

# **PREPARATION AND CHARACTERIZATION OF BIODEGRADABLE CHITOSAN-CALCIUM ALGINATE COMPOSITE MEMBRANE AND ITS ANTIMICROBIAL ACTIVITY**

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## **Abstract**

The pure chitosan membranes, un-modified and modified chitosan-calcium alginate composite membranes were prepared by using casting and autoclaving methods. All prepared chitosan composite membranes have smooth surfaces, highly transparent and pale yellow colour. The physicochemical properties of prepared chitosan composite membranes were determined such as tensile strength, elongation at break and tear strength. According to the mechanical properties of the pure chitosan membranes, 2 % (w/v) chitosan was chosen as optimum condition and 2 % (w/v) chitosan, 0.1 % (w/v) sodium alginate, 0.2 % (w/v) calcium chloride solution were the most suitable for preparing chitosan-calcium alginate membranes. Swelling behaviour and water uptake of these membranes were determined as a function of contact time. Then, modified chitosan-calcium alginate composite membranes were prepared by using various percent of glycerine as a plasticizer. According to the physicochemical properties of modified chitosan-calcium alginate-glycerine composite membranes, optimum ratio for membrane preparation was chosen. It was found that the modified chitosan-calcium-alginate-glycerine composite-1 membrane showed tensile strength 34.2 MPa, elongation at break 30 % and tear strength 55.5 kNm<sup>-1</sup>. The characterization of chitosan membranes, chitosan-calcium alginate composite membranes and modified chitosan-calcium alginate-glycerine composite-1 membrane were studied by FT IR and SEM analyses. From FT IR analysis, the characteristic absorption peaks of modified chitosan-calcium alginate-glycerine composite membrane clearly showed that the two polymers were blended. SEM micrograph of modified chitosan-calcium alginate-glycerine composite membrane showed more homogeneous nature. The antimicrobial activities of all prepared chitosan membranes were tested by agar well diffusion method. From these results, all of the prepared chitosan membranes showed effective and efficient antimicrobial activities. The biodegradable nature of the prepared chitosan membrane, chitosan-calcium alginate composite membrane and modified chitosan-calcium alginate-glycerine composite membranes were studied by soil burial test.

**Keywords:** chitosan, physicochemical, antimicrobial activities, biodegradable nature

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## Introduction

Chitin is the most abundant nitrogen containing biopolymers in nature and it is found widely in the shells of crabs, lobsters, krill and shrimp (Wibowo *et al.*, 2004). It is a linear polysaccharide consisting of ideally (1→4) linked 1-2-acetamido-2-deoxy-β-D- glucopyranose. Chitosan is a linear polysaccharide consisting of ( 1→4) linked 2-amino-2- deoxy -β-D- glucopyranose. Chitosan is produced by alkaline N- deacetylation of chitin. (Mohanty *et al.*, 2002). Chitosan is very similar to chitin. Chitosan is a white to ability light yellow colour, insoluble in water but it is readily soluble in dilute aqueous organic acid such as acetic acid, propionic acid, formic acid and lactic acid. The term biopolymers refer to naturally occurring large polymeric molecules, such as proteins, nucleic acids and polysaccharides, which are essential components of all living systems. The same term is also used to describe synthetic polymer prepared from identical or similar monomers or subunits to those which make up the natural polymers. Natural biopolymers are receiving much attention due to their biocompatibility and biodegradability (Peter, 1995). Biodegradation is the degradation and assimilation of organic polymers and other compounds by the action of living organisms. Sodium alginate is the common commercial algin which is the alkali metal salt of alginic acid. Alkali metal salts of alginic acid as well as the ammonium, magnesium and lower amine salts are water soluble. The soluble alginates are strongly hydrophilic colloids, yielding highly viscous solution at low concentration (Whistler and BeMiller, 1997). Sodium alginate fits in a divalent cation such as calcium leading to the formation of calcium alginate beads (Draget, 2000). The resultant structure of linked chains is called an egg-box model. Calcium alginate beads were synthesized through a cross-linking reaction between sodium alginate and calcium chloride solutions. The effect of calcium concentration on the bead strength and chemical stability were investigated (Gotoh *et al.*, 2004). Composites consist of two (or more) distinct constituents or phases, which when mixed together result in a material with entirely different properties from those of the individual components. Typically, a manmade composite would consist of a reinforcement phase of stiff, strong material, frequently fibrous in nature, embedded in a continuous matrix phase. As a biopolymer, it is readily processible into membranes, hollow fibers and beads as well as sponges from its aqueous acid solution.

Chitosan's unique properties make it useful for a broad variety of industrial and biomedical applications, especially as a chelating agent. In addition, chitosan and its derivatives are effective separation agents for adsorption and removal of transition metal ions, such as Cu (II), Cr (III), Hg (II), Cd (II), Zn (II) and Ni (II) ions.

The main aim of the research work is to study the preparation of modified chitosan-calcium alginate-glycerine composite membrane and to be used as an sorbent for antimicrobial and biodegradable properties.

## **Materials and Methods**

### **Sample Collection**

Chitosan sample was purchased from Shwe Poe Co. Ltd., Hlaing Tharyar Township, Yangon Region.

### **Preparation of Chitosan Membranes**

A series of chitosan membranes, with different concentrations (1.0 %, 1.5 %, 2.0 % w/v) was prepared by solution casting and solvent evaporating method. Chitosan flakes were dissolved thoroughly in 1 % (v/v) acetic acid solution. The above chitosan solutions were left for 30 min with often stirring to obtain clear homogeneous solution. The prepared chitosan solution was poured onto melamine plates and gelling of membranes was allowed to take place at room temperature for about one week. The prepared membranes were neutralized with 1 M NaOH solution and the membranes were washed with water for several times and dried in air. All of the prepared chitosan membranes were kept under dry conditions before further use.

### **Preparation of Chitosan-Calcium Alginate Composite Membranes and Chitosan-Calcium Alginate-Glycerine Composite Membrane**

Chitosan-calcium-alginate composite membrane was prepared by dissolving the chitosan 2.0 % (w/v) in 1 % (v/v) acetic acid solution. The mixture was stirred for 30 min to obtain the clear homogeneous chitosan solution. The calcium alginate gel was prepared by mixing the certain amount of sodium alginate 0.1 % (w/v) and calcium chloride solution 0.2 % (w/v). This mixture was stirred for 30 min. This calcium alginate gel was mixed with

above chitosan solutions and the mixture was stirred for 1 h. The resulting prepared solutions were poured onto melamine plates and membranes were allowed to dry at room temperature for one week to obtain composite membranes. Similarly, modified composite membranes were prepared by using optimum conditions of 2 % (w/v) chitosan, 0.1 % (w/v) sodium alginate, 0.2 % (w/v) calcium chloride solution and the various amount of (0.1 %, 0.2 %, 0.3 %, 0.4 %, and 0.5 % w/v) glycerine were added into the composite solution. The glycerine was also used as plasticizer for flexibility (film strength). The resulting modified composite solutions were autoclaved at a pressure of 0.1 MPa and  $121\pm 1^\circ\text{C}$  for 1 h. The prepared solutions were poured onto melamine plates and membranes were allowed to dry at room temperature for one week. The membranes obtained from this procedure were denoted as composites-1, 2, 3, 4 and 5, respectively.

### **Determination of the Physicochemical Properties of Commercial Chitosan**

The physicochemical properties (pH, degree of deacetylation, molecular weight, viscosity) of commercial chitosan were determined. Then, water uptake and degree of swelling (%) were also determined.

### **Determination of Physicomechanical Properties of Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes**

The mechanical properties of prepared chitosan-calcium alginate-glycerine composite membranes were determined such as thickness, tensile strength, elongation at break and tear strength.

### **Characterization of the Prepared Pure Chitosan Membrane, Un-modified and Modified Composite Membranes**

The FT IR spectra of prepared pure chitosan membrane, un-modified and modified composite membranes were recorded by using Shimadzu, IR Prestige-21, (Japan) FT IR spectrophotometer at Department of Chemistry, University of Yangon. The FT IR spectrometer was calibrated by blank scanning between  $550$  and  $4000\text{ cm}^{-1}$  with resolution of  $1\text{ cm}^{-1}$  and 3 scans/sample.

The surface morphology of the prepared pure chitosan membrane, un-modified and modified composite membranes were studied by SEM micrograph taken on a JSM 5610 LV scanning electron microscope, JEOL Ltd., Japan, at West Yangon University.

### **Antimicrobial Activities of the Prepared Pure Chitosan Membrane, Un-modified and Modified Composite Membranes by Agar Well Diffusion Method**

The prepared pure chitosan membrane, un-modified and modified composite membranes were tested with *Bacillus subtilis*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E. coli* species to investigate the nature of antimicrobial activity. After preparing the bacteriological media, the dried membranes were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. The plates were incubated immediately or within 30 min after incubation. After overnight incubation at 37 °C, the results observed are shown in Table 6 and Figure 6.

### **Determination of Biodegradability by Soil Burial Test**

Biodegradation of different membranes was determined soil burial test by examining the weight loss and morphology changes. The membranes were cut into 1"× 1" dimensions. The membranes were then accurately weighed and buried in soil at the depth of 5 cm. They were taken out from the soil at an interval of one week. Determination of sample appearance on degradation bury study was also daily recorded by photo. The results are shown in Figures 7, 8 and 9.

## **Results and Discussion**

### **Aspect of Physicochemical Measurement of Commercial Chitosan**

The physicochemical properties of commercial chitosan such as viscosity, molecular weight, pH, and degree of deacetylation are shown in Table 1.

**Table 1: Physicochemical Parameters of the Commercial Chitosan**

Sample	Viscosity (cP)	Molecular weight (Da)	pH	DD (%)
Chitosan	90.5	$6.94 \times 10^5$	4.8	82

\*cP =centi Poise, Da =Daltons, DD =Degree of Deacetylation

### **Water Uptake of Prepared Chitosan-Calcium Alginate-Glycerine Composite Films**

The degree of water uptake was investigated with increasing immersion time. The water uptake as a function of time for all of the prepared chitosan-calcium-alginate composite membranes are shown in Table 2 and Figure 1. The water uptake was one of the most significant parameter when a membrane to be used as packaging materials. The water uptake was the amount of water entrapped in the matrix including bound water. Table 2 shows that highest water uptake percent of chitosan-calcium alginate-glycine blended membrane composite-3 has 584 % at 90 min. The water uptake (%) of modified chitosan-calcium alginate-glycerine composite-3 membrane was slightly different from 10 min to 120 min. But modified composite-3 membrane was quite difference within this time intervals. The optimum contact time was found to be 90 mim.

### **Aspect of Degree of Swelling of Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes**

The degree of swelling as a function of immersion time for all of the prepared chitosan-calcium alginate-glycerine composite membranes were shown in Table 3 and Figure 2. From the resulting data, mostly the degree of swelling increased with increasing immersion time. Table 3 shows that the highest swelling percent of chitosan-calcium-alginate-glycerine blended composite-3 membrane has 85 % at 90 min. The swelling (%) of prepared chitosan composite 1, 2, 4, and 5 membranes were not appreciably difference but composite-3 membrane was appreciably difference within contact time from 10 min to 120 min. The optimum contact time was found to be 90 mim.

**Table 2: Water Uptake of the Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes as a Function of Contact Time**

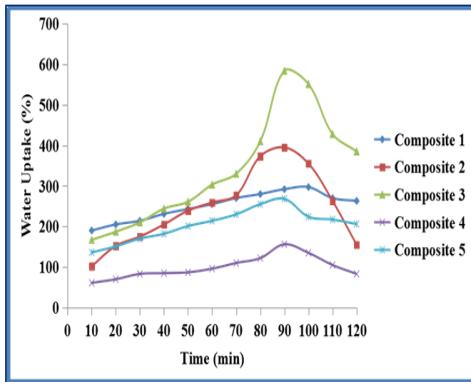
Mem-branes	Water Uptake (%) of the samples at different contact times (mim)											
	10	20	30	40	50	60	70	80	90	100	110	120
Composite 1	190	205	214	231	243	255	270	280	292	297	270	263
Composite 2	102	152	175	205	239	259	277	373	395	355	263	155
Composite 3	167	187	210	244	261	303	330	410	584	551	428	385
Composite 4	61	70	83	85	87	96	110	122	156	135	105	83
Composite 5	136	151	170	182	201	214	230	255	268	224	217	206

Composite 1 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.1 %) w/v  
 Composite 2 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.2 %) w/v  
 Composite 3 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.3 %) w/v  
 Composite 4 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.4 %) w/v  
 Composite 5 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.5 %) w/v  
 Chi = chitosan, Alg = alginate, Gly = glycerine

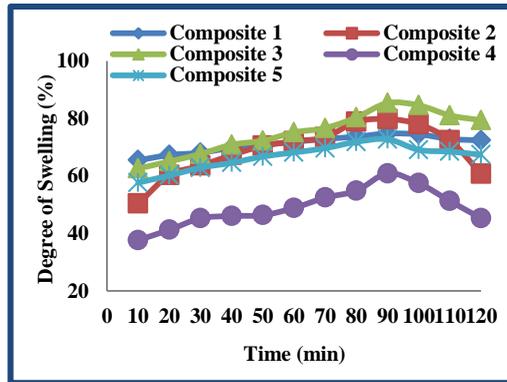
**Table 3: Swelling Behaviour of the Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes as a Function of Contact Time**

Mem-branes	Degree of Swelling (%) of the samples at different contact times (mim)											
	10	20	30	40	50	60	70	80	90	100	110	120
Composite 1	66	67	68	70	71	72	73	74	75	75	73	72
Composite 2	50	60	64	67	71	72	74	79	80	78	72	61
Composite 3	63	65	68	71	72	75	77	80	85	83	81	79
Composite 4	38	41	45	46	46	49	53	55	61	58	51	45
Composite 5	58	60	63	65	67	68	70	72	73	69	69	67

Composite 1 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.1 %) w/v  
 Composite 2 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.2 %) w/v  
 Composite 3 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.3 %) w/v  
 Composite 4 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.4 %) w/v  
 Composite 5 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.5 %) w/v  
 Chi = chitosan, Alg = alginate, Gly = glycerine



**Figure 1:** Water uptake of the prepared chitosan- calcium alginate-glycerine composite membranes as a function of contact time



**Figure 2:** Degree of swelling (%) of the prepared chitosan-calcium alginate-glycerine composite membranes as a function of contact time

### Aspect of Physicomechanical Measurement of the Prepared Chitosan Composite Membranes

The physicomechanical properties such as tensile strength, tear strength and percent elongation at break are important parameters which reveal the nature of membranes. The physicomechanical measurements of the prepared chitosan composite membranes are presented in Table 4 and Figure 3. The more the elongation at break of a certain membrane, the higher stability of the membrane. The more the tear strength of the membrane, the less durable nature of the membrane. This means to point out that composite-1 is the most suitable to prepare desired membrane for biodegradable and wound healing testing. Table 4 shows that the prepared chitosan composite-1 membrane has the highest tensile strength 34.2 MPa, elongation at breaks 30 % and tear strength 55.5 kN/m.

**Table 4: Physicomechanical Properties of the Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes**

Membrane	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kNm <sup>-1</sup> )	Thickness (mm)
Composite 1	34.2	30.00	55.50	0.10
Composite 2	28.9	17.00	92.70	0.10
Composite 3	27.1	19.00	59.10	0.11
Composite 4	22.3	29.00	49.00	0.11
Composite 5	28.2	17.00	35.50	0.10

Composite 1 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.1 %) w/v

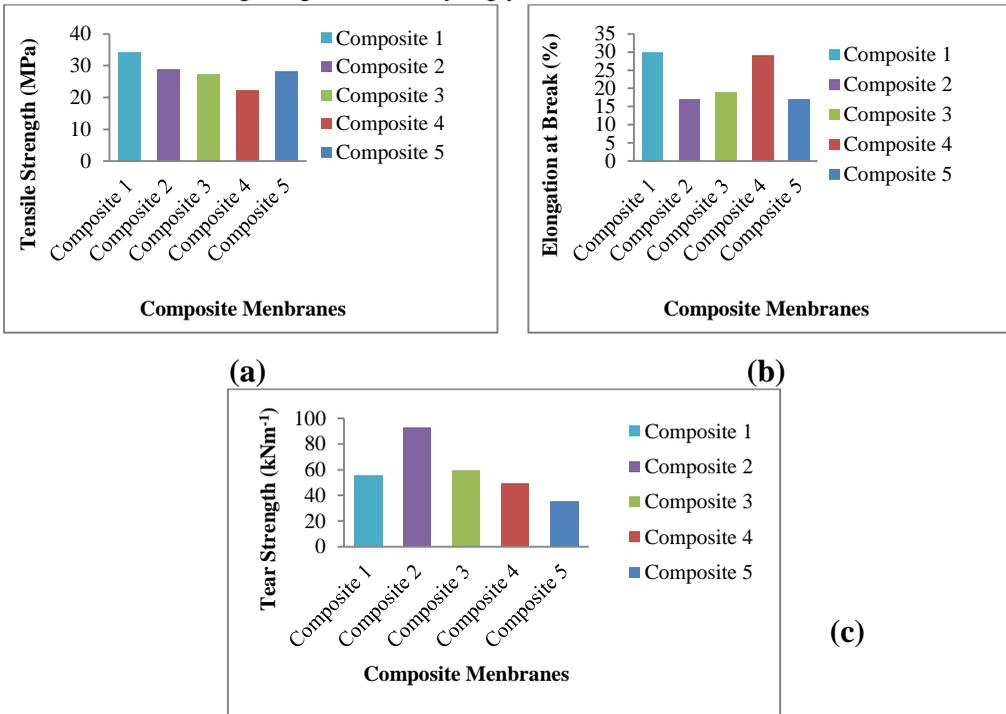
Composite 2 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.2 %) w/v

Composite 3 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.3 %) w/v

Composite 4 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.4 %) w/v

Composite 5 = Chi (2 %) + Alg (0.1 %) + CaCl<sub>2</sub> (0.2 %) + Gly (0.5 %) w/v

Chi = chitosan, Alg = alginate, Gly = glycerine

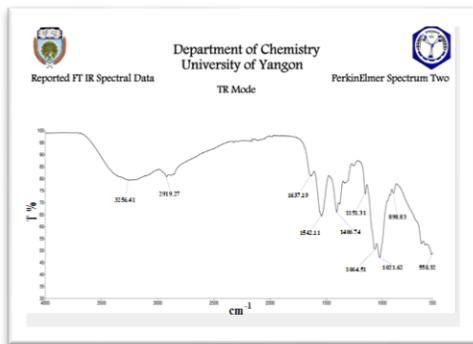


**Figure 3:** Variation of (a) tensile strength (b) elongation at break and (c) tear strength of the prepared composite membranes

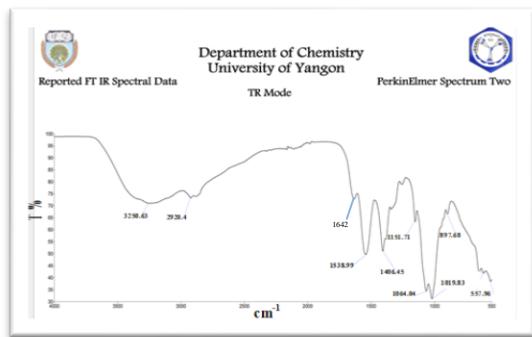
## Characterization of Pure Chitosan Membranes and the Prepared Chitosan-Calcium Alginate-Glycerine Composite Membranes

### (a) FT IR spectral analysis

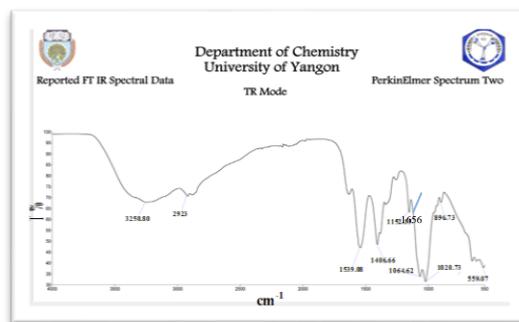
FT IR spectroscopic studies allowed to analyze the characteristic bands corresponding to the vibrations of the hydroxyl, methyl, carbonyl, amide and amine groups. It also provides data about some interactions between polymeric components in the blends. The FT IR spectra of chitosan membranes and prepared chitosan-calcium alginate-glycerine composite membranes are presented in Figures 4 and Table 5. The broad band at 3600-3200  $\text{cm}^{-1}$  range in spectra of all samples is attributed to the OH stretching, which overlap with N-H stretching in the same region. The characteristic chitosan absorption band at 2990-2850  $\text{cm}^{-1}$  range in spectra represents stretching group (CH). The strong peak at 1680-1620  $\text{cm}^{-1}$  is due to the O-H bending vibration. The sharp peak at 1563-1473  $\text{cm}^{-1}$  range in spectra represents N-H bending in secondary amide. The band at near 1425-1390  $\text{cm}^{-1}$  corresponds to vibrations of COO- stretching groups. The band at 1190-1130  $\text{cm}^{-1}$  is due to the C-N stretching in secondary amine groups. The sharp peak at 1060-1020  $\text{cm}^{-1}$  range in spectra represents of C-O-C stretching vibration of primary alcohol and 1000-600  $\text{cm}^{-1}$  corresponds to deformation of C-O stretching vibration of cyclic ether (Muzzarelli and Peter, 1973; Silverstein and Webster, 1998)



(a)



(b)



(c)

**Figure 4:** FT IR spectra of (a) pure chitosan film (b) chitosan-calcium-alginate composite film and (c) chitosan-calcium-alginate-glycerine composite membrane

**Table 5: FT IR Band Assignments of Pure Chitosan Membrane and the Prepared Chitosan-Calcium Alginate Composite Membranes**

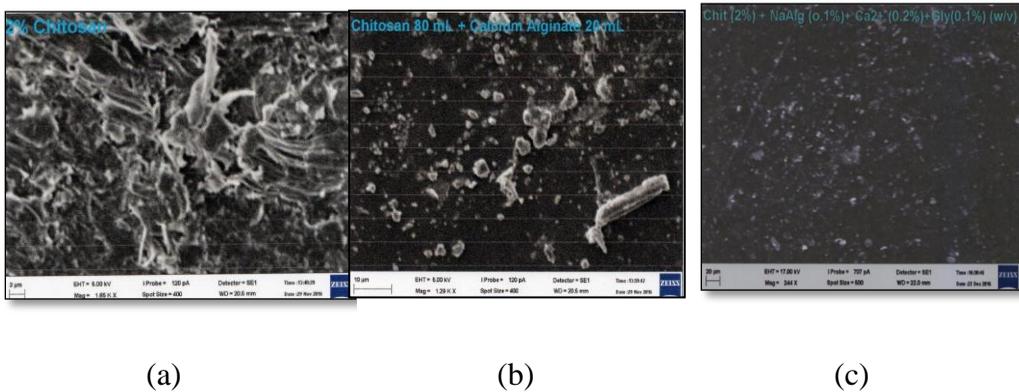
Observed wave number (cm <sup>-1</sup> )			* Literature wave number (cm <sup>-1</sup> )	Remark
Chitosan	Chi-Cal-Alg	Chi-Cal-Alg-Gly		
3256	3250	3250	3600-3200	O-H stretching (or) N-H stretching vibration
2919	2920	2923	2990-2850	C-H stretching vibration
1637	1642	1656	1680-1620	O-H bending vibration
1542	1538	1539	1563-1473	N-H bending in secondary amide
1406	1406	1406	1425-1390	COO- stretching vibration
1151	1151	1152	1190-1130	C-N stretching in secondary amine
1021	1019	1020	1060-1020	C-O-C stretching vibration, Cyclic ether

\*Muzzarelli and Peter, 1973; Silverstein and Webster, 1998

Chi = Chitosan, Cal = Calcium, Alg = Alginate, Gly = Glycerine

### (b) Scanning Electron Microscopic (SEM) Analysis

Figure 5 shows the SEM micrographs of pure chitosan membrane, prepared chitosan-calcium alginate composite membrane and modified chitosan-calcium alginate-glycerine composite membranes. The SEM images revealed that the surface structure of membrane varies with the types of ratios of constituents in the prepared chitosan composite membranes. The SEM micrographs indicated that the prepared chitosan composite membranes showed more sponge-like nature and the hollow structure which could enhance the sorptive properties of membrane. Moreover, it showed orderly surface nature and uniform pores or voids.

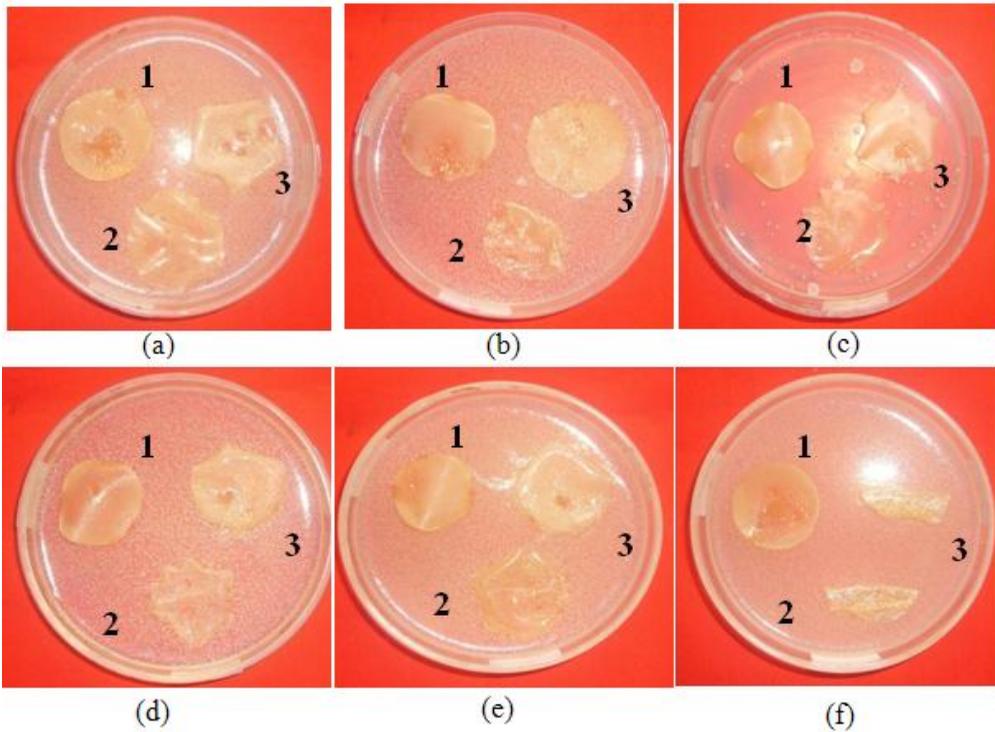


**Figure 5:** SEM micrograph of (a) pure chitosan membrane (b) chitosan-calcium alginate composite membrane and (c) chitosan-calcium alginate-glycerine composite-1 membranes

### Antimicrobial Activity of the Chitosan Membrane, Prepared Chitosan-Calcium Alginate Composite Membranes by Agar Well Diffusion Method

In this research, antimicrobial activity of the chitosan membrane, prepared chitosan-calcium alginate composite membranes were studied. These prepared membranes were tested on six different strains of human pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, and *E. coli*. To strengthen the finding, the antimicrobial activity of the all prepared membranes were determined by agar well diffusion method. Antimicrobial activity of the all prepared membranes were evaluated based on the diameters of clear inhibition zone

surrounding the paper discs. The chitosan membrane displayed higher on six microorganisms while chitosan-calcium-alginate composite membrane indicated lower on six microorganisms. The modified chitosan-calcium-alginate-glycerine composite membrane showed that highest on six microorganisms. The resulting data are shown in Table 6 and Figure 6.



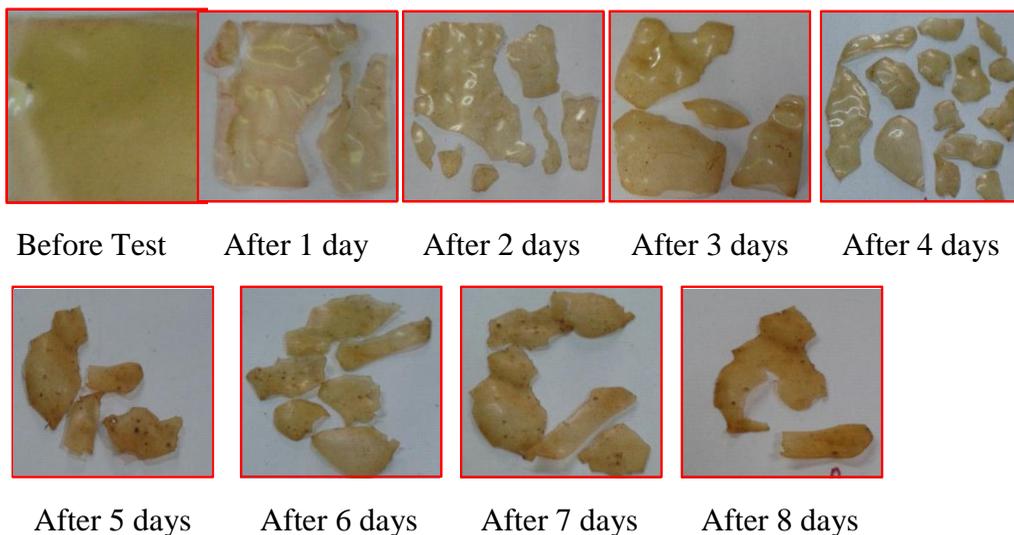
**Figure 6:** Antimicrobial activity of prepared (1) pure chitosan membrane, (2) chitosan-calcium-alginate composite membrane and (3) chitosan-calcium alginate-glycerine composite-1 membranes  
 (a) *Bacillus subtilis*                      (d) *Bacillus pumilus*  
 (b) *Staphylococcus aureus*              (e) *Candida albicans*  
 (c) *Pseudomonas aeruginosa*          (f) *E. coli*

**Table 6: Antimicrobial Activity of Pure Chitosan Membranes and Prepared Chitosan-Calcium Alginate Composite Membranes**

Samples	Inhibition zone diameters (mm) of the samples against different organisms					
	(a)	(b)	(c)	(d)	(e)	(f)
chitosan	+	++	++	++	++	++
chi-cal-alg	-	+	+	+	+	+
chi-cal -alg -gly	+	++	++	+	++	+++
Ager well – 10 mm	*Organisms*					
10 mm ~ 14 mm (+)	(a) <i>Bacillus subtilis</i>			(N.C.T.C-8236)		
15 mm ~ 19 mm (++)	(b) <i>Staphylococcus aureus</i>			(N.C.P.C-6371)		
20 mm above (+++)	(c) <i>Pseudomonas aeruginosa</i>			(6749)		
	(d) <i>Bacillus pumilus</i>			(N.C.I.B-8982)		
	(e) <i>Candida albicans</i>					
	(f) <i>E. coli</i>			(N.C.I.B-8134)		
Chi = Chitosan, Cal = Calcium, Alg = Alginate, Gly = Glycerine						

### On the Aspect of Biodegradation

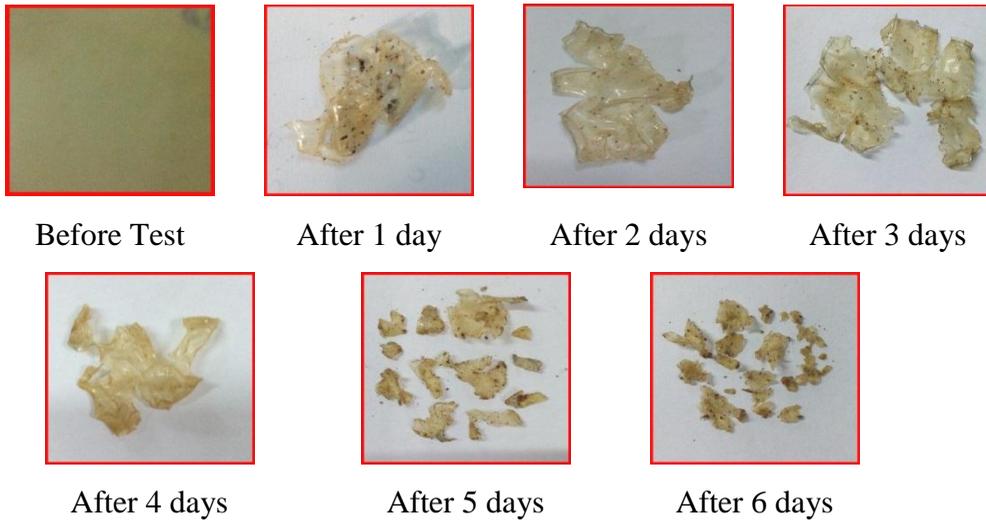
In this work, biodegradation of the prepared chitosan membranes, unmodified and modified chitosan composite membranes were tested by soil burial test. The environmental effects mentioned in this work are moisture and soil which may be favorable conditions for microbial growth. Soil burial test is a traditional way to test samples for degradation because of its similarity to actual condition of waste disposal. Uniformly sized samples were buried in the sandy soil for waste disposal. The biodegradation nature of prepared pure chitosan membranes and chitosan-calcium alginate composite membranes buried in sandy soil for 8 days. The most significant deformation of prepared chitosan-calcium alginate-glycerine composite membranes buried in sandy soil for 6 days. The resulting data are shown in Figures 7, 8 and 9.



**Figure 7:** Physical appearance changes of pure chitosan membrane buried in sandy soil for 8 days



**Figure 8:** Physical appearance changes of chitosan-calcium-alginate composite membrane buried in sandy soil for 8 days



**Figure 9:** Physical appearance changes of modified chitosan-calcium-alginate-glycerine composite-1 membranes buried in sandy soil for 6 days

### Conclusion

In this research, polymer blended membranes consisting of chitosan, sodium alginate, calcium chloride and glycerine were prepared. The various types of pure chitosan membrane and modified chitosan-calcium-alginate composite membranes were prepared by blending, casting and by solvent evaporating technique under autoclaving conditions of 121°C and 0.1 MPa in a time frame of 1 h.

According to the physicomechanical properties such as tensile strength, elongation at break (%) and tear strength, the optimum condition was achieved by using 2 % (w/v) chitosan, 0.1 % (w/v) sodium alginate and 0.2 % (w/v) calcium chloride solution. All prepared chitosan-calcium-alginate composite membranes showed plain, clear, smooth surface, flexible, highly transparent and light yellow colour. According to mechanical properties, chitosan-calcium alginate-glycerine composite-1 membrane was chosen as optimum condition from the other composite membranes for the desired products.

The water uptake and swelling percent of chitosan-calcium alginate-glycerine composite-3 membrane has the highest among the other composite membranes by varying the weight percent of glycerine in the membrane matrix.

From the results of FT IR analysis, the functional groups of modified chitosan composite membranes could be confirmed. FT IR analysis showed that there exist intermolecular interactions between chitosan, calcium alginate and glycerine. According to SEM micrographs, similar pattern of pores are distributed on the surface of modified chitosan composite membranes. The chitosan-calcium-alginate composite membranes showed the sponge like nature and cluster form. This indicates that the membrane has good sorption properties. The surface morphology of modified chitosan-calcium-alginate-glycerine composite membranes showed more homogeneous nature.

According to soil burial test, the significant deformation of modified chitosan-calcium-alginate-glycerine composite membrane was found after 6 days and then chitosan-calcium-alginate composite membrane and chitosan membrane were disappeared after 8 days. However, quite deformation of chitosan membrane was found after two weeks.

The properties of various types of chitosan membrane, chitosan-calcium-alginate composite membranes and modified chitosan-calcium-alginate-glycerine composite membranes were tested on its antimicrobial activities. The pure chitosan membrane and modified chitosan-calcium-alginate-glycerine composite membrane showed most significant on six microorganisms using agar well diffusion method. The modified chitosan-calcium-alginate-glycerine composite-3 membrane is more suitable to use as food and vegetable packaging material due to the high swelling percent and water uptake of this membrane and composite-1 membrane is more suitable to use as wound healing and wound dressing due to the high tensile strength and elongation at break of this membrane. Moreover, it is safe to assume that there were always be a demand for application in medical field.

## Acknowledgements

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