ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF CHITOSAN NANOPARTICLES AND ITS CHARACTERIZATION

S.SANGEETHA., K.KAVITHA

Department of Biochemistry, Vivekanandha College of Arts & Science for Women (Autonomous),

Namakkal, Tamilnadu, India.

Abstract : Chitosen are known to possess antimicrobial and antioxidant activity while chitosan is a biocompatible polymer with antibacterial activity against a many microbe. In this study, antioxidant and antibacterial properties of chitosen nanoparticles were prepared. Antibacterial assays were carried out with a representative gram-negative bacterium and a gram-positive bacterium, the results conferred that antioxidant activity of the chitosan nanoparticles, and the chitosan nanoparticles achieved an antibacterial activity. The results showed that the synthesized ZnNPs have the antioxidant and antimicrobial activity due to the nano size.

IndexTerms **- Nanoparticles, Chitosan**.

1. Introduction:

Chitosan is natural, non-toxic, copolymer of glucosamine and N-acetylglucosamine prepared from chitin. Chitin is a natural polysaccharide synthesized by a great number of living organisms and functions as a structural polysaccharide. Chitosan is the only pseudo natural cationic polymer which has many potential biomedical and other applications. Chitosan has been largely employed in many areas, such as photography, biotechnology, cosmetics, food processing, biomedical products such as artificial skin, wound dressing and contact lens and the system of controlled liberation of medicines as capsules and microcapsules. Chitosan nanoparticles are good drug carriers because of their good biocompatibility and biodegradability, and can be readily modified. Chitin, the second most abundant natural polymer on earth after cellulose, is present in fungal cell walls and in the exoskeleton of arthropods like crustaceans.

Nanoparticles are synthesized by size reduction using either top–down methods such as milling, high-pressure homogenization and sonication or bottom–up processes like reactive precipitation and solvent displacement (Vauthier 2003). Nanoparticles are grouped into organic and inorganic nanoparticles. The inorganic nanoparticles have gained significant importance due to their ability to withstand adverse processing conditions(Whitesides *et al.*,2003). Metal oxide nanoparticles such as titanium oxide, zinc oxide, silver oxides and magnesium oxides are of great interest among inorganic materials due to their tunable optical properties and physical and optical stability (Makhluf *et al*., 2005). Due to the unique electronic, metallic and structural characteristics, organic materials like carbon nanotubes, lipids and polymers have versatile applications (Hatton *et al.,* 2008).

Polymeric nanoparticles can be synthesized from natural and synthetic polymers. They are used owing to their stability and ease of surface modification. Biopolymeric nanoparticles have added advantages, like availability from marine (chitin and chitosan) or agricultural (cellulose, starch, pectin) resources, biodegradability, biocompatibility and nontoxicity. Biodegradable polymers such as chitosan are studied mainly as delivery systems for controlled release of active ingredients, stabilization of biological molecules like proteins, peptides or genetic material (Ghormade *et al*., 2011). The present study is aim to synthesis and characterization of chitosan nanoparticle and also to analyze the in-vitro antioxidant activity and Cytotoxicity activity against the HepG-2 Cell line

2. Materials and methods

2.1 Synthesis of Chitosan Nanoparticles:

Chitosan nanoparticles were prepared using ionic gelation method. Different concentrations of chitosan (1-5 mg) was added to 1 % acetic acid (v/v) and mixed well using magnetic stirrer. The Ch NP were formed by adding 1 % TPP (w/v) drop by drop under magnetic stirring. Then the solution was centrifuged at 10,000 rpm for 10 minutes to remove residual TPP and the particles were freeze dried.

2.2 FT-IR analysis

The nanoparticles were harvested and characterized by FTIR. The FT-IR spectrum was taken in the mid IR region of 400-4000cm1. The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode

2.3 Antibacterial activity:

To perform antimicrobial activity using various bacterial and fungal species were selected viz., *Escherichia coli, staphylococcus aureus, pseudomonas species, bacillus species, klebsiella species* are bacterial cultures.

2.4 Antioxidant activity of the nanoparticles

Antioxidant activity of the Chitosan NPs was evaluated using DPPH radical scavenging activity. A dose of 0.1ml of the colloidal Chitosan NPs was added to 1.0ml ethanol solution of 0.1mM DPPH radical. The mixture was shaken vigorously for 2 min and incubated at 25^0C in the dark for 90min. The absorbance of the sample was measured using UV-2450, Shimadzu Spectrophotometer at 520nm against ethanol blank. A negative control was taken after adding DPPH solution to 0.1ml of deionized water. The percent of DPPH discoloration of the sample was calculated according to the following equation Xu &

Chang [8]. Free radical scavenging activity was expressed as an equivalent of mmol Trolox standard. Linearity range of the calibration curve was 0.1 to 10µmol/ml (Correlation coefficient R2 =0.9).

2.5 RESULTS & DISCUSSION

The results show the synthesis of AgNPs using chitosan. Further, the formation of silver nanoparticles was confirmed by the UVe Vis spectrophotometer and confirmed the formation of silver nanoparticles. However, lack of LSPR suggests the formation of ultra small silver nanoparticles or the silver cluster, which contains a small number of atoms. The synthesis of AgNPs by green route becomes popular because of no use of toxic chemicals, cheap, eco-friendly and suitable for pharmaceutical and biomedical applications. Recently, several kinds of literature proved that bacteria have been resistant to antibiotics and alternative antibi-otics required. The SEM results proved that the variable size silver nanoparticles were synthesized (Fig. 1).

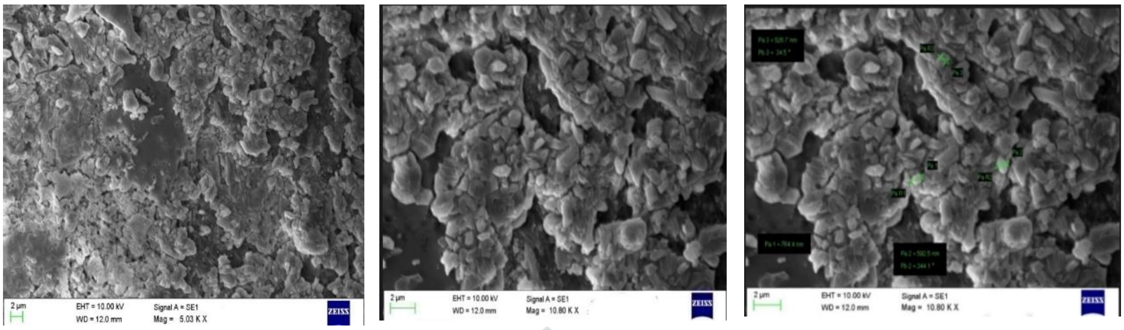

Figure 1: Scanning Electron Microscope

The FTIR results confirmed the various functional group. Fig. 2. Graph represents the percentage of Antioxidant (DPPH), and Free radicals (hydrogen peroxide, hydroxyl and superoxide radical) scavenging activity of silver nanoparticles and vitamin C.A.K. Keshari*et al*. (2018). The antimicrobial properties of chitosan solutions and films against selected bacteria and the effect of chitosan incorporation into gelatin films were studied. The bactericidal effect of chitosan solutions increased with time and temperature of sample incubation. Two psychrotrophic strains *Pseudomonas fluorescens* and *Listeria innocua*were more sensitive to chitosan than mesophilic strains *Escherichia coli* and *Staphylococcus aureus*. The growth of bacteria under chitosan discs was inhibited. In the case of two component gelatin-chitosan films strong antimicrobial effect was also observed. *S. aureus* (ATCC 25923), *Candida albicans* and *Aspergillus niger*are used for the measurement of antimicrobial activity. The antimicrobial activity is probably derived, through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles. It reflects that Chitosan nanoparticles have an excellent anti-bacterial and anti-fungal effect and potential in reducing bacterial, fungal growth for practical applications.

The in vitro antioxidant activity Chitosan was evaluated and compared as radical scavengers against 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH), hydroxyl radical (OH), and superoxide radical (O2) using established methods, and the effect of the molecular weight, the concentration, the newly generated hydroxyl group, the extra introduced positive charge of quaternary ammonium salt group, etc, on the antioxidant activity of these high molecular weight Chitosan is discussed. The data obtained in vitro models exhibited good antioxidant potency and suggested the possibility that high molecular weight Chitosan based films could be effectively employed as natural antioxidant materials for application in the field of food and medicine. This assay has been widely used to test the free radical scavenging ability of various samples. The obtained results indicate that the increasing plant extract concentration such as 20, 40, 60, 80 and 100 µg/mL increases the percentage of inhibition as well. About 100 µg/ml of show better antioxidant protection of 93 % compared to the standard with 94.27 % of same concentration. The IC_{50} value of chitosan and ascorbic acid were found to be 22.5 µg/ml and 15.2 µg/ml respectively. Antioxidant activities according to the DPPH assay and ROS determination tests. Furthermore, undesirable inflammatory reactions were not induced. Due to Chitosan favorable biological properties such as non-toxicity, biocompatibility, biodegradability and antibacterial ability, they are also promising candidates for drug delivery carriers and cell proliferation enhancers. However, most of these studies are still at the laboratory level. Additional studies are necessary before their industrial application. We hope that, this approach could be potentially used to industrial large scale synthesis of ZnNPs. The results showed that the synthesized ZnNPs have the antioxidant, cytotoxic and antimicrobial activity due to the nano size.

REFERENCE

1. Vauthier, C., Dubernet, C., Fattal, E., Pinto-Alphandary, H., Couvreur, P., 2003. Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications. Adv. Drug Deliv. Rev. 55 (4), 519–548.
2. Whitesides, G.M. (2003) The “Right” Size in Nanobiotechnology. Nature Biotechnology, 21, 1161-1165.
3. Makhluf, S., Dror, R., Nitzan, Y., Abramovich, Y., Jelinek, R. and Gedanken, A., Microwave-assisted synthesis of nanocrystalline MgO and its use as a bacteriocide. Advanced Functional Materials, 15, 1708-1715 (2005).
4. Hatton RA, Miller AJ, Silva SRP. Carbon nanotubes: a multi-functional material fororganic optoelectronics. J Mater Chem 2008;18:1183–92430
5. Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. BiotechnolAdv 29(6):792–803
6. GrenhaGhormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. BiotechnolAdv 29(6):792–803
7. Braca, A., N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi and I. Morelli. Antioxidant principles from Bauhinia terapotensis. J. Natl. Prod. 2001; 64: 892-5.
8. I.F. Benzie, J.J. StrainThe ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assayAnal. Biochem., 239 (1996), pp. 70-76
9. Pulido, R., Bravo, L., Sauro-Calixo, F. (2000). Antioxidant activity of dietary polyphenols as determined by a mod-ified ferric reducing/antioxidant power assay, J. Agri. Food chem., 48: 3396-3402. DOI PMid:10956123
10. Garrat DC. (1964) The Quantitative analysis of Drugs. Chapman and Hall Ltd., Japan, 3: 456-458