# Development and characterization of chitosan film

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## *Abstract*

**Chitosan is a natural mucopolysaccharide of marine origin consists of a linear (1-4)) linked 2-amino-2-deoxy-D glucan, can be chemically prepared from naturally occurring chitin by treatment with alkali at elevated temperature. Chitosan is non toxic, biodegradable and biocompatible polymer. Chitosan widely existing in the nature and has antibacterial effect, heavy metal adsorption effect, antioxidation effect and film formability. Chitosan forms viscous solution in various organic acids. These viscous solutions have been used to make functional films. They were readily biodegradable either in sea water or in soil. The cationic properties of chitosan offer the film-maker an additional opportunity to take advantage of electrostatic interactions with other anionic polysaccharides. These films were high modulus, flexible self-supporting and biodegradable, and were advantageous in that all material were derived from petroleum and agricultural products.**

**The main objective of the present research is development and characterization of chitosan films in terms of morphology, physical, mechanical and degradation properties. The morphology of the film was characterized using scanning electron microscope. Degree of swelling ratio and water vapor permeability of pure chitosan films were compared with reinforced chitosan films. Reinforcement of chitosan film with cellulose of coconut fibers enhanced the moisture resistance of the chitosan film The studies on mechanical properties of chitosan films found that reinforcement increased the tensile strength of the film.**

***Key words: Chitosan film, coconut fibers, swelling ratio, tensile strength, water vapor permeability***

## 1. INTRODUCTION

Chitin or poly (β(1-4)-N-acetyl-D-glucosamine) is one of the most abundant polysaccharides found in nature. It can be found in skeletal materials of crustaceans, cuticles of insects, and cell walls of various fungi. Chitosan is prepared by chemical N-deacetylation of chitin. Both of them are observed to have biological functions [1]. Chitin and its derivatives have many properties that make them attractive for a wide variety of applications from food, nutrition and cosmetics to biomedicine, agriculture and environment [2]. Chitosan (CH) is biodegradable, biocompatible and exhibits bioadhesive characteristics. It is a copolymer of glucosamine and N-acetyl glucosamine linked by β 1→4 glucosidic bonds obtained by N-deacetylation of chitin[3].

Chitosan forms good films and membranes. Chitosan film has a potential to be employed for packaging, particularly as an edible packaging. This is due to its excellent oxygen and carbon dioxide barrier properties and interesting antimicrobial properties. For example, biodegradable and edible chitosan films were produced to protect foods from fungal decay and modify the atmospheres of fresh fruits[4]. Chitosan can be complexed with crosslinking agents like sodium citrate, sodium tripolyphosphate etc., which make chitosan a useful polymer for artificial membranes, microspheres, beads, wound dressings and nanoparticles[5]. Chitosan is a semi crystalline polysaccharide that, unlike many biodegradable polymers, is insoluble in water at neutral pH[6].

The film properties of chitosan depend on its morphology, which is effected by molecular weight, degree of N-acetylation, solvent evaporation, and free amine regenerating mechanism[7]. Chitosan films are described as being tough, long lasting and flexible. This presents an opportunity for developing a variety of films using different crawfish chitosans and organic acid solvents. A detailed study of sorption behaviors of such films would allow an efficient selection of crawfish chitosan films that are less sensitive to humidity. Hence, studies were conducted to determine the effects of different chitosans and solvent types on sorption behavior of crawfish chitosan films and to establish models to predict sorption behaviors of such films[8]. The development of new biodegradable packaging material films, for example, chitosan film is needed to find alternatives to petroleum-based plastics because of environmental concerns. Unfortunately, there are some limitations to the application of chitosan film for packaging, because of its high sensitivity to moisture as it has a large number of hydrogen bonds[9]. One strategy to overcome this drawback is to associate chitosan with a moisture-resistant polymer, while maintaining the overall biodegradability of the product[10]. Among the various properties of chitosan, the physicochemical properties of its films are interesting for pharmaceutical applications, the food industry, and membrane separation. In some cases, chitosan films have been used as edible films and coatings in the food industry. For many food applications, the most important functional characteristic of an edible film or coating is its resistance to the migration of moisture. This is because critical levels of water activity must be maintained in many foods if the product, or a certain component of a multiphase food, is to exhibit optimum quality and acceptable safety. In the field of pharmaceuticals, many studies on the permeation of drugs through chitosan membranes have been performed. Among functional properties of edible films, optical properties are essential to define their ability to be applied over a food surface, since these affect the appearance of the coated product, which is an important quality factor. The study is also important to ensure that gloss and transparency of the films are in an adequate range for a specific target application [11].

## 2. MATERIALS AND METHODS

### 2.1 Material:

All the chemicals used are of analytical grade and samples were prepared using deionized water. Chitosan powder was donated by Cochin Institute of Fisheries Technologies, Cochin.

### 2.2 Methods:

2.2.1 Pure chitosan film

Chitosan was added at different concentrations (1 & 2 g ) in 100 ml of known acetic acid solution (1% , 2% , 3% ) in Erlenmeyer flasks, The chitosan powder dissolved in acetic acid by constant continuous stirring, then degassed the solution for 2 hrs. After degassing, the solution poured into the petri plate. This plate was maintained in the oven at 72°C for 20 hrs.

The dry films in glass plates were immersed in 2N sodium hydroxide for more than half an hour at room temperature. Then the wet film were peeled off from the glass plate and washed sufficiently with water. The films were dried at 37°C for 1 week[1].

2.2.2 Preparation of coconut fiber

Cellulose fibers were extracted from coconut fiber by chemical treatment resulting in purified cellulose. Coconut fibers were cut into small pieces. The coconut fibers was soaked in a concentrated sodium hydroxide (10 gram sodium hydroxide was dissolved in 50 ml of distilled) for 2 hrs. The pretreated cellulose fibers was hydrolyzed by hydrochloric acid (Hcl) solution at 80°C for 2 hours and then washed with distilled water repeatedly. The cellulose fibers were treated once more with NaOH solution at 80°C for 2 hours. The alkaline treated fiber was washed several times with distilled water [2]. The treated fibers were grained with help of piston mortal to extract cellulose from it and placed in the oven for 5 hrs at 80°C to dry. Then the fiber was taken out to blend with the chitosan film.

2.2.3 Coconut fiber coated chitosan film

2 gram of chitosan was dissolved in 2% acetic acid solution. The solution was degassed for 2 hrs, and then added a 2 gram of coconut fiber powder. The solution is stirred well until it becomes homogeneous solution.

Then the solution was poured onto a glass plate and dried at 72° C for 20 hrs. The dry films were immersed 2N sodium hydroxide for more than half an hour at room temperature. Then the wet film were peeled off gently from the glass plate and washed sufficiently with water. The wet films were dried at 37°C for 1 week.

## 3. FILM CHARACTERIZATION

The Chitosan film morphology was studied using scanning electron microscopy. The physical and mechanical properties of chitosan film measured are film thickness, swelling ratio, water vapor permeability, tensile strength, % elongation at break and degradation.

### 3.1 Surface Morphology

Scanning electron microscopy:
The morphology of the dry films was studied using SEM techniques. Scanning electron microscope (HITACHI SU 6600 Japan) was used for the studies. Pieces were cut from films and mounted in copper stubs. Samples were gold coated and observed using an accelerating voltage of 20 kV. Using SEM the porosity, particle size, and surface roughness of the chitosan film were analyzed.

### 3.2 Physical Properties

3.2.1 Determination of Thickness
The thickness of the chitosan films was measured using slide clipper by reading accurately at different area of films and averaging it.

3.2.2 Swelling ratio:
The sample films were cut in 1×1 cm size and dried in vacuum at room temperature for a week. After initial weighing, they were kept in beaker with 50ml of distilled water at 37°C for 72 hrs. Then the films were taken out after 72 hrs carefully and removed the excess water from the surface of the films with filter paper[4]. The swelling ratio of the films was calculated using the equation (1).

3.2.3 Water Vapor permeability:
Water vapor permeability of the films was measured by keeping the sample films tightly adhered onto the top of glass vials with an approximate volume of 24 $cm^3$. Each vial was filled with pre-weighed anhydrous calcium chloride, glass vials for control contained small glass beads of approximately identical weights to those of the sample vials and kept in a desiccator with 90±5% RH maintained with a saturated sodium chloride solution at 30°±2°c. The vials were weighted before and after being kept in the close desiccator for 3 days[4]. Rate of water vapor permeability (WVP) was calculated by using the equation (2).

### 3.3 Mechanical Properties

3.3.1 Tensile strength and %Elongation at break
Tensile strength and elongation at break of the films were measured by a Universal Testing Machine (Tinius Oisen H10 ks). The films with a dimension of 4 cm x2 cm with a speed of 50mm/ min and 10N load cell. Tensile strength and % elongation at break were calculated by using the equation (3) and (4), respectively. The film were cut using a Dumbbell Specimens Cutting Die Size 40×6×2 mm[4].

### 3.4 Degradation properties

All the dried samples were weighed ($W_o$) before the experiment. Then the samples were immersed in solvents for different time periods using the area/volume ratio = 0.1 $cm^{-1}$ , following each immersion the sample were carefully removed from the medium and weighed after drying at 40°C and constant final weight ($W_f$) being verified. The degradation index (Di) was calculated based on the mass loss using the equation(5).

## 4. ANALYTICAL METHODS

**Swelling ratio (%) =**

$[(W_s-W_d)/W_d]x100$…………………………(1)

**Rate of WVP (g/ day/ L) =**

$[\{(T_f - T_i)-(C_f - C_i)\}/3v]x1000$………………(2)

**Tensile strength ($N/mm^2$) =** Breaking force (N)/ Cross section area of sample $(mm)^2$ ………..(3)

**Elongation at break (%) =**

(The increase in length at breaking point (mm) /Original length (mm) ×100…………………(4)

**% Degradation =**

$[(W_o-W_f)/W_f]x100$…………………(5)

## 5. RESULTSAND DISCUSSION

### 5.1 Physical properties

The physical properties of pure chitosan film and reinforced chitosan film (cellulose of coconut fibers) were analyzed by measuring the thickness of the film, swelling ratio, and water vapor permeability.

### 5.2 Determination of Thickness

Films prepared in different conditions were analyzed for thickness using slide clipper. The thickness observed is shown in table 1.

Film prepared from 2g chitosan in 2% acetic acid showed thickness of 0.17 mm has high value compared with other films .So 0.17 mm thickness chitosan film was taken as test film and reinforced with coconut fiber cellulose to further increase the film thickness of 0.32mm. The chitosan films were measured for large value of the film thickness and a small degree of acetylating to more easily assess the effect of thermal treatments [1].

### 5.3 Swelling Ratio

The degree of swelling ratio was observed for films prepared in different conditions and are summarized in Table I. The swelling ratio of pure chitosan films and reinforced chitosan film were measured for water uptake (by immersion in water) because of the partial dissolution of chitosan. The swelling ratios of films were calculated from equation (1). The swelling ratio of the chitosan films slightly decreased when the cellulose fibers of coconut were reinforced with chitosan films, which indicates that the cellulose enhanced the moisture resistance of the chitosan film[2].

### 5.4 Water Vapor permeability

The water vapor permeability was observed and shown in TABLE 1. The WVP of chitosan film were calculated from the equation (2). It illustrates the influence of the coconut cellulose fibers with test film on the rate of WVP of chitosan films. It was found that the rate of WVP of chitosan film decreased when the cellulose fibers were incorporated into the chitosan films, corresponding to swelling ratio results. This suggests that the hydrophobic characteristics of the cellulose fibers exhibit resistant properties to water vapor[2].

### 5.5 Mechanical properties

The Mechanical properties measured are tensile strength, % elongation and stress at break.

#### 5.5.1 Tensile strength and % Elongation at break

The tensile strength and % elongation at break of the films were tested in dry states and the results are summarized in TABLE 2. It was found that the tensile strength at break of the dry chitosan film increased but not % elongation at break when the cellulose of coconut fibers was incorporated into the chitosan film. The tensile strengths of the films increased with reinforced chitosan film. Our results proved that the reinforced chitosan film could act as reinforcing fillers of the chitosan matrix to improve the tensile strength of chitosan film. Finally, it indicated that the reinforced chitosan films had higher tensile strength and lower % elongation than the chitosan film in dry state[2].

### 5.6 Surface Morphology:

The surface Morphology of the chitosan films was studied using SEM to measure the porosity and particle size of the chitosan films.

The morphology of pure chitosan and reinforced chitosan film are reported in Fig.1. The scanning electron micrograph for the pure chitosan film revealed that the film is nonporous and the texture is plain without pores. The surface structure of pure chitosan film is smooth and uneven. There were small pores available on the surface in case of low gram chitosan films (Fig.1.a). From the micrograph under magnification, the surface of chitosan film shows a randomly distributed microstructure space that looked like a crack (Fig.2.a). This structure suggested that chitosan film particles might have failed to crystallize. Cellulose fiber film has a “branch” like structure, which has large spaces between the particles. This happened due to longer evaporation time taken by cellulose which enabled it to crystallize (Fig.2.b). From SEM micrographs, it is proven that the surface characteristic of the membrane plays important role to allow water uptake. Blending cellulose fiber had improved the porosity of chitosan film.

Meanwhile, reinforced chitosan film has a rough and uneven structure which disallows more water molecules to be adsorbed. The morphology of reinforced chitosan film are reported in Fig.3.b. SEM micrographs also revealed that the surface structure of chitosan- reinforced chitosan film has much similarity with chitosan film which exhibited amorphous feature[4].

### 5.7 Degradation

Degradation experiments were conducted with chitosan and reinforced chitosan film. The specimens of the hydrogels were soaked in deionized water and HCl. The observed pattern indicated an initial rapid mass loss usually in first 30min of testing that continually reduces. These results are presented in Fig.4.

The results have revealed a strong influence of composite materials on the degradation properties. It was clearly observed that when the pure chitosan film content increased, degradation also increased, whereas when the cellulose content increased, degradation decreased, suggesting higher density of chemical cross-linking among polymer chains the degradation behavior can be influenced by the solution medium utilized on the immersion test. The degradation index decreases for most samples immersed in deionized water compared to HCl. This process can be explained by the higher osmotic pressure caused by a larger concentration gradient in deionized water than in HCl.

## 6. CONCLUSIONS

Physical and mechanical properties of chitosan films can be controlled by choosing appropriate molecular weight of chitosan and solvent system. The result on mechanical properties showed that the tensile properties of the reinforced chitosan films were superior to the free film. The reinforcement of cellulose fiber from coconut into the chitosan film improved thermal stability of the chitosan film. The swelling ratio and water vapor permeability of the chitosan film can be decreased with hydrophobic characteristic of the cellulose fiber as higher the chitosan content, higher the degree of contact angle.

The morphology of the film was characterized using SEM. Film prepared from 2g chitosan in 2% acetic acid showed thickness of 0.17 mm which was high value compared with other films, the reinforced chitosan film further increased the film thickness to 0.32 mm. The swelling ratio and water vapor permeability of pure chitosan films and reinforced chitosan film measured for water uptake, which indicates that the reinforced chitosan film enhanced the moisture resistance of the chitosan film

## ACKNOWLEDGEMENTS

This study was supported by Faculty research grant from National Institute of Technology Calicut. Project No : Dean (C&SR)/FRG10-11/0101 Dated 18.10.10 and Department of science and technology-DST/FTP/CS_68/2007.

## LIST OF SYMBOLS

| | |
|---|---|
| $W_s$ | weight of the swollen films,g |
| $W_d$ | weight of the dry films,g |
| $W_o$ | weight of the film before degradation,g |
| $W_f$ | weight of the film after degradation,g |
| $T_i$ | initial weights of sample vials,g |
| $T_f$ | final weights of sample vials,g |
| $C_i$ | initial weights of control vials,g |
| $C_f$ | final weights of control vials,g |
| v | volume of each vial,$cm^3$ |

**Table 1**

**Physical properties of chitosan and reinforced chitosan film**

| Chitosan (g) | Acetic acid (%) | Thickness (mm) | Swelling ratio (%) | WVP (g/day/l) |
|---|---|---|---|---|
| 1g | 1% | 0.07mm | 40% | 4.8 |
| 1g | 2% | 0.1mm | 10% | 7.9 |
| 1g | 3% | 0.1mm | 21.15% | 5.2 |
| 2g | 1% | 0.15mm | 34.9% | 5.3 |
| 2g | 2% | 0.17mm | 49% | 8.05 |
| 2g | 3% | 0.16mm | 25% | 7.6 |
| 2g + Coconut fiber 2g | 2% | 0.32mm | 35.3% | 8.01 |

**Table 2**

**Mechanical properties of chitosan and reinforced chitosan film**

| Chitosan (g) | Acetic acid (%) | Tensile (Mpa) | Elongation (%) | Stress at break Mpa |
|---|---|---|---|---|
| 1g | 1% | 16.43 | 189 | 2.143 |
| 1g | 2% | 31.5 | 119 | 1.5 |
| 1g | 3% | 43.5 | 116 | 1.5 |
| 2g | 1% | 15.67 | 22.4 | 1 |
| 2g | 2% | 44.12 | 64 | 0.882 |
| 2g | 3% | 38.24 | 56.1 | 0.882 |
| 2g+ Coconut fiber 2g | 2% | 52.34 | 60.2 | 0.468 8 |

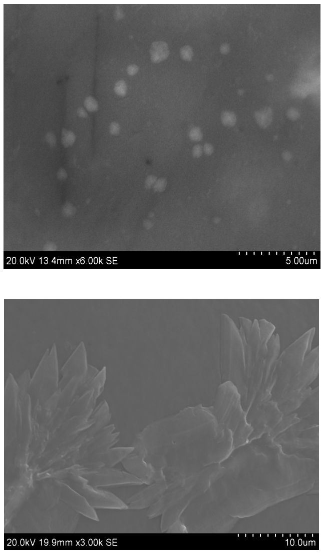

**Fig.1 : SEM images of surface morphology of (a) pure chitosan film, 1g +2%, (Smooth) and (b) reinforced chitosan film (Branchy)**

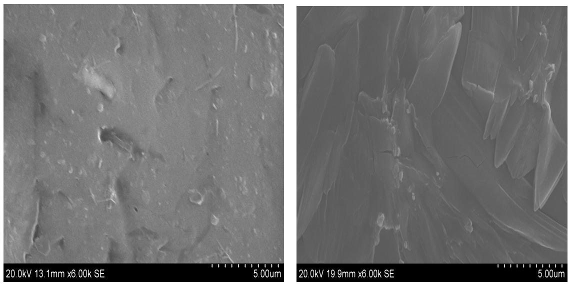

**Fig.2 : SEM images of surface morphology (a) chitosan film, 2 g +2%, (Cracky ) and (b) reinforced chitosan film (Branch)**

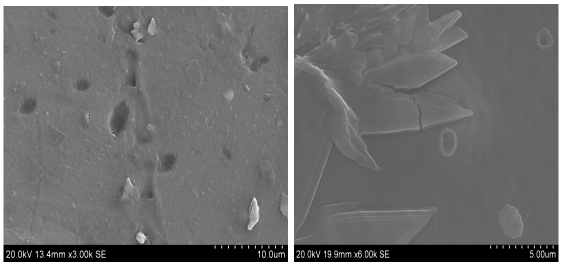

**Fig.3 : SEM images of surface morphology (a)chitosan film, 2 g +2%, ( small pores) and (b)reinforced chitosan film ( no pores)**

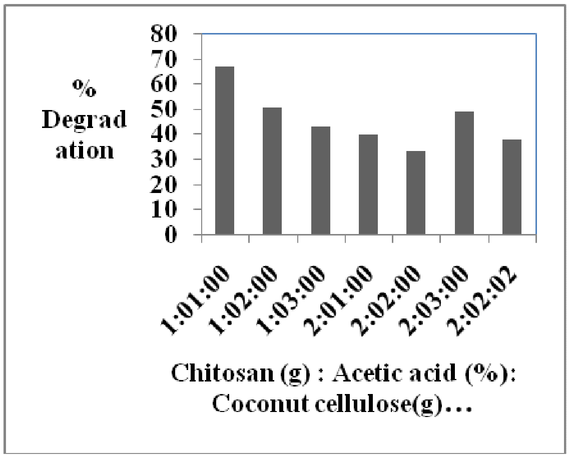

**Fig.4: Degradation % of chitosan and reinforced chitosan film.**

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