

## APPLICATIONS OF CHITOSAN-SULFATHIAZOLE AS ANTIMICROBIAL AGENT

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**Abstract:** Chitosan, a hydrophilic biopolymer industrially obtained by N-deacetylation of chitin, can be applied as an antimicrobial agent. It highlights the applications of chitosan as an antimicrobial agent against fungi, bacteria, and viruses and as an elicitor of plant defense mechanisms. A series of novel chitosan-sulfathiazole nanocomposite (CSFZ) films were prepared by using solvent casting method for wound healing application. Fourier transform infrared spectroscopy (FTIR) was employed to ascertain the interaction between negatively charged sulfathiazole and positively charged chitosan. Moreover, the antibacterial activity of the films was investigated against gram positive and gram negative microorganisms. It was found that all CSFZ films showed good inhibitory activity against all the tested bacteria as compared to control. The above analysis suggested that the CSFZ films could be used as potential candidates for wound healing application.

**Keywords:** Chitosan-sulfathiazole nanocomposite, Escherichia coli, Bacillus subtilis

### 1. INTRODUCTION

Chitin is the second most abundant naturally occurring polysaccharide found in the outer shell of crustaceans and insect exoskeleton. It is also found in mushrooms. It consists of  $\beta$ -(1-4) linked N-acetylglucosamine and is not soluble in most of the solvents. Chitosan is the deacetylated derivative of chitin and consists of  $\beta$ -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine and is widely used in tissue engineering. It is readily soluble in dilute acidic solutions below pH 6.0 at which amines present in them get protonated, and they become water soluble. It is a cationic polysaccharide that can form polyelectrolyte complexes with other polysaccharides. It is cytocompatible with fibroblasts, keratinocytes, myocardial cells, hepatocytes, and chondrocytes. Due to easy applicability, water sorptivity, oxygen permeability, blood coagulating property, and cytokine induction (interleukin-8) which activate the fibroblast migration and proliferation, it is used as wound dressing material [1].

Pathogenic bacterial infection is one of the most crucial problems in wound care management. Infection at wound surface not only changes a normal healing wound into non healing wound or chronic wound but also causes a serious problem to public health. To overcome this problem, scientists are aggressively working to prepare advanced wound dressing materials. Nowadays, natural polymers based wound dressing materials play a decisive role to prevent microbial infection at wound surface. Among them, chitosan is a very promising candidate for wound healing due to its strong antibacterial properties against broad spectrum of gram negative and gram positive bacteria as well. Moreover, it is biodegradable, non-toxic, biocompatible, and hemocompatible that are essential to accelerate wound healing process [1-7]. The N-acetyl glucosamine units present in chitosan and chitin also play a significant role not only in enhancing the reepithelization process but also in repairing wound tissues [8]. Further, chitosan finds a wide variety of applications ranging from tissue engineering to drug delivery to gene delivery to medicine to agriculture to pharmaceutical to wound healing to bone healing application because of its ease of moulding into gel, sponge, nanocomposite, scaffolds, powder, beads, and films [9-12]. Till now various blends of chitosan with natural polymers, synthetic polymers, nanomaterials have been investigated to prepare dressing materials for wound healing applications such as chitosan and collagen artificial skin incorporated with nano particle [12]. The purpose of the present investigation is to show and explain the effect of synthesised chitosan nanocomposite material on the human pathogens bacteria and its growth inhibition effect against them.

### 2. EXPERIMENT

#### Preparation of chitosan-zeolite-ceftriaxone film (CZCF)

The nanocomposite films were prepared by using the above- mentioned method. In brief, 1% (w/v) chitosan (CS) solution, 0.025% (w/v) sulfathiazole (SF) and 0.025% (w/v) zeolite (Z) solution were prepared separately. Then CS solution was added dropwise into CS and Z solution (respectively CS, SF and Z solution) in different ratios under continuous magnetic stirring at 52°C to obtain a solution mixture. Finally, the solution mixture was cast into a petri plate and dried at 60°C to evaporate the solvent for the formation of CZCF film. A schematic representation for the formation of chitosan-sulfathiazole-zeolite nanocomposite film using solvent casting method.

#### **Antibacterial study (Agar disc diffusion method)**

Antibacterial activity of CS and CZCF films was determined by using agar disc diffusion method against gram positive (*B. subtilis*) and gram negative (*E. coli*) bacteria. For antibacterial activity, first of all, the respective nutrient agar medium and nutrient broth medium were prepared by adding 2.8 g of agar and 1.3 g of broth separately in each 100 mL of distilled water and sterilized. The nutrient agar media was then poured into sterilized petri plates. A loopful of each bacterial strain was spread on nutrient agar and incubated at 37 °C for 24 h to produce a single colony. A representative bacteria colony was picked off with a wire loop and then placed in pre-sterilized nutrient broth and incubated overnight at 37 °C for 12 h. After that the cultured bacterial medium *B. subtilis* and *E. coli* suspension of 107-108 CFU/mL were inoculated on petri plates and bacteria was spread evenly over the surface of the agar media. Then each film with a dimension of 10 mm x 10 mm was placed on inoculated agar plates. These plates were incubated at 37°C for 24 h and zone of inhibition around disc was measured to determine the antibacterial activity.

#### **In vitro nonenzymatic hydrolytic degradation**

The degradation of the CZCF films was studied in a phosphate buffered saline solution (PBS) (pH 7.4) at 37°C. The prepared films of equal weights were immersed separately in glass beaker containing PBS and incubated at 37 °C for 3 weeks. Then the CZCF films were removed at predetermined time interval (after one week) from PBS and washed with distilled water to remove residual buffer salts. Finally, weight of each film was measured. All the hydrolytic degradation studies were performed in triplicate and the percentage of hydrolytic degradation was calculated by using the following equation:

$$\text{Hydrolytic degradation (\%)} = [W_i - W_d / W_i] \times 100 \quad (1)$$

where  $W_i$  represents initial weight of the film and  $W_d$  is dry weight of the film.

#### **Hemocompatibility test**

The hemocompatibility of the as-prepared CZCF films were investigated according to the method described elsewhere. In brief, first of all, a fresh anticoagulated blood was prepared by adding 1 mL of anticoagulated acid citrate dextrose solution (ACD) into 9 mL of fresh human blood. Before performing this test, each CZCF film with a dimension of 1 cm x 1 cm was placed in a test tube containing 4 mL of saline water and incubated for 30 min at 37 °C. After that ACD blood (200 µL) was added to the test tube and further incubated for 60 min at 37 °C. Similarly, positive and negative controls were prepared by adding the same amount of ACD blood in 4 mL of distilled water (100% hemolysis) and 4 mL of saline solution (0% hemolysis) respectively. After the incubation, each test sample was centrifuged at 2000 rpm for 5 min. The hemoglobin released during hemolysis was determined by the optical density (OD) of the supernatant at 540 nm using UV-VIS spectrophotometer. The percentage of hemolysis was calculate:

$$\text{Hemolysis (\%)} = [OD_{\text{test}} - OD_{\text{neg}} / OD_{\text{pos}} - OD_{\text{neg}}] \times 100 \quad (2)$$

where  $OD_{\text{test}}$ ,  $OD_{\text{neg}}$ , and  $OD_{\text{pos}}$  denote the absorbance of test sample, negative control, and positive control respectively.

### **3. RESULTS AND DISCUSSION**

#### **Antibacterial study**

Wound generally provides a conducive environment for the growth of microorganisms, which trigger infection and delay the natural wound healing process. Therefore, it is mandatory to conduct an antibacterial study to investigate the efficacy of a dressing material to be used for wound healing application. The antibacterial activity of the CSFZ films were evaluated against gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria and the data of the zone inhibition of bacteria were shown in Table 1. The antibacterial result indicated that CSFZ 4 (40 mm) film showed the highest antibacterial activity against gram positive bacteria (*B. subtilis*) as compared to other CSFZ films namely CSFZ 1 (27 mm), CSFZ 2 (31 mm), CSFZ 3 (35 mm), CSFZ 5 (35.5 mm), and CSFZ 6 (31.5 mm). Furthermore, the result also indicated that the zone of inhibition for pure chitosan increased against *E. coli* from 34 mm to 40 mm (CSFZ 6) with the incorporation of zeolite. Chitosan interacts with the membrane of the cell to alter cell permeability. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth. It also activates several defense processes in the host tissue, acts as a water binding agent, and inhibits various enzymes. Binding of chitosan with DNA and inhibition of mRNA synthesis occurs through chitosan penetration toward the nuclei of the microorganisms and interference with the synthesis of mRNA and proteins. It has been proposed that when chitosan is liberated from the cell wall of bacterial pathogens host hydrolytic enzymes, it then penetrates to the nuclei of bacteria and interferes with RNA and protein synthesis.

Chitosan, however, shows its antibacterial activity only in an acidic medium because of its poor solubility above pH 6.5. Thus, water-soluble chitosan derivatives (soluble in both acidic and basic physiological circumstances) may be good candidates as a polycationic biocide.

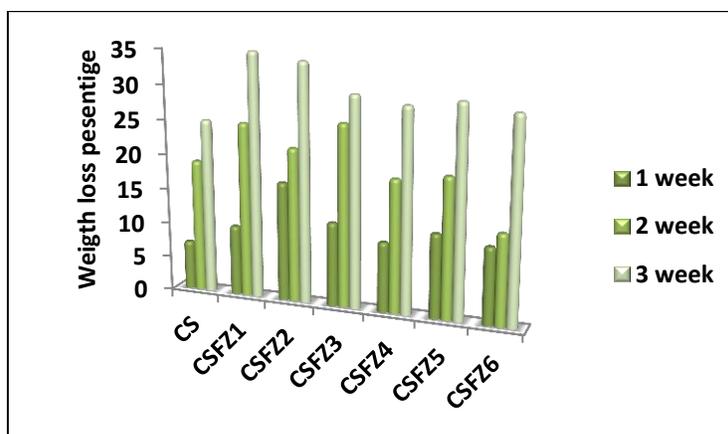
In addition to the formation of gas-permeable films, chitosan has a dual function: (a) to direct the interference of bacterial growth and (b) to activate several defense processes.

**Table 1. Data of zone of inhibition for CSFZ films against Gram positive and Gram negative bacteria.**

Sample	Diameter of zone of inhibition (in mm)	
	Gram positive bacteria	Gram negative bacteria
	( <i>Bacillus subtilis</i> )	( <i>Escherichia coli</i> )
CS	37.6	34
CSFZ 1	27	35.5
CSFZ 2	31	36
CSFZ 3	35	35
CSFZ 4	40	36
CSFZ 5	35.5	34.8
CSFZ 6	31.5	40

### 2.2. In vitro nonenzymatic hydrolytic degradation

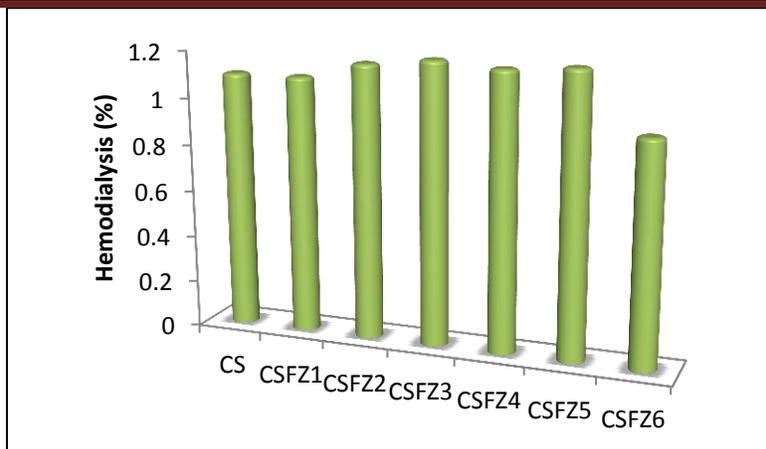
The degradation rate of nanocomposite films was measured at 1st, 2nd, and 3rd week after incubation in PBS (pH 7.4) and was plotted as function of time (Fig. 1). It was found that all the CSFZ films as well as pure chitosan (control) showed 6-17% degradation after one week of incubation. The CSFZ films showed faster degradation after second (22-30%) and third week (30-35%) of incubation. As shown the weight loss was more in the CSFZ films containing larger zeolite content. It might be due to easy removal of loosely bound zeolite particles adhered to the surface of the nanocomposite films during incubation in PBS solution and this phenomenon was also confirmed from the SEM analysis.



**Fig. 1. In vitro nonenzymatic hydrolytic degradation data for CS and CSFZ films.**

### 2.3. Hemocompatibility test

The hemocompatibility test is considered to be very simple and reliable process to measure the hemostatic potential of wound dressings (Fig.2). According to ASTM F material can be divided into three different categories according to hemolytic index (hemolysis %) which are as follows: (1) the materials with percentages of hemolysis over 6% are considered to be hemolytic, (2) the materials with percentages of hemolysis between 2% to 6% are classified as slightly hemolytic, and (3) the materials which show a hemolysis percentage below 2% are considered as non-hemolytic material. Fig. 8 represents hemolysis percentage values of the CSFZ nanocomposite films and the result showed that the hemolysis percentage for all the prepared CSFZ films were below 1.15%, which indicated that the CSFZ films were non-hemolytic in nature.



*Fig. 2. Hemolysis index of pure chitosan and CSFZ films.*

#### 4. CONCLUSION

A series of novel chitosan-sulfathiazole-zeolite nanocomposite films were prepared by using solvent casting method for wound healing application. FTIR spectra confirmed the H-bonding interactions between the hydroxyl groups of zeolite with the hydroxyl and amino groups of chitosan. On the other hand, all the CSFZ films exhibited good antibacterial activity against gram positive (*B. subtilis*) and gram negative (*E.coli*) bacteria as well compared to control. Moreover, hemocompatibility results indicated that all the prepared nanocomposite films were highly blood compatible in nature. Finally, all the results indicated that the as prepared CSFZ nanocomposite films, especially CSFZ 5 film has all the requisite properties for wound healing application. Therefore, it is recommended that the combination of these materials could be deployed for fabricating wound care products.

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