Covenant Journal of Physicl & Life Sciences (CJPL) Vol. 4 No. 2, Dec, 2016

An Open Access Journal available online

Preparation, Release Pattern and Antibacterial Activities of Chitosan-Silver Nanocomposite Films

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Abstract: The present study examined the preparation of chitosan-silver nanocomposite films as carriers for silver release pattern. Chitosan a biopolymer having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications. Chitosan–silver nanocomposite films has been synthesised by reduction method, which is a simple and inexpensive method. The chitosan-silver nanocomposite films was characterized in terms of its surface plasmon resonance and crystalline structure by using UV-Visible spectroscopy, X-ray diffraction, Fourier transform infrared and Scanning electron microscope. Swelling and release studies were carried out on crosslinked and un-crosslinked nanocomposite films. Antibacterial activities of chitosan-silver nanocomposite films were investigated on some pathogens: Staphylococcus aureus, Shigella dysenteriae, Escherichia coli, Salmonella typhii and Klebsiella pneumonia using agar well diffusion method. crosslinked chitosan-silver nanocomposite demonstrated a slower release pattern relative to un-crosslinked chitosan-silver nanocomposite. The crosslinked and un-crosslinked nanocomposite became dislodged and completely released at 120 minutes and 90 minutes respectively. The results of the antibacterial activities revealed that the cross-linked chitosan-silver nanocomposite films has higher antibacterial properties than un-crosslinked chitosan-silver nanocomposite films. This study provides nanocomposite films potentially useful for delivery system.

Keywords: Biopolymer, Carrier, Nanocomposite, Antibacterial.

1. Introduction

In the past years, several synthetic as well as natural polymers have been examined for pharmaceutical applications. A basic requirement for these polymers to be used in humans or

animals is that they have to degrade into molecules with no toxicity to biological environments. In recent years, biodegradable polymers have attracted attention of researchers as carriers for drug delivery system [8].

Chitosan is a polysaccharide that occurs naturally. Its units are composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan interact very easily with bacterium and binds to DNA, glycosaminoglycans and most of the proteins, thereby enhancing the antimicrobial effect of metal nanoparticles [7]. Chitosan properties allow it to rapidly clot blood and have recently gained approval in the United State of America for use in bandages and other hemostatic products. Chitosan is used in pharmaceutical manufacturing as fillers in tablets and to mask bitter tastes in solutions taken by mouth. Films and coatings based on biopolymers functions as barriers against moisture, oxygen, aroma flavor as well as oil are the materials for future applications [3].

Metal nanoparticles embedded into polymer matrix can be used as sensor, materials with solvent switchable electronics properties, optical limiters and filters, and for optical data storage. They can be applied for catalytic applications and antimicrobial coatings. Nanocomposite of polymer/metal nanoparticles has attracted great attention because of its potential applications in the field of catalysis, bioengineering, photonics, and electronics [9]. Polymers are considered good host materials for metallic nanoparticles as well as other stabilizing agents, including citrates, organic solvents, and organometallics [4]. Some examples of metal/polymer nanocomposites being synthesized include poly (vinylalcohol), poly (vinylpyrolidone) [6], chitosan [1], gelatin [6]. This accounts for the different properties of nanocomposites that are synthesized.

2. Experimental

2.1. Materials

All reagents in this work were of analytical grade and were used as received without further purification. AgNO3 (99.98% Sigma -Aldrich) was used as the silver precursor, Sodium citrate, (crosslinking agent), Ammonia solution, Sodium hydroxide and glacial acetic acid (99%) were also obtained from Sigma–Aldrich. All the aqueous solutions were prepared with double-distilled water.

2.2. Methods

2.2.1. Preparation of Crosslinked Chitosan-Silver Nanocomposite Films

5 g of silver nitrate was dissolved by stirring in 100 mL of distilled water. 20 mL of the solution was taken and aqueous NaOH was added dropwise to the vigorously stirred solution till precipitation occurred. The dirty green precipitate formed was dissolved in few drops of dilute ammonia. Two layers were formed after centrifugation and addition of acetic acid. It was decanted and added in chitosan matrix (6 mL, 1% (w/v) in acetic acid). Film was made by casting the solution on the petri dish and dried at room temperature for 24 hours. When the film has been harvested cross-linking was done by dipping the film in a 10 mL solution of sodium citrate (5 % w/v) after adjustment to pH 5. The film

was washed with water to remove excess sodium citrate.

2.2.2. Swelling Studies

The completely dried, pre-weighed chitosan, crosslinked chitosan-silver and un-crosslinked chitosan-silver nanocomposite films were immersed in 250 mL phosphate buffer (pH 7.4) at 25 oC. The water uptake of the films was measured at 20 minutes interval using analytical balance to determine the mass. The swelling ratio (Q) of the films was calculated using the following equation: Q = Ws/Wd,

Where Ws is the weight of the swollen film at different time intervals (20, 40, 60, 80, 100, 120 and 140 minutes) and Wd is the weight of dry film.

2.2.3. Release Study

In Vitro release study of silver from chitosan-silver nanocomposite films was determined. A known weight of the dried nanocomposite films was put in the 20 mL phosphate buffer solution in a 50 mL tube. The temperature and rotation were adjusted to 37 oC and 90 rpm respectively. At predetermined time of 30, 60, 90, 120, 150, 180 and 210 minutes, 10 mL of sample was withdrawn and ultra-centrifuged for 30 minutes. The samples were further analyzed using UV-Visible spectrophotometer. The absorbance of each solution of the nanocomposite films was measured at λmax 425 nm.

2.2.4. Antibacterial activity of Chitosan-silver nanocomposite films

The antimicrobial activities of the synthesized films were tested against human pathogens like Staphylococcus aureus, Shigella dysenteriae, Escherichia coli, Salmonella typhii and Klebsiella pnueumoniae by agar well diffusion method. An overnight culture of each pathogen grown in nutrient broth at 37 oC was diluted to a turbidity equivalent of 3.0×108 cfu/mL (1.0 Mcfarland standard) with a sterile normal saline. The cell suspension was then swabbed on the surface of Mueller-Hinton agar plates. A sterile cork borer of diameter 7 mm was used to make wells on the agar plates. Each well was filled with 50.0 µL of each solution of chitosan-silver nanocomposite. The plates were left for one hour to allow the test materials to diffuse in the agar and then incubated at 37 oC for 24 hours without inversion. The antimicrobial activity was determined by measuring the clear zone (zone of inhibition) around the wells. The diameter (mm) of the zone of inhibition of 1.00 mm or greater was considered as a significant inhibition [11].

2.3. Characterization Methods and Instruments

The synthesized chitosan-silver nanocomposite films were confirmed using UV-visible spectroscopy (UV-visible), X-ray diffraction (XRD), Scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). The UV-visiblespectra of the nanocomposite were detected over the range of 300–700 nm using a Shimadzu (UV.1650) UV-visible spectrophotometer. Crystalline structures of the synthesized chitosan-silver were examined using Rigaku D/Max-2550Pc (Tokyo. Japan). Morphology of the films was studied by SEM through a JEOL JSM 840A.

3. Result and Discussion

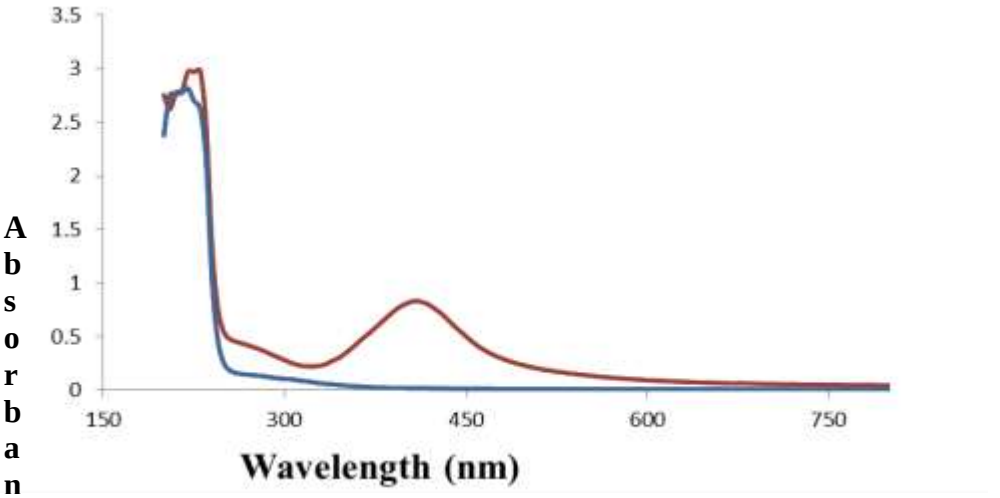

Figure 1: UV-visible spectrum of chitosan-silver nanocomposite films.

3.1 Characterization

The UV-Visible spectrum of chitosan-silver nanocomposite films is shown in Figure 1. The spectrum of chitosan-silver nanocomposite films shows a single peak at maximum wavelength 420 nm which confirmed the presence of silver; this peak arises due to surface plasmon resonance vibrations of silver atoms. The peak at maximum wavelength 230 nm shows the presence of chitosan.

Figure 2 shows the X-ray diffraction pattern chitosan-silver nanocomposite films. Several distinct diffraction peaks at 2θ values of 20.5, 21.0, 27.2, 36.8, 42.8, 50.5, 60.2 and 67.7 (crystalline peaks) is attributed to the presence of silver while the non-crystalline part indicate the presence of Chitosan as shown on the chitosan-silver nanocomposite XRD pattern which confirmed the formation of the nanocomposite.

Figure 3 shows the FTIR spectra of chitosan-silver nanocomposite films. The absorption peaks in the chitosan-silver nanocomposite spectra at 2930.93cm^{-1}and 1342.50 cm^{-1}are characteristic of O-H stretching and O-H bending of chitosan respectively. The stronger the hydrogen bond the longer the O-H bond, the lower the vibration frequency, and the broader and the more intense the absorption band. The peak observed at 1619.29 cm^{-1}indicates hydrogen bonding nature of N-H bending and the peak at 3369.75 cm^{-1} represents H bonded-OH of chitosan, the lower the frequency the stronger the H bond. The SEM image in Figure 4 reveals rough surfaces with whitish particles (silver nanoparticles) surrounded with black particles (chitosan), a clear evidence of swelling on the surface.

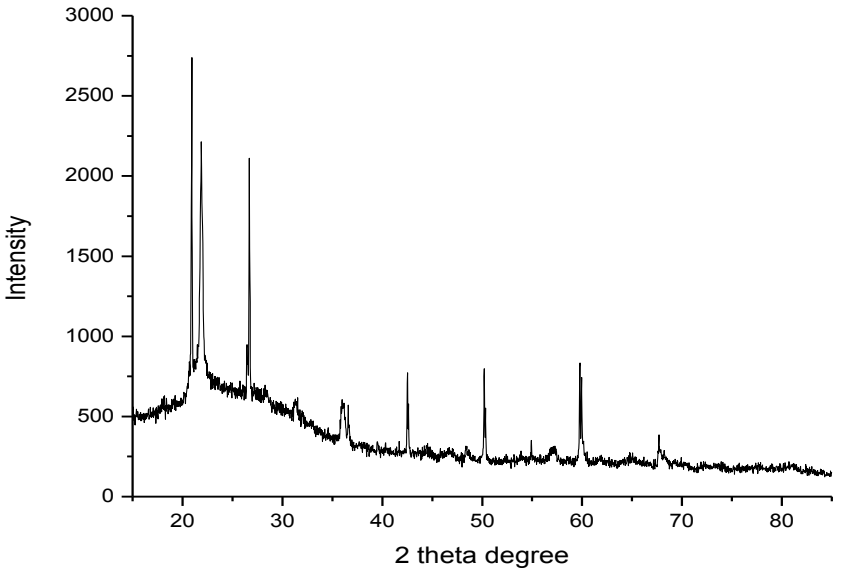

Figure 2: XRD pattern of Synthesized Chitosan – Silver nanocomposite film

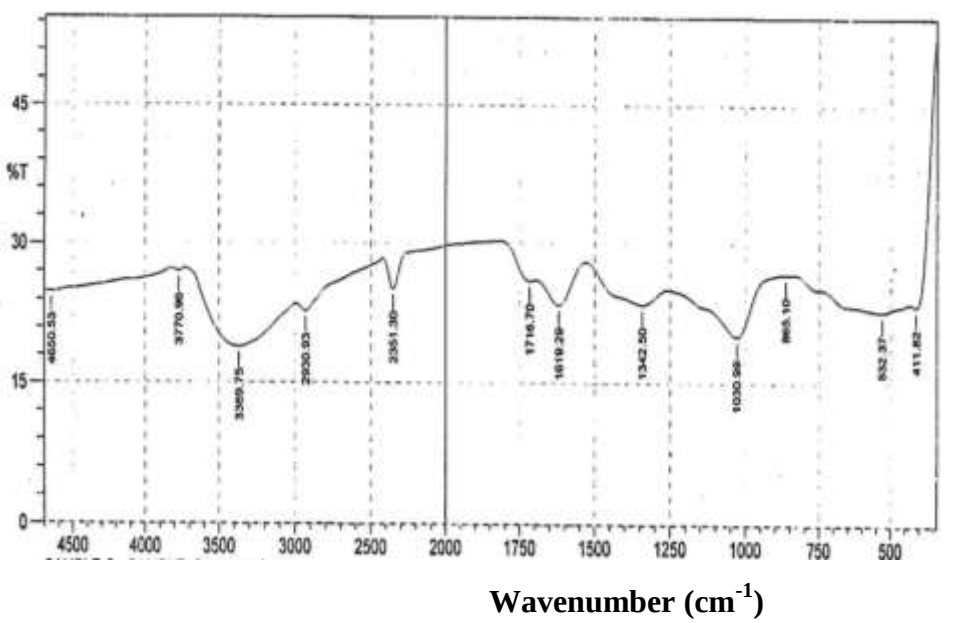

Figure 3: FTIR Spectra of Synthesized Chitosan-silver Nanocomposite film

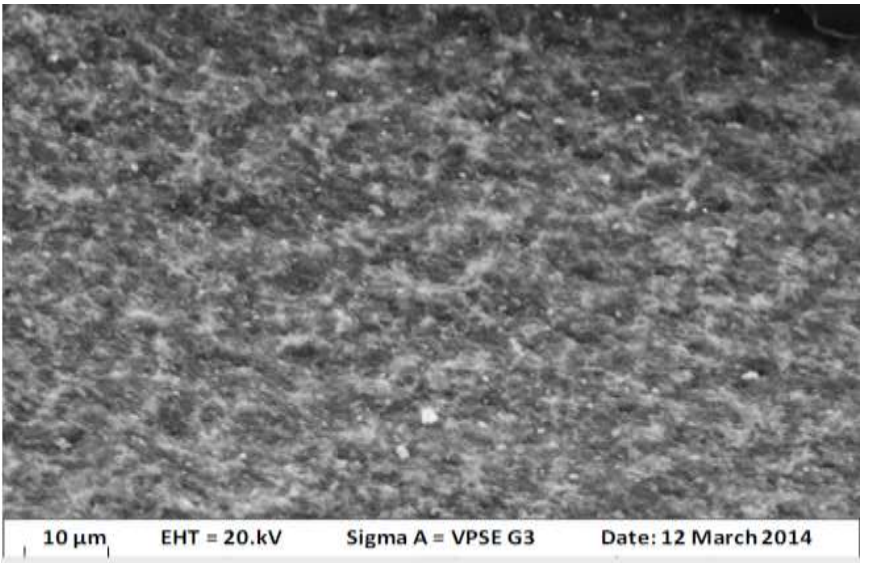

Figure 4: SEM image of chitosan-silver nanocomposite film

3.2 Swelling Test

Swelling test is useful for correlating drug release characteristics from polymeric films and barriers. It can be judiciously used in predicting and modifying drug release from dosage forms. The cross-linked chitosan-silver nanocomposite is known to be dependent on the availability of the cationic sites and the negatively charged species.

3.3 Release Study

Phosphate buffer (pH 7.4) has been considered as release medium which simulates body fluid according to literatures. However, crosslinked chitosan-silver demonstrated a slower silver release pattern relative to un-crosslinked chitosan-silver. Moreover, it was apparently noted from the graphs that almost all the silver nanoparticles within cross linked and uncross-linked became dislodged and completely released at 120 minutes and 90 minutes respectively. This result perfectly agrees with our swelling studies, where swelling property of cross-linked chitosan-silver exhibited onset of dormancy at 120 minutes which made it coincided with un-crosslinked chitosan-silver. From swelling and release studies, it is easy to explain that silver nanoparticles is gradually released into the system (according to figure 6 below). This is a clear evidence that polymer composite like chitosan can serve as carrier in delivery system.

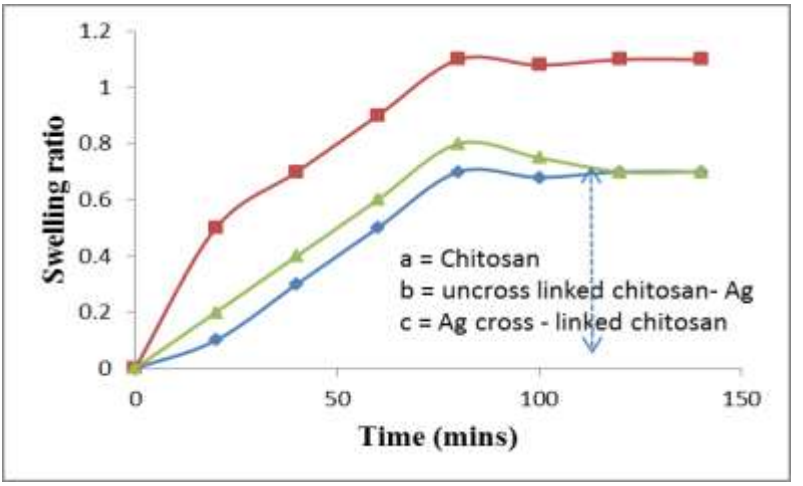

Figure 5: Swelling ratio against time.

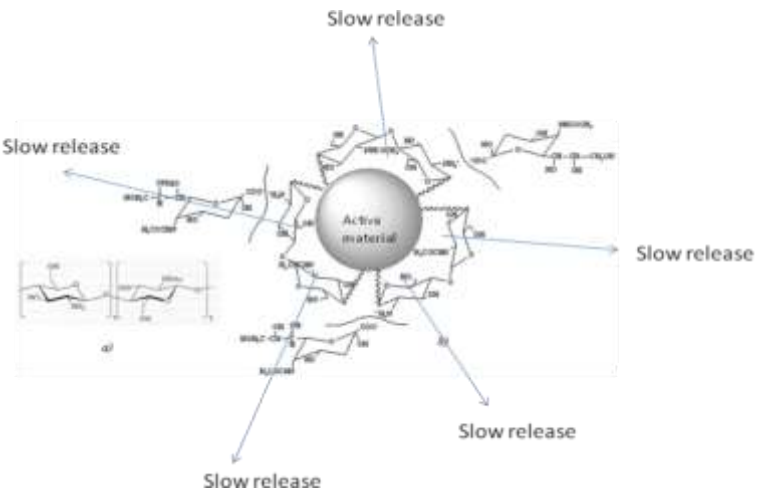

Figure 6: Shows the release of Silver from Chitosan

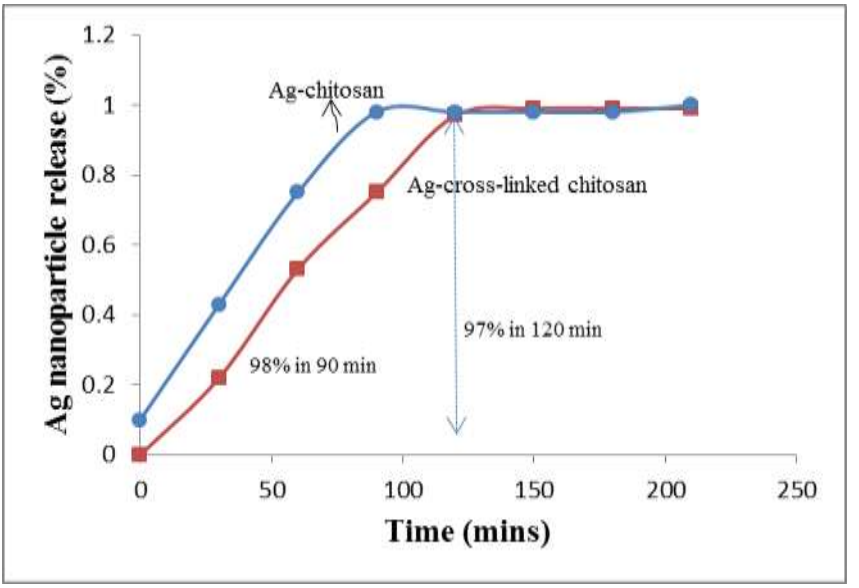

Figure 7: Shows silver released pattern of crosslinked chitosan-silver film compared with un crosslinked chitosan-silver.

Table 1: Antibacterial Activity of Films against test pathogens

Pathogens	**Zones of inhibition (mm)**												
	A (mg/ mL)			**B (mg/ mL)**			**C (mg/mL)**			**D (mg/mL)**			***Control**
	100	50	25	100	50	25	100	50	25	100	50	25	
Staphylococcus aureus	16	10	5	12	8	Nil	12	7	Nil	11	6	Nil	21
Shigella sp.	15	10	6	11	5	10	11	5	Nil	8	Nil	Nil	21
Escherichia coli	10	5	Nil	9	4	Nil	8	4	Nil	7	Nil	Nil	16
Salmonella sp.	9	6	Nil	8	5	Nil	8	4	Nil	6	Nil	Nil	19
Klebsiella sp.	8	6	Nil	9	7	Nil	7	Nil	Nil	Nil	Nil	Nil	21

Control: Ciprofloxacin, Sample A: crosslinked chitosan- silver, Sample B: un-crosslinked chitosan-silver, Sample C: crosslinked chitosan, Sample D: chitosan

The antibacterial activity of the synthesized nanocomposite films was evaluated by agar well diffusion method. The inhibitory growth was measured based on the diameter of the clear inhibition zone. The results of the antibacterial activity of the synthesized nanocomposite films are shown in Table 1. With the exception of Chitosan, films at higher concentration (100mg/mL) showed higher antibacterial activity against *Staphylococcus aureus*, *Shigella sp*. *Escherichia coli*, *Salmonella sp*. and *Klebsiella sp*, but they were not as active as ciprofloxacin (control) which could be due to the concentrations used. The results further revealed that the higher the concentrations of the films, the higher their antibacterial activities against test pathogens.

4. Conclusion

In this study, Chitosan-silver nanocomposite films were synthesized by reduction method. Various characterisation techniques also confirmed the synthesis of chitosan-silver nanocomposite films. Crosslinked chitosan-silver nanocomposite film showed a slow silver release pattern relative to the un-crosslinked nanocomposite. The synthesized nanocomposite demonstrated higher antibacterial activity at higher concentration against some pathogens. Thus, Chitosan-silver nanocomposite films can be useful in different biological research and biomedical applications including delivery system (wound dressing).

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