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# Preparation and characterization of chitosan-bentonite nanocomposite films for wound healing application

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ABSTRACT

A series of novel chitosan-bentonite nanocomposite (CBN) films were prepared by using solvent casting method for wound healing application. The physicochemical properties namely thickness, folding endurance, water absorption capacity, and water vapour transmission rate (WVTR) of the films were studied. Fourier transform infrared spectroscopy (FTIR) was employed to ascertain the interaction between negatively charged bentonite and positively charged chitosan. The surface morphology of the prepared composite films was also studied by scanning electron microscopy (SEM). Results showed that WVTR, water absorption capacity, thickness, and folding endurance of the CBN films were $1093 \pm 20.5$–$1954 \pm 51$ g m$^{-2}$ day$^{-1}$, $1232 \pm 14.58$–$1688 \pm 18.52$, $17.50 \pm 5$–$42.50 \pm 9.75$ μm, and $145.25 \pm 2.21$–$289.50 \pm 0.57$ respectively. Due to strong hydrophilic nature of bentonite, it greatly enhances the water absorption capacities of the prepared nanocomposite films. In addition, the presence of bentonite in the said films also increases the mechanical strength. Moreover, the antibacterial activity of the films was investigated against gram positive and gram negative. It was found that all CBN films showed good inhibitory activity against all the tested bacteria as compared to control. The above analysis suggested that the CBN films could be used as potential candidates for wound healing application.



## 1. Introduction

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 [9–13]. 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-titanium oxide [14], water soluble chitosan with PVA [15], CS with herbal plant extract [16], to name a few. But still, there is a shortcoming of using chitosan as wound dressing material as a consequence of its poor mechanical strength and water absorption capacity as well. Therefore, in this present study, we had selected bentonite as one of the most promising reinforcing materials due to its non toxicity, high cation-exchange capacity, low cost, ease of availability, and antimicrobial activity [17,18]. In addition, it also has the ability to absorb large amount of wound fluid and thus maintains moist environment, which is extremely essential for any wound dressing material [19]. Moreover, it is used for the treatment of various skin problems such as boils, wound, ulcers, and sores [20]. On the other hand, due to polycationic nature of chitosan, it can interact with negatively charged bentonite thus resulting in the formation of a polyelectrolyte complex. The interaction between chitosan and bentonite not only improves the mechanical strength but also enhances the overall wound healing properties of the nanocomposite films.

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**Table 1**
Proportion of chitosan and bentonite in nanocomposite films.

| S. No. | Sample code | Chitosan (mL) | Bentonite (mL) |
|---|---|---|---|
| 1 | CH | 25 | – |
| 2 | BCH11 | 10 | 10 |
| 3 | BCH12 | 20 | 10 |
| 4 | BCH13 | 30 | 10 |
| 5 | BCH14 | 40 | 10 |
| 6 | BCH15 | 50 | 10 |
| 7 | BCH16 | 60 | 10 |

The above-mentioned films were characterized to investigate their physical, mechanical, and biological properties such as surface morphology, thickness, folding endurance, water absorption capacity, WVTR, dressing pH, porosity, antibacterial activity, nonenzymatic hydrolytic degradation, and hemocompatibility in order to obtain a potential nanocomposite film as an advanced wound care product.

## 2. Experimental

### 2.1. Materials

Chitosan (CS) with a molecular weight of 100,000 and a degree of deacetylation 79% was obtained as a gift sample from Central Institute of Fisheries and Technology, Cochin. Bentonite (BN) was purchased from CDH India. All chemicals were used as received without further purification.

### 2.2. Preparation of chitosan film

First of all, 1% (w/v) chitosan solution was prepared by dissolving 1 g of chitosan in 100 mL of 1% (v/v) aqueous acetic acid under continuous magnetic stirring. Then the solution was allowed to keep at room temperature for one or two days to remove the air bubbles formed during stirring so as to obtain a clear solution. After that the clear solution was cast evenly into petri plate and allowed to dry at 60 °C in an oven. After drying, the chitosan film was peeled off and stored in desiccator for further study.

### 2.3. Preparation of CBN films

The nanocomposite films were prepared by using the above-mentioned method. In brief, 1% (w/v) CS solution and 0.5% (w/v) bentonite (BN) solution were prepared separately. Then CS solution was added dropwise into BN solution in different ratios under continuous magnetic stirring at 52 °C to obtain a solution mixture (Table 1). Finally, the solution mixture was cast into a petri plate and dried at 60 °C to evaporate the solvent for the formation of CBN film. A schematic representation for the formation of chitosan-bentonite nanocomposite film using solvent casting method is shown in Scheme 1.

### 2.4. Characterization of CBN film

Fourier transform infrared (FTIR) spectra have been recorded using Thermoscientific Nicolet 380 with KBR in the range between 400 and 4000 $cm^{-1}$. Scanning electron microscopy (SEM) analysis was carried out on HITECHI (Model No. S-3700N)

### 2.5. Folding endurance

Folding endurance of the CBN films has been measured to find the flexibility, which is needed to handle the films comfortably, safely, and carefully on wound surface. Folding endurance is determined manually by repeatedly folding the film at the same point until it breaks or folds up to 300 times [21]. The number of times of folding without any breakage gives the exact value of folding endurance of the CBN film. It was performed in triplicate for each film to obtain an average value.

### 2.6. Thickness and mass measurement of the CBN films

Thickness is a major factor that greatly affects the functional properties of wound dressing materials. The thicknesses of the CBN films were measured using a screw gauge (NISCO Company) by randomly selecting four positions on each film. The mean was used to express the thickness of the films. To ascertain the mass uniformity of each CBN film, four pieces of equal sizes were cut from different positions of each CBN film followed by weighing their respective masses with electronic balance and eventually, these values were used to calculate the average mass.

### 2.7. Water absorption capacity

For water absorption capacities, the preweighed CBN films (25 mm × 25 mm) were individually immersed in 20 mL of freshly prepared 0.9% saline solution at room temperature. Then, the soaked films were withdrawn from the medium at pre-determined time interval. The swollen weights of the CBN films were determined after removing excess surface water on the films with filter paper followed by placing them back into the same saline solution. Percentage swelling of the CBN films was calculated according to the following equation [22]:

$$\text{Swelling}\ (\%) = [W_f - Wi/W_i] \times 100$$

where $W_f$ is the weight of the wet film and $W_i$ is the initial weight of the film.

### 2.8. Water vapour transmission rate

Water vapour transmission rate (WVTR) was determined gravimetrically according to the modified ASTM E96-95 standard method [23]. Succinctly, a CBN Film of a suitable dimension was mounted on circular opening of a WVT cup containing 15 mL of distilled water. The WVT cup was accurately weighed and placed in an incubator at 25 °C. Then the WVT cup was re-weighed periodically at 24, 48, and 72 h and then a rate of change in mass was plotted as function of time for each CBN film. Finally, the WVTR was calculated using the equation as follows:

$$\text{WVTR}\ (g\,m^{-2}\,day^{-1}) = [M \times 24/T{\cdot}A]$$

where M represents change in weight, T is time during which the change in weight occurred, and A is the exposed area of the film ($m^2$).

### 2.9. Dressing pH

First of all, the CBN films were separately immersed in normal saline solution till it reached equilibrium. After that each of the CBN film was removed from the normal saline solution and the same solution was taken into consideration for determining dressing pH of the said films using digital pH meter. All tests were performed in triplicate and average values were recorded.

### 2.10. Porosity measurement

The porosity of the CBN films was determined using liquid displacement method, as described elsewhere [24]. In brief, the weight of each CBN film with a dimension of 1 cm × 1 cm was recorded and then the film was put into a beaker containing 5 mL of absolute