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Synthesis of Chitosan Silver nanoparticles from Chitin of Crustacean shells and Its Applications

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Abstract

Synthesis of nanoparticles is an emerging branch of nanotechnology. Chitosan is an ecofriendly, biodegradable and biocompatible nanoparticles with good sensing capability have gained importance. Chitosan- silver nanoparticles is one such system which possesses all the above mentioned qualities. In the present study chitosan based silver nanoparticles is synthesized, by using marine shells such as prawn and crab shells. The prepared nanoparticles were characterized using UV and SEM analysis. The SEM analysis shows that the synthesized silver nanoparticles are of about 96nm-130nm. The analysis confirms the presence of nanoparticles. The nanoparticles were subjected to antibacterial activity and were found to have maximum inhibition zone on *Streptococcus* sp. The synthesized nanoparticles were used as edible coating on fruits and vegetables. The shelf life period was measured on the coated fruit and vegetables, it was found to be no damage at room temperature after 28 days and 30 days of incubation, which increases the shelf life of post harvested fruits and vegetables.. Silver nanoparticles are selected for the present study has been known for its inhibitory and bacterial effects. So the present study clearly provides applications of silver nanoparticles on antibacterial and edible coating on fruits and vegetables.

Keywords: Silver nanoparticles, SEM, Antimicrobial activity.

Introduction

Chitin, poly-β-(1-4)-N-acetyl-D-glucosamine, the main component of shells of crustacea such as crabs, shrimps and various insects is the most widely occurring natural biopolymer along with cellulose (Martinou, et. al., 1995). Chitin is clustered with calcium carbonate and proteins whereas chitosan is prepared from chitin by deacetylating its acetamide groups with a strong alkaine solution (Shigemasa, et al., 1996). Since chitin and chitosan have various bioactivities and functionalities, they attract much attention and are studied in various fields of applications (Minamisawa, et. al., 1996), (Peter, M G, et. al., 1995).

Some desirable properties of chitosan are that it forms films without the addition of additives, exhibits good oxygen and carbon dioxide permeability, as well as excellent mechanical properties. Chitosan not only acts as a chelator in biological systems, but also exhibits antimicrobial activity against bacteria, yeasts and molds (Lucera, et.al., 2012). Promising results and applications are already being developed in the areas of nutrient delivery systems through bioactive nanoencapsulations, biosensors to detect and quantify pathogens organic compounds, other chemicals and food composition alteration, and even edible film to preserve fruit or vegetables (Duran and Marcato, 2012). Consequently, natural biopolymer-based nanocomposite packaging materials with biofunctional properties have a huge potential for applications in the active food packaging industry (Rhim and Perry, 2007). Silver nanoparticles (AgNPs) have been known for its inhibitory and bactericidal effects in the fast decades (Cho, KH, et.al., 2005).

Searching for new therapeutic agents capable to work against resistant bacterial strains is one of the most important challenges for nowadays science. Application of nanotechnology in creating new biomaterials provides new solutions mainly because of small dimensions of the created systems. One of the most effective promising materials are nanocomposites based on silver particles and chitosan (Ragiel and Kyziol, 2013). The present study reveals the green synthesis of silver nanocomposites and its characterization through UV Spectrophotometer and SEM. The synthesized nanocomposites were used as antibacterial agents. The self life period was also measured for fruits and vegetables using the synthesized nanocomposites.

Materials and Methods

Collection of sample

Crab and prawn shells were collected from Kundapura beach, Udupi District, Karnataka during August 2015. The prawn and crab shells cleaned and washed it with water until proteins are removed and dried at 60°C. Chemicals such as Sodium hydroxide, Hydrochloric acid and Silver nitrate were purchased from Durga Research Laboratories, Mangalore.

Microbial culture

Escherichia coli, Streptococcus sp., *Klebsiella* sp., *Bacillus* sp., *Salmonella* sp. microorganisms were maintained in laboratory of PG department of Biotechnology, Alva's College, Moodbidri. Pure cultures which were maintained were sub cultured on nutrient medium and used.

Synthesis of chitosan from Crab and prawns shells

The dried Crab and prawns shells were grinded using mortar and crusher. Powdered shells were treated with 1% NaOH at 60ºC for 8 hour to remove chitin. Higher solid to alkali solution ratio was preferred. The powder was filtered and demineralized using 5% Hydrochloric acid at 45ºC. It was then washed and decolourized in direct sunlight for about 10 hour. Chitin observed was deacetylated by treating it with 50% NaOH at 85ºC for 2 hours. Finally chitosan was synthesized from crustacean shells.

Synthesis of silver nanocomposites in various concentrations

0.05g of silver nitrate and 0.25% of glucose were added with 10ml of distilled water, autoclave at 121°C for 20 minutes and cooled at room temperature again centrifuged at 3000 rpm for 20 minutes. Supernatant was stored at 4°C for overnight. Next day chitosan was added in various concentration (10mg, 20mg, 30mg and 40mg) in 1ml of supernatant, centrifuged at 6000 rpm for 10 minutes, dried at 70°C hot air oven for 2 hours, nanocomposites was obtained.

Characterization of silver nanopartlicles

The formation of AgNPs was monitored by visual inspection of the solution, as well as by periodical recordings. UV-visible spectroscopy widely used techniques for structural characterization of silver nanoparticles. In SEM analysis size of chitosan Ag nanocomposites determined.

Antimicrobial activity by well diffusion method

To determine the antibacterial activity of Ag-NPs from chitosan, nutrient agar medium was sterilized and sterile media was poured aseptically and the plates were seeded with appropriate micro organisms *Escherichia coli, Streptococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Salmonella* sp. By evenly on to the surface of the medium with a sterile cotton swab. Wells were cut out and filled with 0.1ml of the each synthesized silver nanoparticles solution in respective wells. Then the plates were incubated at 37^0C for 24 hours. Zones of inhibition were measured with a measuring scale. This experiment was carried out in doublet for their confirmation.

Fruits and vegetable coating

Fruits and vegetables were coated in low molecular chitosan solution and low molecular chitosan AgNPs, by using sterile cotton swab thrice. Each coating has been done after drying of fruits and vegetables in equal interval of time period, and stored at room temperature. Samples were stored in aseptic condition for 28 days for further analysis.

Results

Characterization of silver nanocomposites Ultraviolet(UV)- visible studies

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. Silver nanocomposites Plasmon peak was observed at nearly 450nm in size from the below given peak. It was clearly indicating the presence of silver nanocomposites. Figure 1 shows the absorbance spectrum of synthesized silver nanocomposites from chitosan.

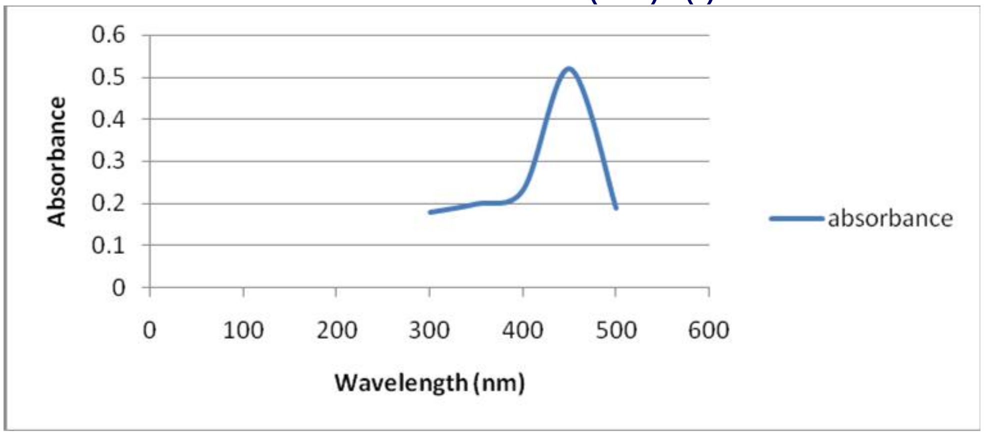

Figure 1: UV-Visible spectroscopy, absorption of Plasmon peak of Ag nanocomposites from chitosan

SEM

Size of chitosan Ag nanocomposites was determined by scanning electron microscope. The size of silver nanocomposites was found to be minimum of 96.3nm shown in figure 2.

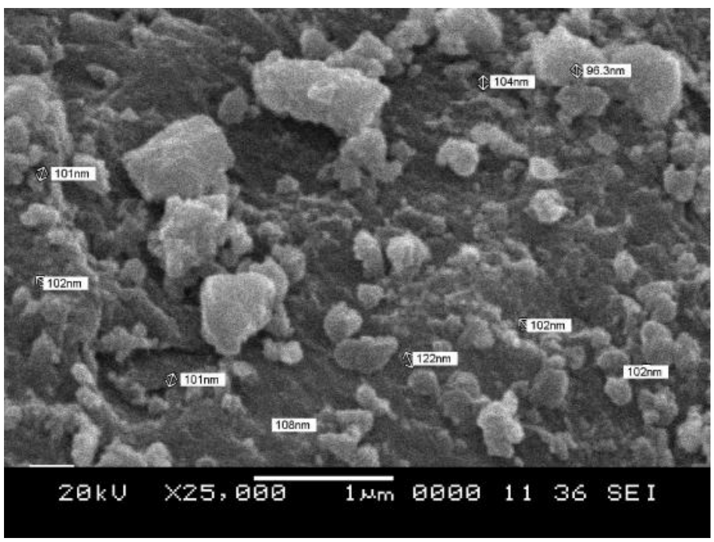

Figure 2. SEM analysis of chitosan Ag nanocomposites

Antimicrobial activity of Ag nanocomposites against microorganism

Antimicrobial test were performed against *Escherichia coli, Streptococcus* sp., *Klebsiella* sp., *Bacillus* sp., *Salmonella* sp*.* On nutrient plates treated with different concentrations of Ag nanocomposites (10mg, 20mg, 30 mg). It is further concluded that *Streptococcus sp* shows higher activity. The zone of inhibition are reported in table.1 and figure 3.

Table 1: Antimicrobial activity of different concentration of chitosan Ag nanocomposite

Organism name	Zone of inhibition (diameter in mm)			
	10µl	20µl	30µl	Streptomycin disc
E.coli	3	4	3	4
Streptococcus sp.	5	6	7	4
Klebsiella sp.	2	2	4	4
Bacillus sp.	3	2	1	1
Salmonella sp.	2	3	4	4

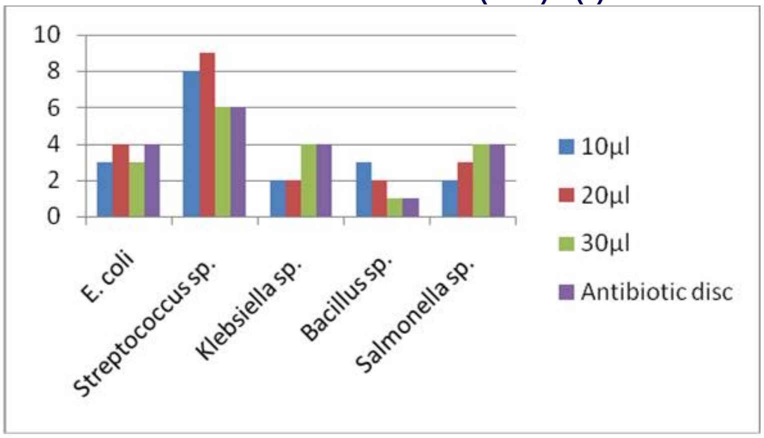

Figure 3: Antimicrobial activity of different concentration of chitosan Ag nanocomposite

Application of edible fruit and vegetable coating

Vegetable coating

Ag nanocomposites were coated in the outer layer of Vegetables (Brinjal, Tomato and Chilly) done under the sterile condition at room temperature. The results conclude that when compared with non-coating, the coated vegetables showed the good shelf life period from 21 to 30 days at room temperature. For tomato the shelf life of 21 days for brinjal 30 days and for chilly it was 23 days figure 4.

Figure 4: Vegetables coated with chitosan and chitosan nanocomposite

Fruits coating:

Ag nanocomposites coated in the outer layer of Fruits (Apple and Sapota) were done under the sterile condition at room temperature. It is concluded that when compared with non-coating, the coated fruits and vegetables showed the good shelf life period from 20 to25 days at room temperature. For apple shelf life period was about 25 days and for sapota it was 25 days figure 5.

Figure 5: Fruits coated with chitosan and chitosan nanocomposite

Conclusion

In this study, silver nanoparticles were synthesized by biological method and characterized by various techniques such as UV- visible Spectroscopy, SEM which also confirms the presence of chitosan Ag nanocomposites. The antibacterial effects of nanocomposites against *Escherichia coli, Strptococcus* sp, *Klebsilla* sp., *Bacillus* sp., *Salmonellla* sp were examined and it was proved that the chitosan Ag nanocomposites had excellent antibacterial performance. Typically, the chitosan nanocomposite can be used as a surface coating on perishable fruits and vegetables to enhance microbial safety and extend food shelf life. Therefore the presence study clearly provides novel antimicrobial material which is potentially useful in food packing.

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