Synthesis of Chitosan-Silver Nanocomposites and their Antibacterial Activity

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Abstract— The present study explores the *in situ* fabrication of chitosan-silver nanocomposites in view of their increasing applications as antimicrobial packaging, wound dressing and antibacterial materials. Chitosan/Silver nanocomposites were prepared by embedding of silver nanoparticles in Chitosan polymer. Synthesis of nanocomposites was confirmed Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD) analysis and Differential Scanning Calorimetry (DSC) etc. In addition, the formed nanocomposites have an average particle size of ~10-15 nm as observed by Transmission Electron Microscopy (TEM). Their antibacterial activity was assessed by zone of inhibition method against *Staphylococcus aureus* MTCC 1809, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella entrica* MTCC 1253 *in vitro*.

Keywords— Silver nanoparticles, Chitosan, Nanocomposites, antibacterial

1 Introduction

CONVENTIONAL food packaging systems are supposed to passively protect the food, that is to say, to act as a barrier between the food and the surrounding environment. Antimicrobial food packaging systems have received considerable attention since they help control the growth of pathogenic and spoilage microorganisms on food surfaces, where microbial growth predominates [1]. Antimicrobial nanocomposites systems are particularly interesting, since materials in the nanoscale range have a higher surface-to-volume ratio when compared with their micro scale counterparts. Nanomaterials are thus more efficient, since they are able to attach more copies of microbial molecules and cells [2]. Nanoscale materials have been investigated for antimicrobial activity as growth inhibitors [3], killing agents [4] or antibiotic carriers [5].

Chitosan is the second most plentiful natural biopolymer and is relatively cheap [6]. It has attracted considerable interest due to its biological properties, such as antimicrobial activity, antitumor activity, and immune enhancing effect. The proposed mechanism for its antimicrobial action is binding to the negatively charged bacterial cell wall, with consequent destabilization of the cell envelope and altered permeability, followed by attachment to DNA with inhibition of its replication [7].

The antibacterial activity of chitosan is influenced by a number of factors, including the species of bacteria, concentration, pH, solvent and molecular mass [8]. Due to excellent antimicrobial property, chitosan film may be used in food packaging [9]. Recently, a chitosan-starch film has been prepared by using microwave treatment which may find potential application in food packaging. The recent review on antimicrobial and antioxidative activities of Chitosan in food also expresses the hopefulness of the different findings of the researchers for further progress to improve microbial food safety and food quality [10].

Du *et al* (2004) found that antimicrobial properties of chitosan were enhanced by loading chitosan with various metals. They evaluated *in vitro* antibacterial activity of chitosan nanoparticles and copper-loaded nanoparticles against various microorganisms. Copper ions were adsorbed onto the chitosan nanoparticles mainly by ion-exchange resins and surface chelation to form copper-loaded nanoparticles [11]. Among all antimicrobial metals, silver is well known for its strong toxicity to a wide range of microorganisms besides some processing advantages such as high temperature stability and low volatility [5]. In fact, the most common nanocomposites used as antimicrobial films for food packaging are based on silver nanoparticles, whose antimicrobial activity has been ascribed to releasing antimicrobial Ag^+ ions by dissolution of silver nanoparticles [12-14]. Ali *et al* (2010) studied the antimicrobial activity of chitosan/silver nanocomposites against bacteria *S. aureus* [15]. The ultimate objective of this communication was to study the synthesis of Ch/Ag (Chitosan/ Silver) nanocomposites and evaluate their antibacterial activity *in vitro*. Synthesis of nanocomposites was confirmed by various characterisation techniques i.e. FTIR, XRD, DSC, TEM etc. In order to evaluate and compare their antibacterial activities, *Staphylococcus aureus* MTCC 1809, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella entrica* MTCC 1253 were chosen as tested bacteria.

Chitosan, Tri-sodium citrate purified LR and Acetic acid was obtained from S. d fine-chem Limited, India. Silver nitrate Extrapure (M.W.-169.87) was purchased from Sisco Research Laboratories, India. The test strains *Staphylococcus aureus* MTCC 1809, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella entrica* MTCC 1253 were procured from IMTECH, Chandigarh.

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2 MATERIALS AND METHODS

2.1 Synthesis of Nanocomposites

The silver nanoparticles were prepared by chemical reduction method, in which sodium citrate was used as reducing agent of $AgNO_3$. After the addition of trisodium citrate (1% w/v) into $AgNO_3$ solution, the mixture was stirred for 2h, then, treated with sonication at 1.5 kW for 30 min. The prepared silver sol was added into the chitosan solution (1% w/v in 1% acetic acid) and stirred for 12 h. The thick white nanocomposites gel was obtained after loading of Ag nanoparticles into chitosan solution. The suspension was subsequently centrifuged at 12,000g for 10 min at 4°C. The precipitates were suspended in water, centrifuged again, and then freeze dried (Lyophilized). The freeze-dried Ch/Ag nanocomposites were suspended in water for characterization or directly used for other experiments.

2.1 Characterisation

The lyophilized nanocomposites samples were pelletized with KBr in the weight ratio 1/100 and IR spectra was observed using a FTIR spectrophotometer (Spectrum BX11, Perkin–Elmer) in the range 4600 – 400 cm^{-1} and resolution ± 4 cm^{-1}.

Ch/Ag nanocomposites were characterized using X-Ray diffraction analyser (RIGAKU-miniflex Desktop X-ray-Diffraction using CuK_α radiation λ = 1.54016 Å) at 30 KV over the range of 20–80°. Phase purity and grain size were determined.

Thermal stability of nanocomposites was analysed by TA instrument (DSC Q10 V 9.0 build 275). For this analysis, the sample was weighed in an aluminium DSC pan and a lid was crimped on. The reference was an empty DSC pan with lid.

The morphology & size of Ch/Ag nanocomposites was characterized using Transmission Electron Microscopy (TEM) using JEOL 2100F TEM instrument operating at an accelerating voltage of 100 kV. Ch/Ag nanocomposites were suspended in double distilled water and homogenized by ultrasonication just before observation. Samples were prepared by placing a drop of homogeneous suspension on a copper grid with a lacey carbon film and allowing it to dry in air.

For antibacterial activity tests, 20 ml nutrient agar was poured in well-rinsed, autoclaved petri plates and allowed to solidify. 1.0 ml of fresh bacterial culture was homogeneously spread on the solidified agar plates and 500 µl of homogenized solution of Ch/Ag nanocomposites filled in deep blocks, prepared by cutting the agar by gel puncture. The plates were incubated at 37°C for 24 h. The zone size was determined by measuring the radius of the zone of inhibition by scale and divider.

3 RESULT AND DISCUSSION

3.1 FTIR spectra

IR spectra of chitosan and Ch/Ag nanocomposites are shown in figure 1. In the IR spectrum, a broad band 3500 - 3200 cm^{-1} is observed. Its shape changes – toward lower frequencies it becomes more asymmetric which indicates the presence of OH and NH_2 groups[16].Changes occur also at the frequencies 2900 and 2880 cm^{-1}; peaks corresponding to CH_2 groups tend to equalization, the height of a peak which occurs for a smaller wave number decreases. Peak for the frequency 1680 cm^{-1} in Fig. 1(a) was due to oscillations of C=O in amino groups and provide an evidence of the presence of acetyl amino groups, i.e. a partly deacetylated form of chitin. Disappearance of peak 1680 cm^{-1} in Fig1 (a) and occurrence of new peak 1581 cm^{-1} (characteristic of NH_2 amino groups) in Fig 1(b), indicates its involvement in complexation[17]. The presence of CH_2OH groups is very probable, as the peak for the frequency 1420 cm^{-1} disappears. The decrease of this peak was proportional to increase of adsorbed Ag^+ ions. Changes related to C-CH_3 deformations in amide groups were observed at the frequency 1384 cm^{-1} (Fig. 1b). It seems that the saccharide structure changes – after adsorption of Ag ions the peak 1050 cm^{-1} becomes more visible. Changes are also reported in the range of low frequencies from 650 to 400 cm^{-1}. These changes may be a result of the reducing action of chitosan and precipitation of metallic silver [18].

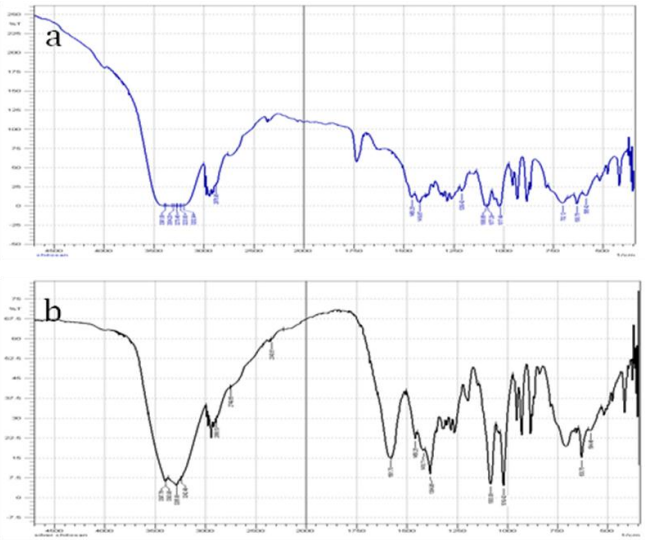

Fig.1 IR spectra of (a) chitosan nanoparticles and (b) chitosan/Ag nanocomposites – complete frequency range.

3.2 X-Ray diffraction studies (XRD)

In diffraction patterns of pure chitosan granules there are mainly two peaks 2θ =10° and 20°, which according to literature might indicate crystalline form II [19]. Modrzejewska *et al* (2009) also reported that after the adsorption of Ag^+ ions, the crystalline structure changes [18]. From the X-ray analysis it follows that after adsorption of Ag metal ions the degree of ordering of the tested sample is reduced. This can provide an evidence of a dependence of the ordering degree, i.e. supramolecular structure of chitosan on the adsorption of Ag metal ion [20]. We can observe from figure 2 (a and b), disappearance of the peak at the angle 2 θ = 20°, broad bands appear at the angle 2 θ = 35°, which indicates that the structure is ordered in the amorphous part. This is in agreement with the reported results (Yin *et. al*. 2004) [20].

3.3 Morphological studies

Transmission electron microscopy (TEM) was used to investigate the surface morphology of Ch/Ag nanocomposites. Micrograph of figure 3 shows that Ch/Ag nanocomposites has uniform, very well capped particle structures less than 10-15 nm in size. There is no agglomeration of nanoparticles (may be due to presence of the chitosan as capping agent) and the surface was somewhat rough. It is noteworthy that the particles are uniformly mixed in a chitosan matrix.

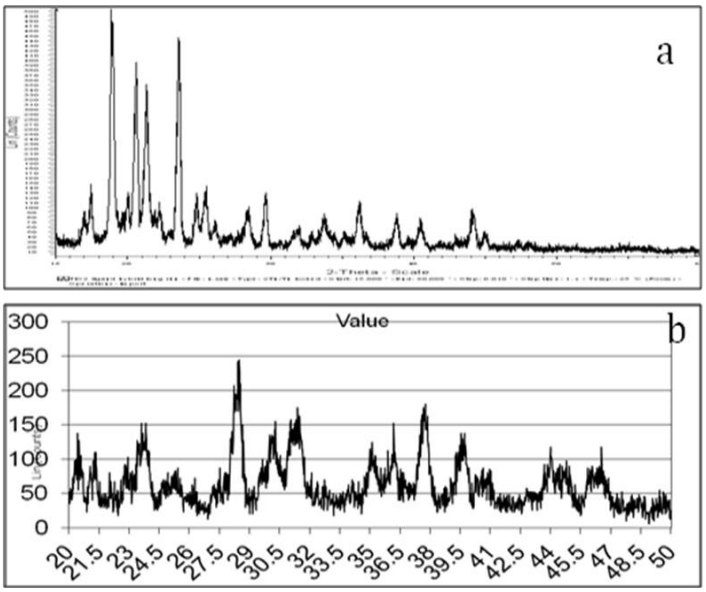

Fig.2. XRD spectra of (a) chitosan nanoparticles and (b) chitosan/Ag nanocomposites

Table1: Data analysis of TG-DSC analysis

DSC spectra			
samples	*TR(°C)	#DT(°C)	Area(J/g)
Chitosan	142.56-335.51	152.65, 167.82, 269.48, 312.97	15.15, 248.1, 281.3, 75.91
Ch/Ag	41.07-.189.29	71.62,155.29,164.82,169.48	61.50, 26.18, 38.03, 78.06

*Temperature range, #Decomposition Temperature

3.4 DSC spectra

Figure 4 (a and b) show DSC spectra for chitosan and Ch/Ag nanocomposites, respectively. It is clear from thermal analysis data (Table 1) that, dehydration endotherm at 41- 355°C has moved. The thermograms of chitosan exhibited endothermic peak at 167.82 °C with enthalpy of fusion 248.1J/g corresponding to its melting point, indicating its crystalline nature (Fig 4a); on the other hand Ch/Ag nanocomposites exhibited peak at 71.62°C with enthalpy of fusion 61.50J/g for (Fig. 4b). Thermal decomposition temperature of chitosan moved to lower temperature in chitosan-metal complexes which indicates that chitosan chain of complexes can be broken more easily. The last endotherm of complexes belongs to metal salt [20].

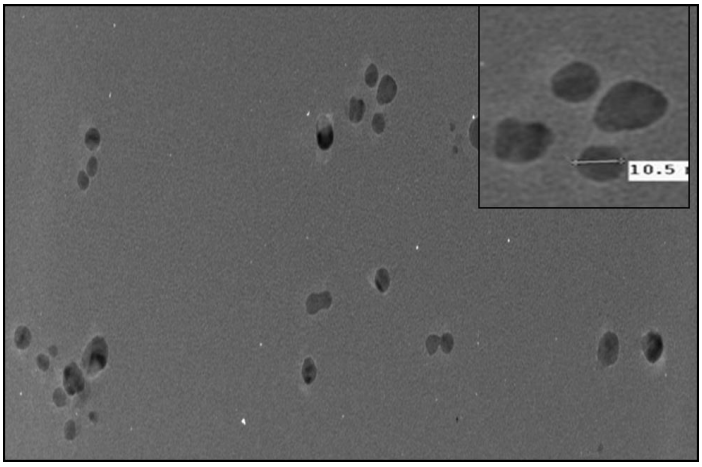

Fig. 3 Transmission electron micrographs of chitosan/silver nanocomposites

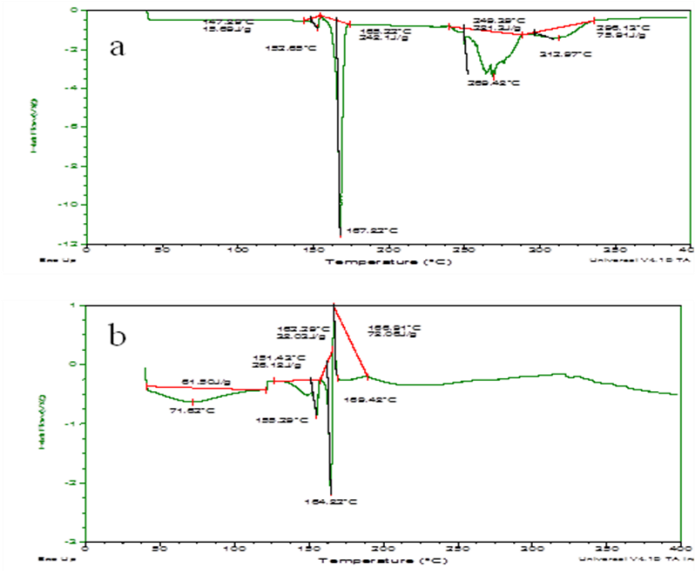

Fig.4 DSC spectra of (a) chitosan nanoparticles and (b) chitosan/Ag nanocomposites

3.5 Antimicrobial activity

The ability of chitosan and chitosan/Ag nanocomposites to inhibit growth of the tested strains is shown in Fig. 5. The inhibitory activity was measured based on the diameter of the clear inhibition zone. If there was no clear zone surrounding, it was assumed that there was no inhibitory zone.

In terms of surrounding clearing zone, our results have revealed that chitosan/silver nanocomposites clearly show greater inhibitory effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella entrica* in comparison to chitosan. Cao *et.al* (2010) also reported antibacterial activity of Silver/chitosan nanocomposites against *S. aureus* and *E. coli*, in which they showed similar results [21].

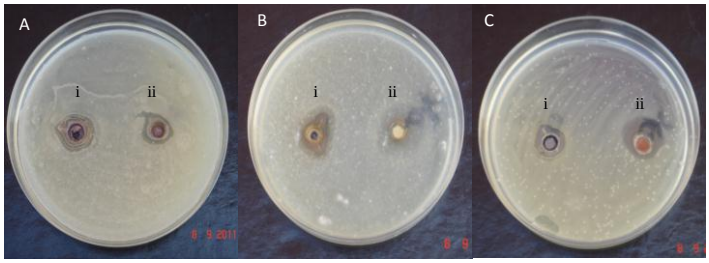

Fig.5. Antibacterial activity of chitosan (i) and Ch/Ag (ii) against (a) *Staphylococcus aureus* MTTC 1809, (b) *Pseudomonas aeruginosa* MTTC 424 and (c) *Salmonella entrica* MTCC 1253 *in vitro*.

4 Conclusion

In this study, silver nanoparticles were synthesized by chemical reduction method and embedding them into Chitosan matrix to form Ch/Ag nanocomposites. Various characterisation techniques also confirmed the synthesis of Ch/Ag nanocomposites.

Results showed that antibacterial activity of chitosan nanoparticles was significantly enhanced when loaded with silver nanoparticles. Therefore, the present study clearly provides novel antimicrobial material which is potentially useful in food packaging.

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