the crystals are looking like cluster of molecule including Zinc (Srivastava *et al.,* 2013).

The EDAX of Chitosan/ ZnO nanoparticles are shown in figure 10. The presence of Zinc metal is confirmed by observing two small peaks at 8 and 9 K Ev and a large peak at 1 K Ev. The other promi-

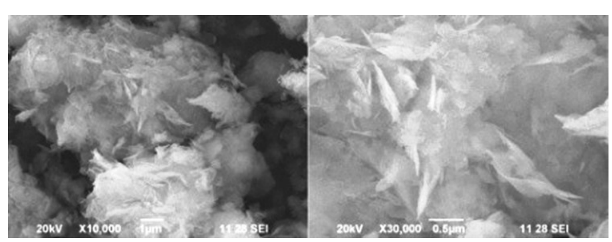


Fig. 9. SEM image of Chitosan/ZnO nanoparticles

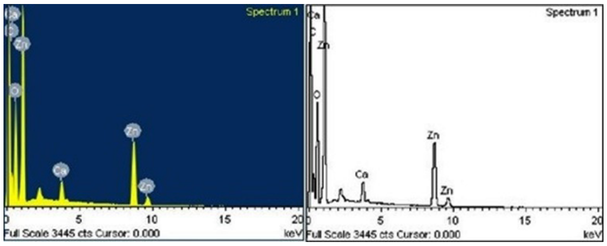


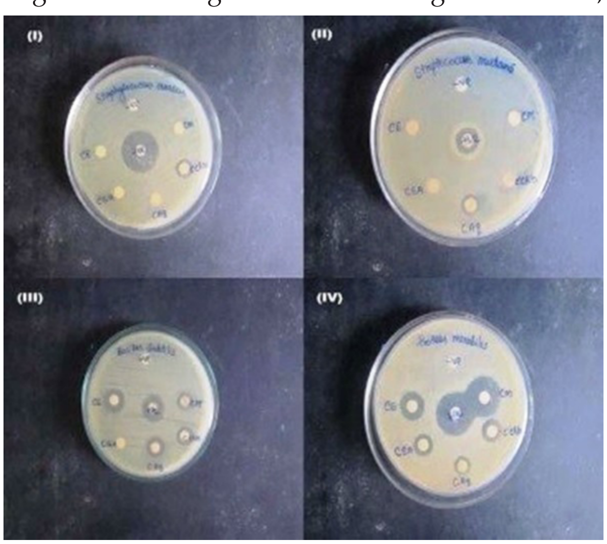
Fig. 10. EDAX of Chitosan/ ZnO nanoparticles

nent peaks showed the existence of Calcium. Few impurities such as Cl , K and Oxygen are also recorded (Roselina and Azizan, 2012).

Antogonistic activity of Chitosan/ ZnO nanoparticles

The figure 11 showed the result of antogonistic activity of Chitosan/ ZnO nanoparticles against Gram positive microorganisms. The present study revealed that *Proteus mirabilis* is the highly sensitive organism because it forms 19mm and 18mm zone of inhibition for methanol and aqueous mixture of Chitosan/ ZnO nanoparticles. *Staphylococcus aureus* was also sensitive to the chitosan/ ZnO nano particles followed by *Proteus mirabilis*. *Streptococcus mutans* was considered as a resistant stain for the synthesised nanoparticles, lacks zone of inhibition.

The figure 12 shows the result of antogonistic activity of Chitosan/ ZnO nanoparticles against Gram negative microorganisms. According to the result, aqueous solution of chitosan/ zinc oxide nanoparticle shows 16 mm and 15 mm zone of inhibition against *E.coli* and *Proteus vulgaris* respectively. Hence the strains were considered as sensitive strains and others are considered as moderately sensitive based on the zone of inhibition. Similarly, the zinc oxide nanoparticle exhibit 19, 31 and 23 mm zone of inhibition against *Staphylococcus aureus*, *E.coli* and *Pseudomonas aeruginosa* (Namasivayam *et al.*, 2015). *Klebsiella pneumoniae* is sensitive to all the five solvent mixture. *Enterococcus faecalis* and *Staphylococcus aureus* is the highly resistant organisms because it shows zone of inhibition only for chloroform mixture of Chitosan encapsulated zinc nanoparticles, Javid and Ghaemi (2016).



(I) *Staphylococcus aureus* (II) *Streptococcus mutans* (III) *Bacillus subtilis* (IV) *Proteus mirabilis*

Fig. 11. Antibacterial Activity of chitosan/ ZnO nanoparticles against Gram positive microorganisms

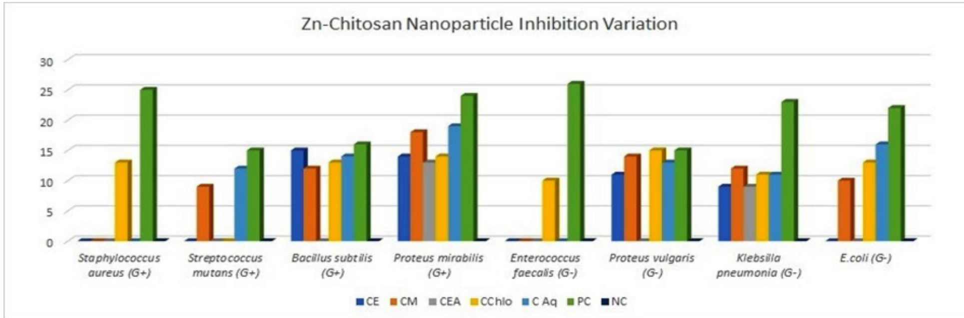
(V) *Enterococcus faecalis* (VI) *Proteus vulgaris* (VII) *Klebsiella pneumoniae* (VIII) *Escherichia coli*

Fig. 12. Antibacterial Activity Result of Gram Negative Microorganisms

Figure 13 showed the zone of inhibition variation among the gram positive and negative strains. According to the results, gram positive strains are observed as more susceptible to the synthesized chitosan/ ZnO nanoparticles than Gram negative strains. *Enterococcus faecalis* and *Staphylococcus aureus* are observed as resistant strains for the synthesized chitosan/ ZnO nanoparticles.

Conclusion

Green synthesis and characterization of Chitosan/ ZnO nanoparticles were investigated. In this study, Chitosan can be used as a biodegradable polymer as well as a strong capping agent. Zinc is an essential



Zn E - Zinc ethanol ; Zn M – Zinc methanol; ZnEA–Zincethylacetate; Zn Chlo Zinc chloroform; ZnAq – Zinc Aqueous; PC - Positive Control (Streptomycin 25μg); NC-Negative Control (Sterileplaindisc)

Fig. 13. Chitosan/ zinc oxide nanoparticles Inhibitory zone variation against bacteria

trace element for human, animals, plants and bacterial growth. In-spite of this, ZnO nanoparticles are toxic to many fungi viruses and bacteria. Chitosan/ ZnO nanoparticles synthesized fron the present study may be used for treating wounds, ulcers and many microbiological infections in near future. Moreover, Chitosan-Nps can be used as a drug carrier in cancer therapy. However the obtained Nps is one of the safe antibacterial drug. Although Chitosan/ ZnO Nps have various applications in different areas such as science, medicine and technology. From all these results, Chitosan encapsulated ZnO Nps prove that it is an environment friendly anti-biomolecule. But, further invivo studies are required to determine the toxicity of these Nps.

Conflict of Interest: None

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