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# PREPARATION, CHARACTERIZATION AND APPLICATION OF PVA-RICE STARCH-CHITOSAN BLENDED FILMS

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## Abstract

Chitosan was prepared from shrimp shells and rice starch were extracted from rice grain. The polymeric films of PVA-rice starch-chitosan and PVA-rice starch-chitosan-glycerol-citric acid with various ratios were prepared by casting methods. The physicomechanical properties such as thickness, tensile strength, elongation at break**,** and tear strength of the prepared films were determined by mechanical testing. PVA-rice starch-chitosan-glycerol-citric acid films showed the potent antimicrobial activity on seven tested microorganisms such as *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens and Staphylococcus aureus* by the agar disc diffusion method. Medicinal application of PVA-rice starch-chitosan-glycerol-citric acid films with various ratio was studied by using Wistar rats. After 15 days, the wound healing activity of the PVA- starch-chitosan-glycerol-citric acid film with the ratio of 2: 1: 2:0.5:1 was found to be effective on treated lesions of Wistar rats.

**Keywords**: chitosan blended films, physicomechanical properties, antimicrobial activity, wound healing activity

## Introduction

The development of environmental friendly polymer materials can be classified into two categories based on the raw material used: degradable synthetic polymers and renewable natural polymers. Renewable natural polymer resources include starch, cellulose and chitosan. Starch is the most attractive candidate because of its low cost, ready availability, its inherent biodegradability and potential for mass production from renewable resources. However, the poor mechanical properties and relatively hydrophilic nature of starch compared to most petroleum-based polymers prevent its use in widespread applications. Recently, several attempts have been made to blend starch with biodegradable synthetic polymers. Starch itself is very brittle and has poor mechanical properties for plastic applications. This condition necessitates the addition of plasticizers. Plasticizers include polyols such as glycerol and polyethylene glycol. In this study glycerol is used as plasticizer because of its ready availability. In addition, citric acid was used as the additive in this study. Citric acid is rated as nutritionally harmless since it is a nontoxic metabolic product of the body.

PVA is a synthetic water-soluble and biodegradable polymer (Chiellini *et al*., 2003). PVA has excellent mechanical properties and compatibility with starch, PVA/starch blend is assumed to be biodegradable since both components are biodegradable in various microbial environments. The biodegradability of blends consisting of starch, PVA, Glycerol and urea is performed by bacteria and fungi isolated from the activated sludge of a municipal sewage plant and landfill, which indicate that microorganisms consumed starch and the amorphous region of PVA as well as the plasticizers (Tudorachi *et al*., 2000). Because both starch and PVA are polyols, starch, will form continuous phase with PVA during blending.

Starch and chitosan are abundant naturally occurring polysaccharide. Both of them are cheap, renewable, non-toxic, and biodegradable. Starch is totally biodegradable in a wide variety

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environment. It can be hydrolyzed into glucose by microorganisms or enzymes, and then metabolized into carbon dioxide and water. Carbon dioxide will recycle into starch again by plants and sunshine. Starch itself is poor in processability, also poor in the dimensional stability and mechanical properties for its end products (Choi *et al*., 1999). Therefore, native starch is not used directly.

## Materials and Methods

### Extraction of Rice Starch

Rice starch was extracted from rice grain. Rice grains were ground in a grinding machine and the rice powder was passed through 250 mesh screens. Rice powder sample (100 g) was placed in a 2 L steel pot. Distilled water (1500 mL) was added to the rice powder, stirred manually for 1 h and the liquid detained was centrifuged for 30 min. The supernatant was decanted off and the residue was extracted with 1liter distilled water for 24 h. The rice sample residues were put into a Petri dish and air dried in the laboratory (Valgadde *et al*., 2015). The yield of extracted rice starch was calculated. (Appendix-I)

### Preparation of Chitosan

The shrimp shells (100 g) were deproteinised by using 5% NaOH with a 1: 8 (w/v) ratio and refluxed at 60°C for 2 h. Then the product was collected with 300 µm sieve, washed and dried under the fume hood to constant weight. The deprotonised product was then decolourised with acetone for 24 h, and was demineralised with 1 % HCl with 1:10 (w/v) for 24 h at room temperature. The light brown powder was obtained as chitin (Hossain and Iqbal 2014). Then, the chitin was deacetylated with a 1: 5 (w/v) ratio of 5 % NaOH at 100 °C for 10 h (Kandile *et al*., 2018). Finally, the prepared chitosan was washed with distilled water and dried under a fume hood to yield a constant weight. The yield of chitosan was 69.03 %.

### Preparation of PVA-Starch-Chitosan Blended Films

PVA, starch, and chitosan films with various ratios of 2:1: 2 (PVA: starch: chitosan), 2:1:3, 2:1:4, and 2:1:5 were also prepared. Starch (1.0 g) was added into 250 mL beakers. Next, 100 mL of distilled water were added to the beakers. The solution mixtures were stirred for 10 min. The chitosan (2.0 g) was added to the starch solution, and the solution mixtures were stirred for 10 min. After that, 2.0 g of PVA was added. The solution mixture was stirred by using a magnetic stirrer at 150 ˚C for 30 min at a rate of 1000 rpm and then autoclaved at 0.1 MPa, 121 ˚C for 20 min. The polymeric solution was cast on the melamine plate and oven-dried for 2 h 30 min, and air dried for 2.5 days to obtain the PVA, starch, and chitosan films with a PVA, starch, and chitosan films ratio of 2:1:2. Similarly, the films with different ratios of PVA, starch, and chitosan; 2:1:3, 2:1:4, and 2:1:5 were prepared using the procedure as mentioned above (Brandelero *et al*., 2019).

### Preparation of PVA-Rice Starch-Chitosan-Glycerol-Citric Acid Films

The films with PVA, rice starch, chitosan, glycerol, and citric acid ratios of 2:1:2:0.5:0.5 2:1:3:0.5:1, 2:1:4:0.5:1.5, and 2:1:5:0.5:2 were also prepared. Rice starch (1.0 g) was added into 250 mL beakers. Next, 100 mL of distilled water was added to the beakers. The solution mixtures were stirred for 10 min. The chitosan (2.0 g) was added to the rice starch solution and the solution mixtures were stirred for 10 min. After that, 2.0 g of PVA, 0.5 g of glycerol, and 0.5 g of citric acid were added. The solution mixture was stirred by using a magnetic stirrer at a temperature of 150 ˚C for

30 min at a rate of 1000 rpm and then autoclaved at 0.1 MPa, 121 ˚C for 20 min. The polymeric solution was cast on the melamine plate and oven-dried for 2 h 30 min and air-dried for 2.5 days to obtain the PVA-rice starch-chitosan-glycerol-citric acid film with a ratio of 2:1:2:0.5:0.5. Similarly, films with varying ratios of 2:1:3:0.5:1, 2:1:4:0.5:1.5, and 2:1:5:0.5:2 were prepared as the procedure mentioned above (Brandelero *et al*., 2019).

**Determination of Physicomechanical Properties of PVA-Rice Starch-Chitosan Films and PVA-Rice Starch-Chitosan-Glycerol-Citric Acid films**

Physicomechanical parameters of PVA-rice starch-chitosan films and PVA-rice starch-chitosan-glycerol-citric acid films, such as tensile strength, elongation at break, and tear strength were tested at the Development Centre for Rubber Technology, Yangon. Dog bone-shaped test specimens were cut from the PVA-rice starch-chitosan films and PVA-rice starch-chitosan-glycerol-citric acid films. Mechanical testing for tensile strength properties was performed by a testing machine. The tensile strength, elongation at break, and tear strength of PVA- rice starch-chitosan films, and PVA-rice starch-chitosan-glycerol-citric acid films were calculated**.** (Appendix– II)

**(a) Determination of thickness**

The thickness of the prepared PVA-rice starch-chitosan films was measured by using NSK micrometers. The thickness of the films were measured at five locations (1 centre and 4 corners) using a micrometer.

**(b) Determination of tensile strength and elongation at break**

Dog-bone shaped test specimens were cut from the films. Both ends of the test pieces were firmly clamped in the jaw of the test machine. One jaw was fixed, and the other was moveable. The rate of the moveable jaw was bold 100 mm/min. This procedure for tensile strength was repeated three times.

**(c) Determination of tear strength**

Test specimen was cut off by a die from the above films. The specimen was cut with a single nick (0.04 mm) at the entire inner concave edge by a special cutting device using a razor blade. The clamping of the specimen in the jaw of the test machine was aligned with the travel direction of the grip at 100 mm/min. The recorder of the machine was shown the highest force to tear from a specimen nicked. The procedure was done in triplicate.

**Investigation of Antimicrobial Activity**

Antimicrobial activities in PVA-rice starch-chitosan-glycerol-citric acid (PSCGC II) film was determined by applying agar well diffusion method against seven microorganisms such as *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens and Staphylococcus aureus* at Chemistry Depatrment, Meiktila University.

A 18 g of nutrient agar powder was suspended in 100 mL of distilled water. The mixture was heated while stirring to well dissolve all components. The dissolve mixture was placed in the autoclave at 121 ˚C for 45 min. Then the flasks were cooled down at room temperature and 0.2 mL of spore suspension were added into it and poured into the sterilized Petri dish. The agar was allowed to set for 2 h and then 8 mm plate agar disc was made with the help of sterilized cork borer. After that about 5 mm of the sample was introduced into the agar-disc and incubated at 37 ˚C for 24 h. The inhibition zone (clear zone) which appeared around the agar disc, indicated the presence of antimicrobial activity.

### Wound-Healing Activity of Prepared Films tested by Animal Experiment

The adult male Wistar rats from Laboratory animal services division, Department of Medical Research (Lower Myanmar) DMR-LM were included in this study to determine the proper healing agent in the treatment of incision wound. All Wistar rats were admitted to the study approximately 4 months of age and between 250-300 g of body weight. Free access was allowed to use standard diet (pellet food), water, temperature, humidity and light period. The Wistar rats received only clean water wash and the treatment groups received PVA-rice starch-chitosan-glycerol-citric acid (2: 1: 2: 2: 0.5: 1) film one time /day for 14 days.

## Results and Discussion

### Sample Preparation

Rice starch 32.48 g was extracted from 100 g of rice grain and yield percent was found to be 32.48 %. Chitosan 69.03 g was prepared from 100 g of shrimp shells and yield percent was found to be 69.03 %.

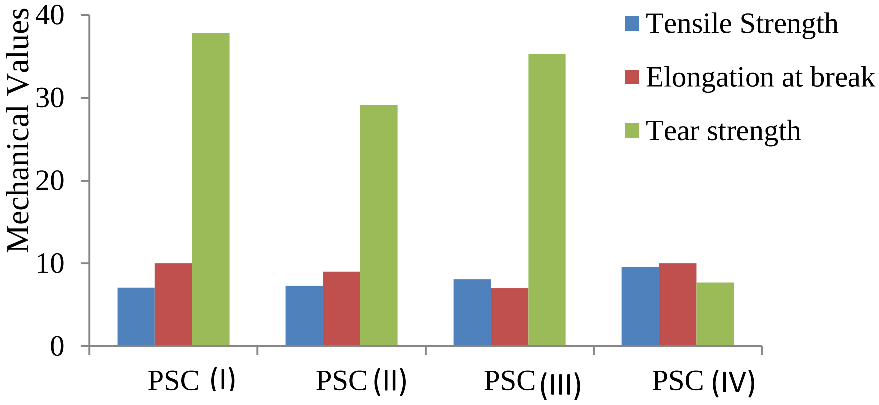
### Physicomechanical Properties of PVA-Rice Starch-Chitosan Films

PVA, rice starch and chitosan films were prepared with four different ratios of 2: 1: 2, 2: 1: 3, 2: 1: 4, and 2: 1: 5 respectively. PVA-rice starch-chitosan film PSC (I) with the ratio of (2:1:2) was found to have good physicomechanical properties, such as tensile strength (7.1 MPa), elongation at break (10 %), and tear strength (37.8 kN/m), as shown in Table 1 and Figure 1.

**Table 1. Physicomechanical Properties of PVA-Rice Starch-Chitosan Blended Films**

| No | Sample | Weight ratio of PVA, rice starch and chitosan | Tensile strength (MPa) | Elongation at break (%) | Tear strength (kN/m) |
|---|---|---|---|---|---|
| 1. | PSC(I) | 2: 1: 2 | 7.1 | 10 | 37.8 |
| 2. | PSC(II) | 2: 1: 3 | 7.3 | 9 | 29.1 |
| 3. | PSC(III) | 2:1: 4 | 8.1 | 7 | 35.3 |
| 4. | PSC(IV) | 2: 1: 5 | 9.6 | 10 | 7.7 |

Thickness = 0.29 mm
PSC(I) = 2% PVA + 1% rice starch + 2% chitosan
PSC(II) = 2% PVA + 1% rice starch + 3% chitosan
PSC (III) = 2% PVA + 1% rice starch + 4% chitosan
PSC(IV) = 2 % PVA + 1% rice starch + 5% chitosan




**PSC = PVA-rice starch-chitosan**

**Figure 1.** Histogram of some mechanical values of prepared PVA- rice starch-chitosan blended films

**Physicomechanical Properties of PVA-Rice Starch-Chitosan-Glycerol-Citric Acid Films**

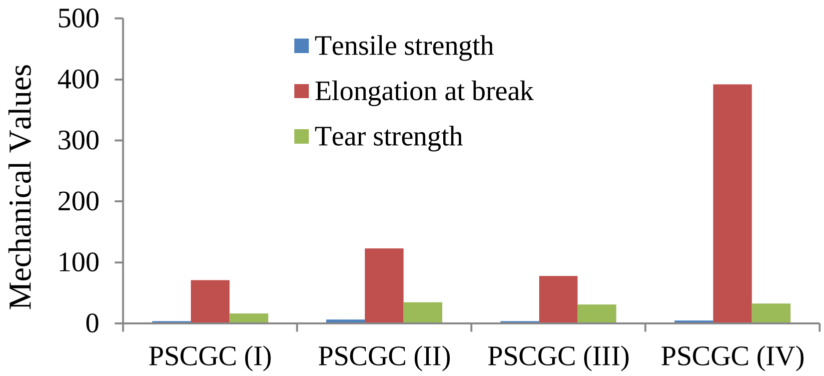
Effect of citric acid on the PVA-rice starch-chitosan films was studied by the addition of 0.5, 1, 1.5, 2 g of citric acid into the mixture of PVA, rice starch, chitosan, glycerol with the ratios 2: 1: 2: 0.5.

Citric acid was added as an additive. It has two functions i.e., as a cross linker and as a plasticizer. The mechanical properties are not only related with the plasticizer. Generally, the tensile strength increased and the elongation at break increased as the percentage of cross linker increased. The citric acid acted both as the cross linker and the plasticizer in the PVA-rice starch-chitosan composites. So, the different functions of citric acid exhibited different amount of citric acid added. The tensile strength and elongation at break as the function of citric acid concentration are shown in Table 2 and Figure 2. The tensile strength of PSCGC (II) was 6.6 MPa which was found to be higher than 4.8 MPa of PSCGC (IV). But adding more citric acid to the blends as a plasticizer made the macromolecules interact less, which led to decrease in tensile strength (4.8 MPa). And thus, elongation at break increased to 392. From the experimental data, the film with the PVA: rice starch: chitosan: glycerol: citric acid ratio of 2: 1: 2: 0.5: 1 (PSCGC II) was found to have the best physicomechanical properties.

**Table 2. Physicomechanical Properties of PVA-Rice Starch-Chitosan-Glycerol-Citric Acid (PSCGC) Films**

| No | Sample | Weight ratio of PVA, rice starch, chitosan, glycerol and citric acid | Tensile strength (MPa) | Elongation at break (%) | Tear Strength (kN/m) |
|---|---|---|---|---|---|
| 1. | PSCGC(I) | 2: 1: 2: 0.5: 0.5 | 3.7 | 71 | 16.5 |
| 2. | PSCGC (II) | 2: 1: 2: 0.5: 1.0 | 6.6 | 123 | 35.0 |
| 3. | PSCGC(III) | 2: 1: 2: 0.5: 1.5 | 4.0 | 78 | 31.0 |
| 4. | PSCGC (IV) | 2:1 :2: 0.5: 2.0 | 4.8 | 392 | 32.9 |

Thickness = 0.38 mm
PSCGC (I) = 2 % PVA+ 1 % rice starch+ 2 % chitosan+ 0.5 % glycerol+ 0.5 % citric acid
PSCGC (II) = 2 % PVA+ 1 % rice starch+ 2 % chitosan+ 0.5 % glycerol+ 1 % citric acid
PSCGC (III) = 2 % PVA+1 % rice starch+ 2 % chitosan+ 0.5 % glycerol+ 1.5 % citric acid
PSCGC (IV) = 2 % PVA+ 1 % rice starch+ 2 % chitosan+0.5 % glycerol+ 2 % citric acid

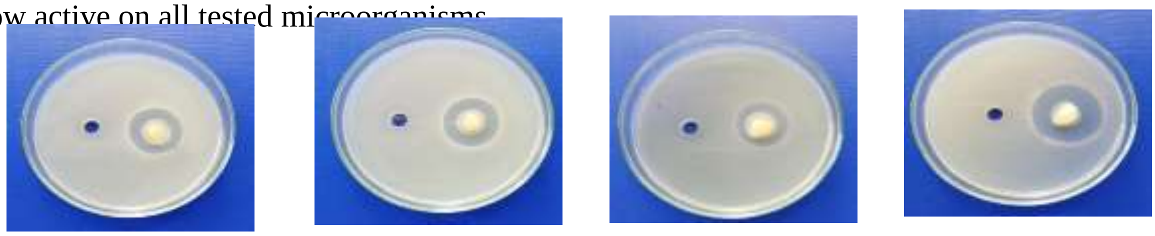



PSCGC = PVA-rice starch-chitosan-glycerol-citric acid

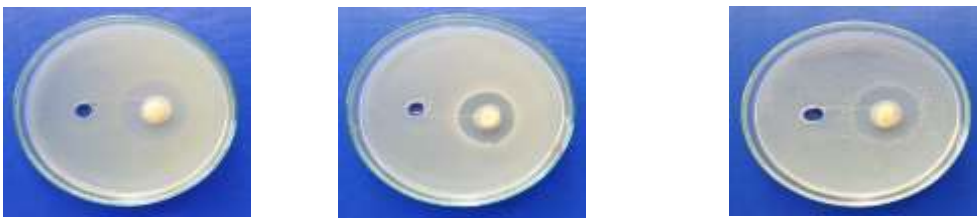
**Figure 2.** Histogram of some mechanical values of prepared of PVA- rice starch-chitosan-glycerol-citric acid films

**Antimicrobial Activity of the PVA-Rice Starch-Chitosan-Glycerol-Citric acid Films**

Antimicrobial activity of the selected PVA-rice starch-chitosan-glycerol-citric acid films (PSCGC II) was tested by agar-well diffusion method on seven microorganisms such as *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens and Staphylococcus aureus*. The results are shown in Table 3 and Figure 3. PVA-rice starch-chitosan-glycerol-citric acid films (PSCGC II) were found to show active on all tested microorganisms.



***A. tumefaciens*** ***B. pumilus*** ***B. subtilis*** ***C. albicans***



***E. coli*** ***P. fluorescens*** ***S. aureus***

**Figure 3.** Antimicrobial activity of PVA-rice starch-chitosan-glycerol-citric acid (PCSGS II)Films

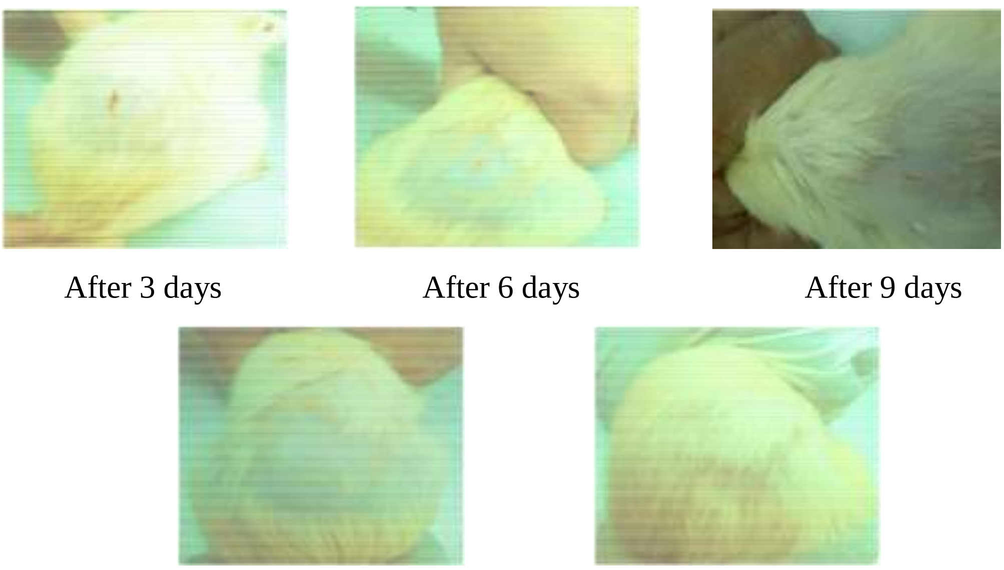
**Table 3. Results of Antimicrobial Activity of PVA-Rice Starch-Chitosan-Glycerol-Citric Acid (PSCGC II) Films**

| No. | Microorganisms | Inhibition Zone Diameters (mm) |
|---|---|---|
| 1. | *A. tumefaciens* | +++ |
| 2. | *B. pumilus* | +++ |
| 3. | *B. subtilis* | +++ |
| 4. | *C. albicans* | +++ |
| 5. | *E.coli* | +++ |
| 6. | *P.fluorescens* | +++ |
| 7. | *S.aureus* | +++ |

Agar-well – 8 mm
8 mm-12 mm (+) (mild activity)
13 mm-17 mm (++) (moderate activity)
18 mm above (+++) (potent activity)

**Wound-Healing Activity of Prepared Film**

The skin incision of Wistar rats was treated with PVA-rice starch-chitosan-glycerol-citric acid film (PSCGC (II)) (Kornblum *et al.*, 1973). After 15 days of treatment, the wound healing places of the rat closed, and their hair regrew. The photographs recorded during the study process are shown in Figure 4. The appearance of the skin treated with the cross-linker PVA-rice starch film (15 days) was observed to be nearly the same as the normal skin (Sharif and Yamaura, 2002).



After 3 days After 6 days After 9 days

After 12 days After 15 days

**Figure 4.** Wound-healing progress after one time per day treated with skin incision wounded on Wistar rats

## Conclusion

This research deals with the preparation and some physicomechanical properties of rice starch-chitosan blend films. Chitosan was prepared from shrimp-shells and yield percent was 69.03 %. Rice starch was extracted from rice grain was investigated. Yield percent of rice starch was 32.48 %. PVA-rice starch-chitosan films were prepared by varying the amount of chitosan, rice starch and PVA concentration were kept at 1 % and 2 % film made up of PVA-rice starch-chitosan with a ratio of 2: 1: 2 showed high tensile strength (7.1 MPa), high elongation at break (10 %) and the highest tear strength (37.8 kN/m). The addition of citric acid to PVA-rice starch-chitosan-glycerol-citric acid films decreased their tensile and tear strength, revealing the film (PSCGC II) with a 2:1:2:0.5:1 ratio with the highest tensile strength (6.6 MPa) and elongation at break (123 %). The PVA-rice starch-chitosan-glycerol-citric acid blend film showed potent antimicrobial activity on all tested microorganisms *vis*., *A. tumefaciens, B. pumilus, B. subtilis, C. albicans, E. coli, P. fluorescens, and S. aureus*. Furthermore, the biomedical application of the prepared film has better and faster wound healing activity on incision Wistar rats. From these results the PVA-rice starch-chitosan-glycerol-citric acid film ratio of 2: 1: 2: 0.5: 1 can be used as a wound healing agent.

## Appendix-I

### Yield of Extracted Rice Starch

$$\text{Yield \%} = \frac{\text{Weight of Rice Starch}}{\text{Weight of Rice Powder}} \times 100$$

## Appendix-Ii

### Determination of Physicomechanical Properties of Rice Starch-Chitosan-PVA Films

(a) Elongation (%) = $\frac{\Delta L}{\mathrm{L}} \times 100$

Where, $\Delta L$ = Difference in length

$L$ = Original length

(b) Tensile strength= $\frac{\text{(load of break)}}{\text{Original width} \times \text{original thickness}}$

(c) Tear strength = $\frac{F}{t}$

Where, F = Force (kN)

t = Specimen thickness (m)

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