Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5542

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 1080-1084 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Synthesis of Chitosan Mediated Nanoparticles and its Antimicrobial Activity

Kulkarni C. P.*

Department of Chemistry,

Kirti M. Doongursee College of Arts, Science and Commerce, Dadar (W), Mumbai - 400028, India

*Corresponding Author E-mail: hortsuren@gmail.com

Received: 28.08.2017 | Revised: 30.09.2017 | Accepted: 4.10.2017

ABSTRACT

In this study, chitosan nanoparticles were synthesized from chitosan polymer by ionic gelation method. The chitin was first extracted from crab shell and then deacetylated to chitosan. The presence and characterization of chitosan nanoparticles was investigated by UV-Visible Spectroscopy (UV) and scanning electron microscopy (SEM). The antimicrobial property of these chitosan nanoparticles were studied against E.coli, S.aureus, S.typhi, P.aureginosa, C.albicans and A.niger and compare with chitosan polymer. CSNPs showed higher antimicrobial activity than chitosan. The study is thus a good demonstration of the applicability of chitosan nanoparticles as an effective antimicrobial agent.

Key words: *Chitosan, Nanoparticles, crab shell, SEM, antibacterial activity*

INTRODUCTION

Today, nanotechnology is found in a wide range of applications in the pharmaceutical industry. Due to new advances in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways. Injectable nano particulate carriers have important potential applications even though conventional carriers can generally be used to reduce the number of administration doses and improve delivery efficiency while decreasing the adverse effects of drug toxicity. In order to achieve this aim, mono dispersed biodegradable nanospheres were developed that could be freeze-dried and easily re-dispersed, without additives, in aqueous solutions.

Chitosan (CS) is a polysaccharide obtained from deacetylation of chitin. CS is composed of deacetylated β(1−4) 2-amino-2-deoxy-β-D-glucan monomers and monomers with N-acetyl groups in place of amino groups that are linked by glycosidic bonds. Chitosan has received great attention in both the medical and pharmaceutical fields. Chitosan, a biodegradable and biocompatible polymer, is a modified natural carbohydrate and the second most abundant polysaccharide in nature. Chitosan is available in a wide range of molecular weights and deacetylation degrees. Due to its characteristics, chitosan has gained increasing attention in the pharmaceutical field.

Cite this article: Kulkarni, C. P., Synthesis of chitosan mediated nanoparticles and its antimicrobial activity, *Int. J. Pure App. Biosci.* **5(5):** 1080-1084 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5542

Copyright © Oct.-Nov., 2017; IJPAB

In addition, chitosan presents mucoadhesive, immune stimulating, antimicrobial and wound-healing properties. Moreover, it has been regarded as a promising polymer for the formulation of vaccine delivery systems.

On the other hand, the evaluation of chitosan as an adjuvant for parenteral vaccination studies was reported together with the results of intranasal or oral vaccination studies, making the possible value of chitosan as an adjuvant for parenteral routes less noticeable in the scientific literature.

Chitin is a characteristic compound found in fungi and some animals. In animals, chitin mainly exists in the shells of crustaceans and mollusks, in the backbone of squids and in the cuticle of insects. Chitosan is widely recognized for its potent antimicrobial activity with, broad spectrum, and high killing rate but low toxicity toward mammalian cells. Chitosan acts as water binding agent and inhibits various enzymes. Various factors play role in antimicrobial activity of chitosan[1].

In the present study, we have synthesized and characterized ChNP by ionic gelation method. The antibacterial activity of ChNP against *E. coli*, *S. aureus*, *S. typhi*, *P. aureginosa*, *C. albicans* and *A. niger* was evaluated.

MATERIALS AND METHODS

Collection of sample:

The crab shell was collected from Mira road (E), fishery shop. Washed thoroughly 2-3 times under running water and once with sterile distilled to remove extraneous matter present on the surface. Shells were autoclaved and air dried under sunlight then grinded in small particles.

Preparation of chitosan:

Chitin extraction from crab shells was carried out as described previously for other crustacean shells by an alkali-acid treatment with minor modifications of the treatment conditions. DPMCA (deproteinization + demineralization + decolorization + deacetylation) was taken as the traditional processing method.

Microorganism: The microorganisms namely *E.coli*, *S.aureus*, *S.typhi*, *P.aureginosa*, *C.albicans* and *A.niger* were procured from Department of Biotechnology, The D.G. Ruparel College of Arts, Science and Commerce, Matunga, Mumbai, India.

Isolation of chitosan from crab shells

Crab shells were suspended in 1 mol l M NaOH solution (1 : 30 w/v) and autoclaved at 121°Cfor 15 min. Alkali-insoluble fractions were collected after centrifugation at 12 000 g for 15 min, washed with distilled water and recentrifuge to a neutral pH (pH 7). Further extracted the residues using 2% acetic acid (1 : 40 w/v) at 95°C for 8 h. Centrifuged the extract slurry at 12 000 g for 15 min and insoluble acid were discarded.

The pH of supernatant fluid were adjusted to 10 with 2 M NaOH, the solution was centrifuged at 12 000 g for 15 min and washed the precipitated chitosan with distilled water, 95% ethanol (1: 20 w/v) and acetone (1: 20 w/v), respectively and dried at 60°C to a constant weight.

Synthesis of Chitosan Nanoparticles

Chitosan solution was prepared of 2.5 mg/ml by dissolving the polymer in 1% (w/v) acetic acid aqueous solution for 0.5 hrs under magnetic stirring. The pH of solution was adjusted to 5.0-6.0 using 1 mol/L NAOH. Chitosan solution was stirred for 0.5 hr at room temperature. Finally, dissolved sodium tripolyphosphate (TPP), the counter ion in pure water to prepare a 1mg/ml solution, added in to the chitosan solution under mild magnetic stirring to form chitosan nanoparticles. Centrifuged the nanoparticles solution at 18000 rpm and 4°C for 30 minutes, after which the nanoparticles were collected at the bottom, extensively washed 3 times with water to remove the TPP and the acetic acid, and finally lyophilized and Stored at 4°C- 8°C *(Yu-Lan H et al., 2011)*.

Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were characterized by SEM (Scanning Electron Microscopy) by Philips XLD 3D model, CIRCOT, Matunga East, Mumbai, to examine the particle size and surface morphology. Where CSNPs are coated with gold metals film and magnified under 15000X.

Antimicrobial Activity of Chitosan and chitosan Nanoparticles

The antimicrobial activity of chitosan and chitosan nanoparticles is studied using agar diffusion method. For this petriplates containing 20 ml Muller Hinton medium are seeded with 24 hrs. old culture of bacterial strains (viz., *Escherichia coli, Staphylococcus aureus, Salmonella typhi*) wells are cut and 100 microliter of above solutions are added. The plates are often incubated at at 37 C for 24 hours. The antibacterial activity is assayed by measuring the diameter of the inhibition zone formed around the well[4].

The antifungal activity is studied using disc diffusion method against fungus- *Aspergillus niger* and yeast-*Candida albicans*. Potato Dextrose Agar plates are prepared and the test culture is inoculated by swabbing the cuture suspension. Filter paper discs approximately 6mm in diameter are soaked with dilutions of above mentioned solutions and placed in the previously prepared agar plates. Each disc is pressed down to ensure complete contact with the agar surface. The agar plates are then incubated at RT. After 16-18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition are measure[3].

RESULTS AND DISCUSSION

Chitosan synthesis from Crab shell

In this study chitosan has been successfully prepared from *Crab shell*. The synthesis of chitosan involves various chemical steps. Pretreatment methods were done using 1N NaOH and 2% acetic acid. The alkali and acid treatments remove proteins and minerals from chitin respectively and deacetylates simultaneously. These methods give advantages for obtaining of higher quality chitosan. Chitin is not soluble but chitosan, the deacetylated product of chitin, is soluble in very dilute acids like acetic acid, lactic acid, formic acid etc. the deacetylation experiment using 2 N NaOH was done to reduce acetyl group from molecular structure, because the presence of acetyl group prevents to make the solution of chitosan. 10 gm (dry weight) of *Saccharomyces cerevisiae* gives 0.9gm chitosan and percentage yield of chitosan is 0.81%.

Preparation and characterization of chitosan nanoparticles

Chitosan nano particles can be prepared using many methods such as ionic gelation method, emulsion cross- linking, and spray drying. In this study, ionic gelation method was applied because the method is easy and fast to be carried out. This simple technique involves electrostatic interaction between positively charged amino group of chitosan and negatively charged polyanions. Formation of nanoparticles occurs spontaneously through the formation of intra- and intermolecular cross- linkages under a constant stirring at room temperature[3].

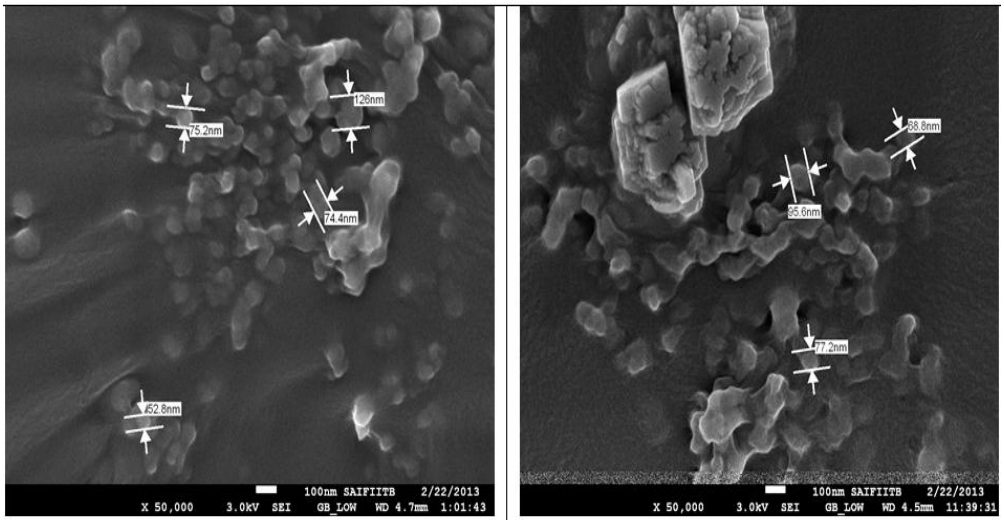

Fig. 1: Scanning electron microscopyphotograph of chitosan nanoparticles

The chitosan nanoparticles prepared in the experiment exhibit a white powdered shape and are soluble in deionized water. The synthesized chitosan nanoparticles were characterized by scanning electron microscopy. SEM was used for the determination of the particle size and the morphological structure of the prepared polymer matrix. The coating of chitosan nanoparticles were done by gold metal and magnified under 15000X. It was observed that chitosan/ TPP has average particle size of 60-110 nm. Fig.1 shows the size of chitosan nanoparticles. Particle size of chitosan nanoparticles is depending on concentration of chitosan and TPP, their mass ratios, and drying methods. Fig.1 shows the SEM picture of chitosan nanoparticles.

Antimicrobial activity of chitosan and CSNPS

When antimicrobial activity of CSNPs and chitosan was compared, CSNPs showed more potent activity against all these cultures used *E. coli*, *S. aureus*, *S. typhi*, *P. aureginosa*, *C. albicans* and *A. niger.*

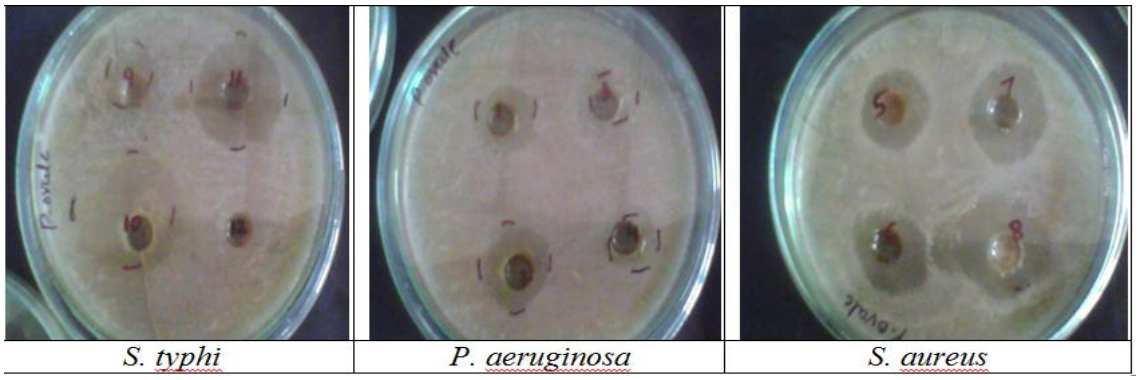

Fig. 2: Antimicrobial activity of chitosan and chitosan nanoparticles

Table 1: The results of agar well diffusion and the disc diffusion methods

Microorganisms	Zone of inhibition (mm)					
	Concentration Of Chitosan Nanoparticles (%)			Concentration Of Chitosan (%)		
	0.5	1.0	1.5	0.5	1.0	1.5
E. coli	22	24	26	-	-	12
S. aureus	16	18	22	-	12	15
S. typhi	14	16	20	14	15	17
P. aeruginosa	12	15	17	17	19	23
C. albicans	14	17	20	11	13	14
A. niger	15	17	22	13	14	17

These results therefore suggest that chitosan solution was less effective as an antimicrobial agent compared with chitosan nanoparticles. This finding coincides with the previous reported study by Qi *et al*, 2004 which demonstrated that chitosan nanoparticles exhibited higher antimicrobial activity due to their special characters of the nanoparticles such as small and compact particle as well as high surface charge. This could be explained by the fact that the fact that the negatively charged plasma membrane is the main target site of polycation. Therefore, the polycationic chitosan nanoparticles with high surface charge will interact more effectively with the fungus compared with free form of chitosan polymer.

Furthermore, chitosan particles have a higher affinity to bind to microbial cells nanosized CSNPs contribute to a larger surface area and cause nanoparticles to be able to absorb more tightly onto the surface of microbial cells and disrupt the membrane integrity. Also cellular uptake of chitosan

nanoparticles into cells is higher than that of chitosan molecules as the bulk chitosan molecules were located extracellularly. This suggests that chitosan nanoparticles might be able to diffuse into microbial cell and hence disrupt the synthesis of DNA as well as RNA. This could explain a better microbial activity of chitosan nanoparticles compared to its free polymer or solution form[4].

CONCLUSION

This study also found that the CNPs show antibacterial activity on both Gram positive and Gram negative bacteria and should be explored further for antimicrobial applications. Being of cationic character, chitosan is able to react with polyanions giving rise to polyelectrolyte complexes. Hence chitosan has become a promising natural polymer for the preparation of microspheres/ nanospheres and microcapsules. In addition, since the chitosan microspheres offer highly convenient and flexible systems for different application, it is believed that the biosynthesized novel CNPs can be considered for different purposes particularly biomedical application.

REFERENCES

1. El-Diasty, E.M., Eleiwa, N. Z., Aideia A.M., Using of chitosan as Antifungal Agent in Kariesh Cheese.*New York Science Journal* **5(9):** 5-10 (2012).
2. Gould, W., Grahame, Preservation, Past, Present and Future. *British Medical Bulletin*, **56:** 84-96 (2000).
3. Ling, YienIng, Zin, N. M., Sarwar, A., Katas, H., Antifungal Activity of Chitosan Nanoparticles and Correlation With Their Physical Properties. *International journal of Biomaterials*, 1-9 (2012).
4. Lifeng, Q., Zirong, X., Xia, J., Caihong, H and Xiangfei, Z., Preparation and antibacterial activity of chitosan nanoparticles. *Elsevier Carbohydrate Research*, **339:** 293-2700 (2004).
5. Mahae, N., Chalat, C. and Muhamud, P., Antioxidant and antimicrobial properties of chitosan- sugar complex. *International Food Research Journal*, **18(4):** 1543-1551 (2011).
6. Pochanavanich, P. and Suntornsuk, W., Fungal chitosan production and its characterization. *Letters in Applied Microbiology*, **35:** 17–21 (2002).
7. Raafat, D., Bargen, K. V., Has, A., Sahl, H.G., Insights into the Mode of Action of Chitosan as an Antibacterial Compound. *Applied and Environmental Microbiology*, **74 (12):** 3764-3773 (2008).