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INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF SILVER AND COPPER NANOPARTICLES ENCSPSULATED IN CHITOSAN

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ABSTRACT :

Nanosized silver and copper were prepared and the particles were encapsulated with the biopolymer chitosan. Nano silver and copper and the corresponding chitosan coated nanoparticles were subjected to UV visible absorption studies showing in the presence of the metals intact in the nano form in the coated material. The chitosan and nano silver and copper coated with chitosan were subjected to IR-spectral studies. Morphology of the chitosan coated nano silver and copper were observed using SEM studies, which showed the formation metals in the nano form and the capping of silver and copper with chitosan. All the five materials nano silver and copper, chitosan and chitosan capped nano silver and copper showed antimicrobial properties among which chitosan capped nano silver and copper showed enhanced antimicrobial properties.

INTRODUCTION :

Infections caused by bacteria are very common. Water is the best growth medium for all bacteria as they form solutions with all nutrients. About 80% of the diseases in India are due to bacterial contamination. When the water is suspected to be contaminated with bacteria such as coliform etc., immediate removal or inactivation of the organism should be done. Usually physical agents such as UV irradiation and chemical agents such as chlorine, silver nitrate etc., are commonly used (Droste, 1997).

In the past few decade's synthetic ion exchange resins, polymer films, metal ions such as Au, Ag, Cu etc., have been used to disinfect water (Feng et al., 2000; Mclean et al., 1993; Chen et al., 2003). Several investigations have been carried out on the bactericidal effect of nanoparticles and their applications in the plastics, health, textile, and paint industry (Zuhuang, 2003; Chen and Chen, 2002; Lee et al., 2003; Fechner and Zimmer, 2003; Reimer and Fleischer, 1999).

Among the various metals, silver is considered to be one of the best bactericidal agents. The US Environmental Protection Agency (EPA) has approved registration of copper as an antimicrobial agent able to reduce specific harmful bacteria linked to potentially deadly microbial infections (European copper Institute, 2008). When silver is made as nanosized particles, due to large surface area and high functionalism they break the cell wall of the bacteria. Emptying the cytoplasm (Prashant jain and Pradeep, 2004). Nanoscale silver materials are also extremely chemical durable (Toshikazu 1999). Similar property is expected in nanosized copper too.

Chitosan is a natural polysaccharide. It is obtained by the partial deacetylation of chitin (N-acetyl –D- Glucosamine). Shrimp and Crab shells, microbial cell walls are the rich sources of chitin. It is a potentially useful material in pharmaceuticals due to its excellent biocompatibility (Sosaku et al, 2005). Chitosan also promotes wound-healing (Biagini et al., 1992) and has bacteriostatic effects (Felt et al., 1999; Liu et al., 2001). Finally, chitosan is very abundant, and its production is of low cost and ecologically interesting (Peter, 1995). Hence in the present investigation, chitosan encapsulated Ag and Cu nanoparticles were prepared and they were tested for their antibacterial properties.

MATERIALS AND METHODS :

Chitosan was obtained from Central Institute of Fisheries Technology, Cochin, Silver nitrate (AgNO3), Copper sulphate $CuSO_4$[Aldrich] were used as received.

Preparation of silver nano particles :

Silver nanoparticles were prepared adopting the method of Panigrahi, et al., (2004). All the reagents were of analytical reagent grade. Silver nitrate (AgNO3), [Aldrich] was used as received. Aqueous solutions (10)2 M) of silver nitrate salt was used as stock solution. Glucose was purchased from S. D. Fine Chemicals, India. Milli-Q water was used throughout the experiment. All absorption spectra were recorded in a Shimadzu UV-160 spectrophotometer (Kyoto, Japan) taking the solutions in a 1 cm quartz cuvette.

A series of solutions were prepared dissolving 0.2 g of sugar in 3.9 ml water in each set and then 100 ml (10^{-2} M) of the silver nitrate salt solution was added to it, so that the final volume of the solution is 4 ml. Glucose was used individually as reducing agent for the synthesis of silver nanoparticle. The concentration of the metal salt was 2.5 χ 10^{-4} M in the final solution. The solution was heated on a hot water bath.

The temperature of the hot water bath was 70 – 75^{o}C. After sometime the solution turned yellow indicating the formation of the silver nanoparticles. The heating was continued until the solution evaporated to dryness. The total time required to evaporate the solution to dryness take 2 h time. Then 4 ml of water was again added to it and the solution was sonicated for 30 minutes.

Preparation of Copper nano particles :

An aliquot of 100 ml water solution of $CuSO_4$ (2 × 10–4 mol. dm–3) was taken in a conical flask and purged with N_2 for 10 min to remove the dissolved oxygen. Then 1 ml ice-cold $NaBH_4$ (0.1 mol. dm–3) solution was added into the $CuSO_4$ solution with stirring.

The metal particles are formed immediately, visualized by the appearance of a yellow colourMilli-Q water was used throughout the experiment. All absorption spectra were recorded in a Shimadzu UV-160 spectrophotometer (Kyoto, Japan) taking the solutions in a 1 cm quartz cuvette.

Preparation of chitosan solution :

Chitosan was obtained from Central Institute of Fisheries Technology, Cochin, which is 89% deacetylated. Chitosan solution was prepared by dissolving 10 grams of chitosan in 100ml of 1% acetic acid solution.

Preparation of chitosan capped copper nanoparticles :

To five ml of nano copper solution in water 10 ml of 10% chitosan solution was added. The mixture was cooled to room temperature with constant stirring and air-dried.

UV –visible spectra :

The copper nano particles and chitosan capped copper nanoparticles were analysed using UV- visible spectrophotometer, Shimadzu model, 1601.

FT – IR spectra :

FT – IR spectrum of chitosan and chitosan capped copper nanoparticles were analysed using IR-8460model of Shimadzu made.

Scanning Electron Microscopy (SEM) :

The morphology of the films was examined on their fractured surfaces, obtained after cutting dried film samples with a paper-cutter, using a scanning electron microscope (JSM-6400 scanning microscope, JEOL).

The samples of chitosan film, nano copper chitosan films were sputter-coated with gold and carbon respectiveyusing a microscope sputter coater (emscope, SC500, Quorum Technologies, East Sussex, U.K.) for 1 min at 20 mA and 15 kV, prior to examination.

Evaluation of Antimicrobial Activity :

The antimicrobial activity of nano copper and chitosan coated nano copper particles were tested qualitatively by an inhibition zone method. In both methods, four different food pathogenic bacteria including *Staphylococcus aureus* ATCC-14458*, Pseudomonas aeruginosa*, *Salmonella typhimurium* ATCC-14028, and *Escherichia coli* O157 were used for testing the antimicrobial activity of the films.

The cells of *S. aureus* were grown on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) and incubated at 37 °C for 2 days. Both strains of *S. typhimurium* and *E. coli* O157:H7 were cultivated on tryptic soy (TS) agar (Difco Lab) at 30 °C for 2 days.

All of the stock cultures were stored at 4 °C. For the qualitative measurement of antimicribial activity, the film samples were punched to make disks (diameter - 6 mm), and the antimicrobial activity was determined using a modified agar diffusion assay (disk test).

The plates were examined for possible clear zones after incubation at 37°C for 2 days. The presence of any clear zone that formed around the film disk on the plate medium was recorded as an indication of inhibition against the microbial species.

RESULTS AND DISCUSSION :

Nanoparticles have unique physicochemical properties that are not found in their parent materials. In general, they have much higher reactivity, and because of their ultra small size, they can easily penetrate skin or cells, rapidly distribute in human body, and even directly interact with organelles within cells. Their huge surface area to mass ratio increases the chemical activities and therefore allows them to become efficient catalysts.

The novel properties of nanomaterials, however, are a two-sided sword. The same properties that allow the wonderful beneficial uses also imply potentially devastating adverse health effects. Rapid uptake through skin and epithelial cells, capability to translocate along neurons, plus the known toxicity of some parent materials warrant careful environment, health and safety evaluation side by side with every nanomaterial application study.

In the present work nanosilver and nanocopper particles and to increase their surface area further and also to find the changes in the properties they are coated with chitosan solution. The resultant particles were characterized using different studies.

Figures – 1, 2, 3 and 4 represent the UV spectra of nano silver, nanocopper and chitosan coated nano silver particles and chitosan coated nano copper particles respectively.

UV Spectrum of silver nanoparticles showed a peak at 420nm. In Chitosan coated Ag nanoparticle the peak was observed at 419nm showing that Ag nanoparticles did not undergo any change after the addition of chitosan. Similarly UV Spectrum of copper nanoparticles showed a peak at 590nm. In Chitosan coated Cu nanoparticle the peak was observed at 569nm showing that Cu nanoparticles did not undergo any change after the addition of chitosan.

The IR spectra of the chitosan **(Figure - 5)** shows prominent peaks at 3789.8741cm^{-1}, 3429.0552 cm^{-1} corresponding to O – H stretching, strong polymerization, at 2928.1464 cm^{-1} for aliphatic C – H stretching. Peaks at approximately 2369.1045 cm^{-1}, 2345.3484 cm^{-1} represent N – H stretching vibration. Peaks at 1637.4352 cm^{-1} and 1438.2275 cm^{-1} represent N –H bending and a peak at 1020.8597 represents C – N vibration in aliphatic compounds .

The IR spectra of the Chitosan capped Nano silver **(Figure - 6)** shows prominent peaks at ≅3788 cm^{-1}, 3427.4005 cm^{-1} corresponding to O – H stretching, strong polymerization, at 2928.1733 cm^{-1} for aliphatic C – H stretching. Peaks at approximately 2369.0387 cm^{-1}, 2345.3187 cm^{-1} represent N – H stretching vibration. Peaks at 1637.5845 cm^{-1} and 1389.4649 cm^{-1} represent N –H bending and a peak at 1026.6957 represents C – N vibration in aliphatic compounds. A is also observed at 617.7611 cm^{-1} showing the presence of inorganic metal ions (silver ions).

The IR spectra of the Chitosan capped Nano copper **(Figure – 7)** shows prominent peaks at 3432.35cm^{-1} corresponding to strong polymerization. Peaks at approximately 2044.52

cm^{-1} represent N – H stretching vibration. Peaks at 1633.03 cm^{-1} represent N –H bending and a peak at 1384.37 cm^{-1} represents O - H bending and C – H stretching. A peak is also observed at 1091.66 cm^{-1} showing C – O – C stretching in polysaccharide of chitosan. Finally the presence of inorganic metal ions (copper ions) is shown by a peak at 463.13 cm^{-1}.

Figures – 8 and 9 represents the SEM image chitosan coated nano silver and chitosan coated nano copper. From the figures chitosan coated silver and copper showed island-sea morphology; that is, the nanoparticles were dispersed throughout the chitosan matrix.

Antimicrobial activity of the methanol extract of chitosan, nano copper and chitosan capped nano copper by well method

S. No.	Name of the Organism	Chitosan	Nano Copper	Chitosan capped nano Copper	Nano Silver	Chitosan capped nano silver
1.	*Staphylococcus aureus* ATCC 25923	-	+	++	+	++
2.	*Salmonella typhimurium ATCC-14028*	+	+	++	+	++
3.	*Pseudomonas aeruginosa*	+	+	++	+	++
4.	*E.coli*	+	++	++	++	++

- No inhibition; + - Clear zone of 6-8mm; ++ - Clear zone of 8 – 10 mm

Nano silver and nano copper showed inhibition for all the microbial species such as *Staphylococcus aureus* ATCC 25923*, Salmonella typhimurium ATCC-14028, Pseudomonas aeruginosa* and *E.coli*. The antimicrobial effect was increased when the nano silver and copper were coated with chitosan. This is because chitosan itself is a good antimicrobial agent which is shown from the above table, and when the nano silver was coated with chitosan there is a synergistic effect of both the materials found showing higher levels inhibition for the three microorganisms.

The extremely small size of nanoparticles means they exhibit enhanced or different properties when compared with the bulk material. The extremely small size of nanoparticles results in the particles having a large surface area relative to their volume. In the case of silver nanoparticles this allows them to easily interact with other particles and increases their antibacterial efficiency.

This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundred of square metres of substrate material. In order to understand how silver nanoparticles kill pathogens, an understanding of how bacteria, viruses and fungus live and grow is required. All bacteria use an enzyme as a form of 'chemical lung' in order to metabolise oxygen. Silver and Copper ions cripple the enzyme and stop the take up of oxygen. This effectively suffocates any bacteria, killing it within 6 minutes and leaving surrounding tissue or material unaffected (Maribel G. Guzmán, 2008). The results thus confirm that the nanometallic particles of silver and copper and their chitosan encapsulated forms show antimicrobial properties. As chitosan also has antibacterial activity its combination with the metals show enhanced antimicrobial effect.

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