

CHARACTERIZATION OF MODIFIED CHITOSAN-ALGINATE-STARCH-GLYCEROL/ SORBITOL COMPOSITE MEMBRANES AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

The chitosan-alginate-starch composite membranes (CAS 1- 4), modified chitosan-alginate-starch-glycerol composite membranes (CASG 1- 4) and modified chitosan-alginate-starch-sorbitol composite membranes (CASS 1- 4) were prepared by using casting and autoclaving methods. These membranes have smooth surfaces, highly transparent and pale yellow colour. The mechanical properties such as tensile strength, elongation at break and tear strength of these prepared membranes were determined. Based on the mechanical properties of the prepared composite membranes: CAS-3, CASG-2 and CASS-2 composite membranes were chosen for further studies. The selected composite membranes were characterized by FT IR and SEM analyses. SEM micrographs of composite membranes showed their significant nature of surface morphologies. The antimicrobial activities of these membranes were tested by agar well diffusion method as to apply these membranes for biomedical applications.

Keywords: composite membranes, mechanical properties, antimicrobial activities

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 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 *et al.*, 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

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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 processed 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. In the present study, modified chitosan-alginate-starch cross-linked composite membranes were prepared to be used for medical purposes.

Materials and Methods

Sample Collection

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

Starch was prepared from maize grain, *Zea mays* L. and this sample was procured from Insein Market, Yangon Region.

Preparation of Chitosan-Alginate-Starch (CAS) Composite Membranes

Chitosan-alginate-starch (CAS) composite membrane was prepared by following procedure. Firstly, chitosan 1.5 % (w/v) was dissolved in 1 % (v/v) acetic acid solution and stirred for 1 h to obtain the clear homogeneous chitosan solution. Secondly, sodium alginate 3 % (w/v) and starch solution 0.3 % (w/v) were prepared individually and then they were mixed and stirred for 30 min. Finally, chitosan solution was added to these mixture solution and 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 the respective composite membranes. By using various concentrations of starch [0.1 %, 0.2 %, 0.3 % and 0.4 % (w/v)], chitosan-alginate-starch (CAS) composite membranes were prepared and denoted as CAS-1, CAS-2, CAS-3 and CAS-4, respectively.

Preparation of Chitosan-Alginate-Starch-Glycerol (CASG) Composite Membranes

Modified chitosan-alginate-starch-glycerol (CASG) composite membranes were prepared by using optimum amounts of 1.5 % (w/v) chitosan, 3 % (w/v) sodium alginate, 0.3 % (w/v) starch solution and 0.10 % (w/v) glycerol. The glycerol was also used as plasticizer for flexibility of membranes. The resulting modified composite solutions were autoclaved at a pressure of 0.1 MPa and 121 ± 1 °C for 1 h. The CASG composite membranes were prepared by using various percentages of glycerol 0.05 %, 0.10 %, 0.15 % and 0.20 % and the prepared membranes were denoted as CASG-1, CASG-2, CASG-3 and CASG-4, respectively.

Preparation of Chitosan-Alginate-Starch-Sorbitol (CASS) Composite Membranes

Modified chitosan-alginate-starch-sorbitol (CASS) composite membranes were prepared by using optimum amounts of 1.5 % (w/v) chitosan, 3 % (w/v) sodium alginate, 0.3 % (w/v) starch solution and 0.10 % (w/v) sorbitol. The sorbitol was also used as plasticizer for flexibility of membranes. The resulting modified composite solutions were autoclaved at a pressure of

0.1 MPa and 121 ± 1 °C for 1 h. The CASS composite membranes were prepared by using various percentages of sorbitol 0.05 %, 0.10 %, 0.15 % and 0.20 % and the prepared membranes were denoted as CASS-1, CASS-2, CASS-3 and CASS-4, respectively.

Mechanical Properties

Determination of thickness

Thickness of the prepared CAS, CASG and CASS composite membranes was measured by using NSK Micrometer. The thickness of the membranes was measured at 5 points (center and 4 corners) using digital micrometer.

Determination of tensile strength and elongation at break

The prepared CAS, CASG and CASS composite membranes were cut off according to JIS K 7127 (1987) and the shape and dimension of test pieces were obtained. The both ends of test pieces were firmly clamped in the jaw of testing machine. One jaw was fixed and the other was moveable. The rate of moveable jaw was hold 100 mm/min. The resulting data was shown at the recorder. This procedure for tensile strength was repeated for three times. The resulting data are presented in Tables 1, 2, 3 and Figures 1, 2, 3.

Determination of tear strength

The specimen was cut off by using die-cutting. Specimen was cut with a single nick (0.05 mm) at the entire of the inner concave edge by a special cutting device using a razor blade. The clamping of the specimen in the jaw of test machine was aligned with travel direction of the grip in 100 mm/min. The order of the machine showed the highest force to tear from a specimen nicked. The procedure was repeated three times for each result. The resulting data are described in Tables 1, 2, 3 and Figures 1, 2, 3.

FT IR analysis

The selected composite membranes: CAS-3, CASG-2 and CASS-2 were analyzed by using Shimadzu, IR Prestige-21, (Japan) FT IR spectrophotometer at the Department of Chemistry, University of Yangon. The FT IR spectrometer was calibrated by blank scanning between 1000 and 4000 cm^{-1} with resolution of 1 cm^{-1} and 3 scans/ sample. The membranes were directly mounted in the light path of the spectrometer for scanning. The FT IR spectral assignment data of prepared membranes are given in Table 4 and Figure 4.

SEM analysis

The selected composite membranes CAS-3, CASG-2 and CASS-2 were characterized by scanning electron microscope, (JEOL Ltd., Japan). The sample was attached to double-sided carbon tap on the same holder and coated with 200 nm platinum with a sputter coater JFC- 1600 auto fine coater. An accelerated voltage of 20 kV and secondary electron was used, then the micrograph was taken with a JEOL Super Mini Cup Ex-54143 MSK. SEM micrographs are shown in Figures 5.

Screening of Antimicrobial Activities of the Selected Composite Membranes by Agar Well Diffusion Method

The selected composite membranes : CAS-3, CASG-2 and CASS-2 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 5 and Figure 6.

Results and Discussion

Aspect of Film Preparation

In the present work, the modified chitosan membranes were prepared by solution-casting from solution of chitosan in dilute acetic acid, sodium alginate solution and starch solution in various concentrations. In this process, chitosan was used as the base polymer. Chitosan as such do not have enough mechanical strength required for a wound dressing material, however, it has antimicrobial property as well as wound healing capacity. To give better tensile strength to the end product, sodium alginate and starch were added and to give flexibility to the composite glycerol and sorbitol were added. Both chitosan and sodium alginate have wound healing ability but their abilities were decreased when these two were mixed. So, starch solution was used to upgrade the healing ability. The gel forms by chemical reaction when the sodium alginate was dissolving in water. The special property of alginates is the ability to form films of sodium alginate. The colour of the chitosan-sodium alginate-starch film varied slightly to yellow with increasing chitosan content.

Mechanical Properties

The mechanical properties such as tensile strength, tear strength and elongation at break are important parameters for showing the nature of membranes. The mechanical properties of CAS 1-4, CASG 1-4 and CASS 1-4 composite membranes are shown in Tables 1, 2, 3 and Figures 1, 2, 3. The more the tensile strength of membrane, the higher is the elasticity of the membrane. This means to point out that CAS-3, CASG-2 and CASS-2 composite membranes are more flexible and more elastic than the others.

Table 1 Mechanical Properties of the Chitosan-Alginate-Starch (CAS) Composite Membranes Containing Various Concentrations of Starch

Membrane	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kNm ⁻¹)
CAS- 1	12.50	19.00	68.00
CAS- 2	18.30	10.00	21.00
CAS- 3	19.50	38.00	32.90
CAS- 4	9.80	25.00	44.30

CAS- 1= Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.1 %) w/v

CAS- 2= Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.2 %) w/v

CAS- 3= Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) w/v

CAS- 4= Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.4 %) w/v

Thickness = ~ 0.10 mm

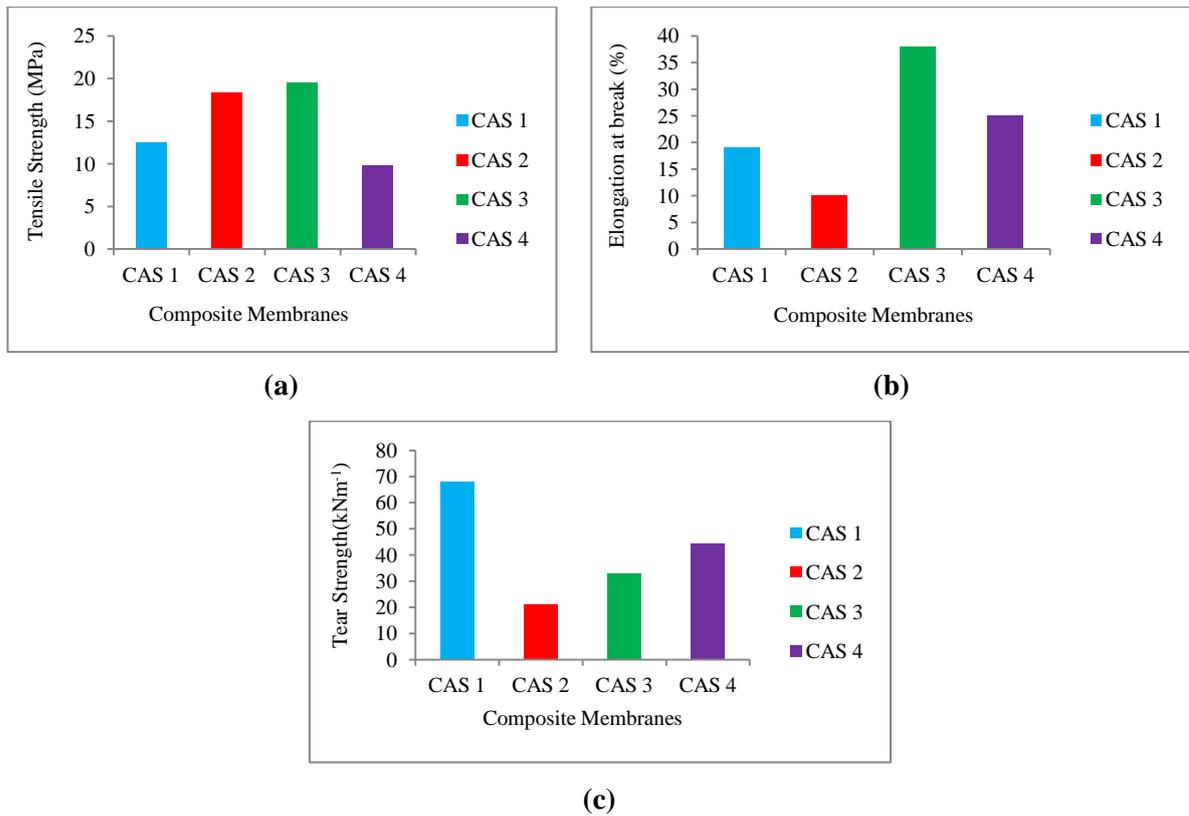


Figure 1 Mechanical properties of the chitosan-alginate-starch (CAS) composite membranes (a) tensile strength (b) elongation at break and (c) tear strength

Table 2 Mechanical Properties of the Chitosan-Alginate-Starch-Glycerol (CASG) Composite Membranes Containing Various Concentrations of Glycerol

Membrane	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kNm ⁻¹)
CASG- 1	16.00	13.00	15.70
CASG- 2	20.50	18.00	42.50
CASG- 3	13.20	15.00	20.00
CASG- 4	9.20	32.00	14.40

CASG- 1 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.05 %) w/v
 CASG- 2 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.10 %) w/v
 CASG- 3 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.15 %) w/v
 CASG- 4 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.20 %) w/v
 Thickness = ~ 0.10 mm

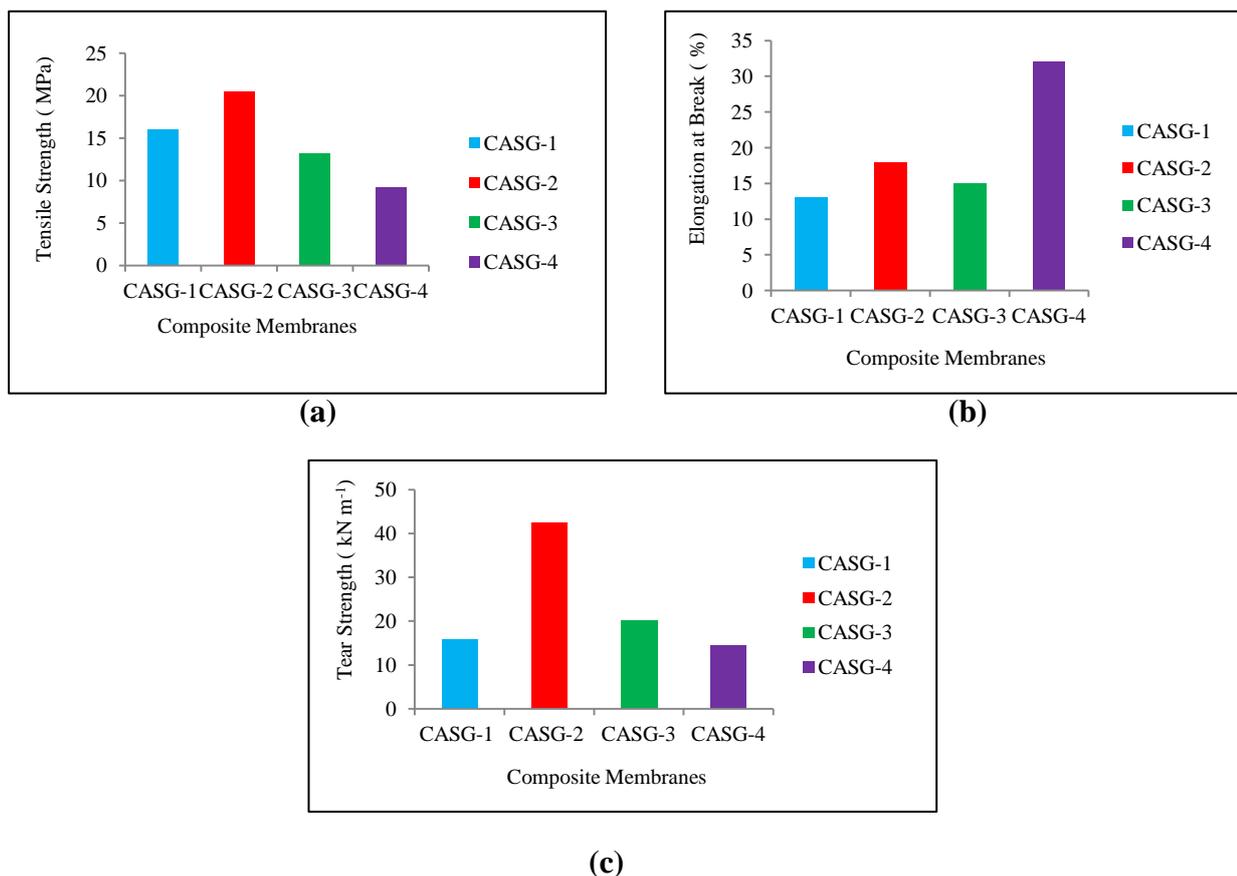


Figure 2 Mechanical properties of the chitosan-alginate-starch-glycerol (CASG) composite membranes (a) tensile strength (b) elongation at break and (c) tear strength

Table 3 Mechanical Properties of the Chitosan-Alginate-Starch-Sorbitol (CASS) Composite Membranes Containing Various Concentrations of Sorbitol

Membrane	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kNm ⁻¹)
CASS- 1	18.80	18.00	9.00
CASS- 2	30.00	17.00	16.00
CASS- 3	10.80	16.00	17.00
CASS- 4	26.30	25.00	7.00

CASS- 1 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) + Sorbitol (0.05 %) w/v

CASS- 2 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) + Sorbitol (0.10 %) w/v

CASS- 3 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) + Sorbitol (0.15 %) w/v

CASS- 4 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) + Sorbitol (0.20 %) w/v

Thickness = ~ 0.10 mm

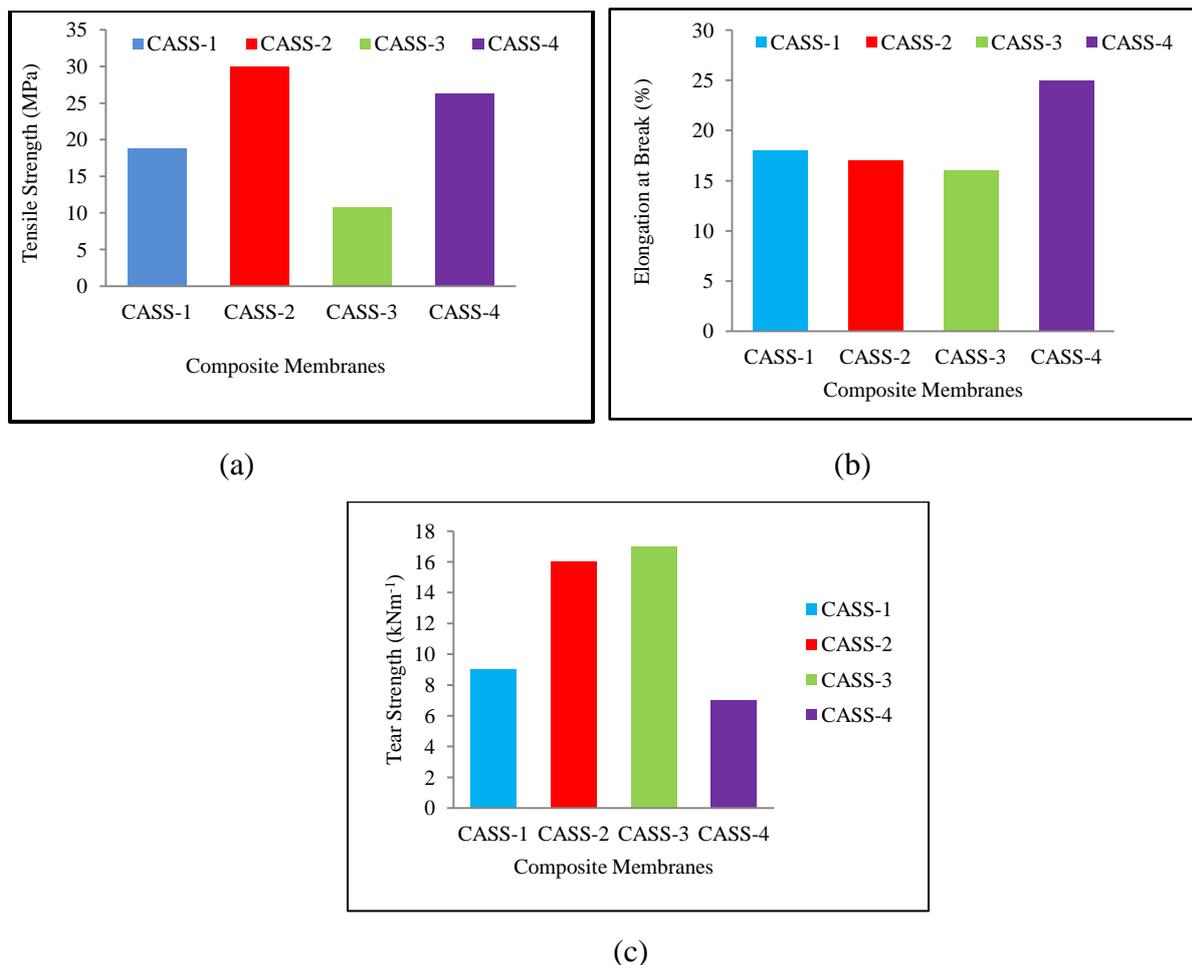


Figure 3 Mechanical properties of the chitosan-alginate-starch-sorbitol (CASS) composite membranes (a) tensile strength (b) elongation at break and (c) tear strength

Characterization of the Selected Composite Membranes

FT IR analysis

FT IR spectroscopic studies allowed to analyze the characteristic bands corresponding to the vibrations of the hydroxyl, carbonyl, amide, amine and ester groups. The FT IR spectra of selected composite membranes: CAS-3, CASG-2 and CASS-2 are shown in Figure 4 and Table 4. The broad bands at 3264, 3291, 3307 cm^{-1} in spectra of all samples are attributed to the OH stretching, which overlap with N-H stretching in the same region. The peaks at 2921, 2923 cm^{-1} are due to the C-H stretching vibration. The strong peaks at 1641, 1632, 1640 cm^{-1} are due to the O-H bending vibration. The sharp peaks at 1544, 1555, 1553 cm^{-1} range in spectra represent N-H bending in secondary amide. The sharp peaks at near 1408, 1322, 1413 cm^{-1} correspond to vibrations of COO- stretching groups. The sharp peaks at 1019, 1028, 1087 cm^{-1} range in spectra represent the C-O-C stretching vibration of primary alcohol and cyclic ether (Muzzarelli and Peter, 1973; Silverstein and Webster, 1998).

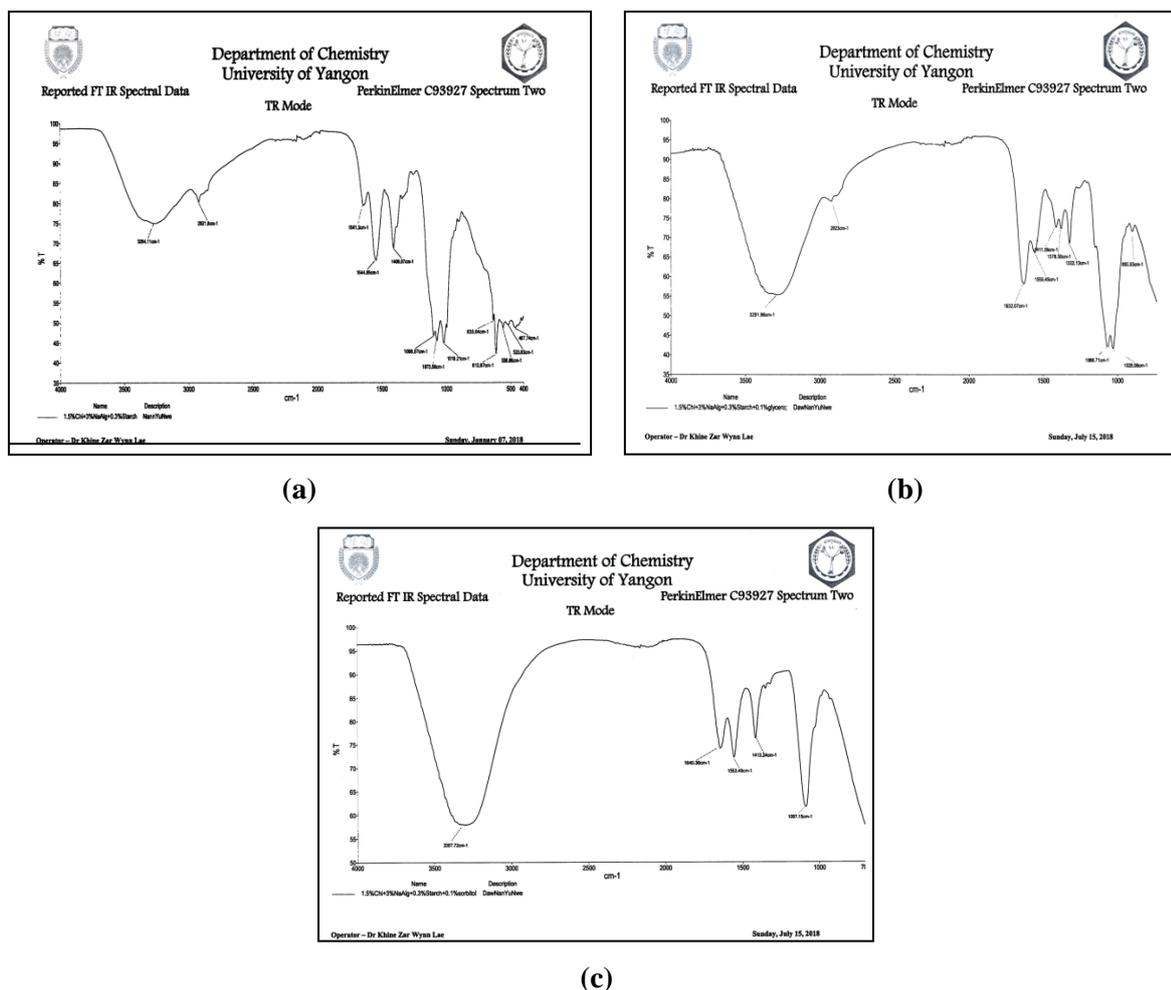


Figure 4 FT IR spectra of the selected composite membranes (a) CAS-3 (b) CASG-2 and (c) CASS-2

Table 4 FT IR Band Assignments of the Selected Composite Membranes: (CAS-3, CASG-2 and CASS-2)

Observed wave number (cm ⁻¹)			* Literature wave number (cm ⁻¹)	Remark
CAS-3	CASG-2	CASS-2		
3264	3291	3307	3450-3225	O-H stretching and N-H stretching vibration
2921	2923	-	2940-2820	C-H stretching vibration
1641	1632	1640	1870-1650	O-H bending vibration
1544	1555	1553	1625-1430	N-H bending in secondary amide
1408	1322	1413	1425-1390	COO- stretching vibration
1019	1028	1087	1045-1015	C-O-C stretching vibration of CH ₂ OH, cyclic ether

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

(b) SEM analysis

The SEM images revealed that the surface morphologies of membranes vary with the ratios of constituents in the selected composite membranes. SEM micrographs of CAS-3, CASG-2 and CASS-2 composite membranes are shown in Figure 5. The SEM micrographs indicated that CAS-3 composite membranes showed more sponge like nature and cluster form which enhances sorption property of membrane and CASG-2 composite membrane showed surface nature uniformly pores or voids and homogeneous nature and CASS-2 composite membrane showed scabrous and patchy surface morphologies.

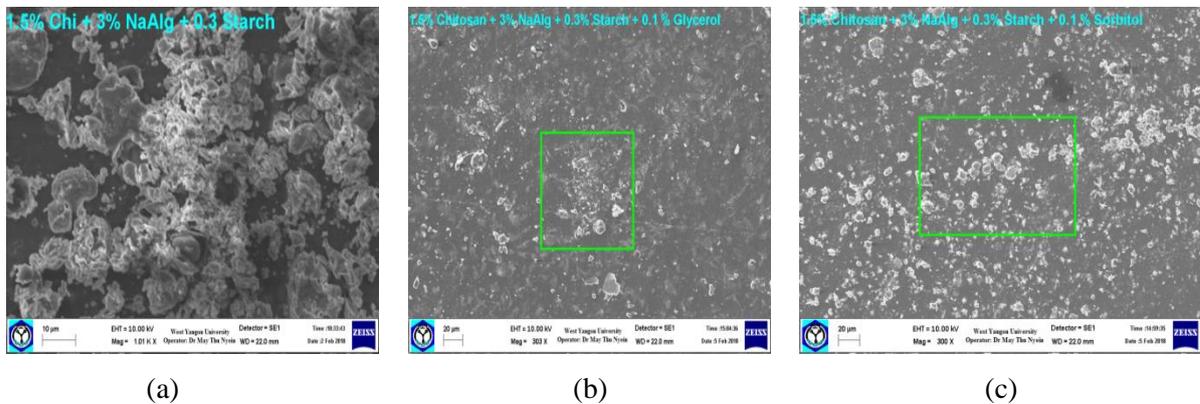


Figure 5 SEM micrographs of the selected composite membranes: (a) CAS-3 (b) CASG-2 and (c) CASS-2

Antimicrobial Activity of the Selected Composite Membranes by Agar Well Diffusion Method

Antimicrobial activities of the selected composite membranes: CAS-3, CASG-2 and CASS-2 were studied. These selected 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 selected membranes was determined by agar well diffusion method. Antimicrobial activities of the all selected membranes were evaluated based on the diameters of clear inhibition zone surrounding the agar well. The selected CAS-3, CASG-2 and CASS-2 composite membranes showed medium antimicrobial activities in the range of inhibition zone diameters (13~19 mm). Among them, the modified CASG-2 composite membrane showed that highest activity against six microorganisms (14~19 mm). The resulting data are shown in Table 5 and Figure 6.

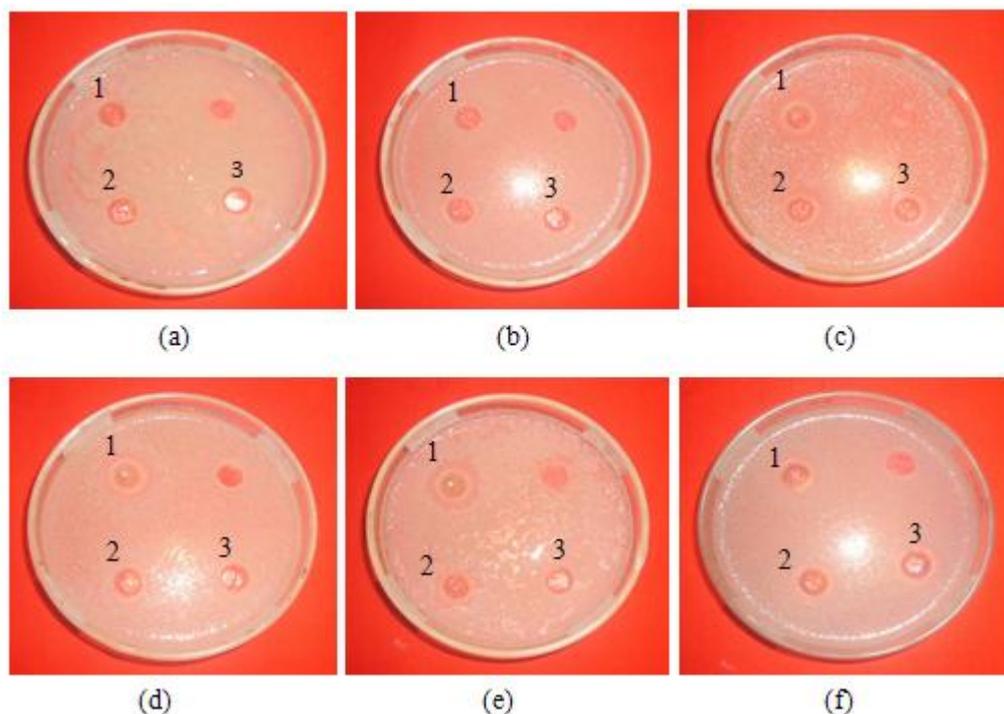


Figure 6 Antimicrobial activities of the selected (1) CASG-2 (2) CAS-3 and (3) CASS-2 composite membranes

- (a) *Bacillus subtilis* (d) *Bacillus pumilus*
 (b) *Staphylococcus aureus* (e) *Candida albicans*
 (c) *Pseudomonas aeruginosa* (f) *E. coli*

Table 5 Antimicrobial Activities of the Selected Composite Membranes: CASG-2, CAS-3 and CASS-2

Membranes	Inhibition zone diameters (mm) of the samples against different organisms					
	(a)	(b)	(c)	(d)	(e)	(f)
CASG-2	14(+)	15(++)	17(++)	18(++)	19(++)	16(++)
CAS-3	13(+)	14(+)	17(++)	16(++)	18(++)	16(++)
CASS-2	13(+)	14(+)	16(++)	14(+)	14(+)	15(++)
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)			

Conclusion

In this study, polymer blended membranes consisting of chitosan, sodium alginate, starch and glycerol/ sorbitol solution were prepared. The various types of chitosan-alginate-starch composite membranes and modified chitosan-alginate-starch-glycerol/ sorbitol composite membranes were prepared by blending, casting and solvent evaporating technique under

autoclaving conditions of 121 °C and 0.1 MPa in a time frame of 1 h. These membranes showed clear, smooth surface, flexible, highly transparent and light yellow colour.

Based on the mechanical properties such as tensile strength, elongation at break (%) and tear strength, the optimum condition was achieved by using 1.5 % (w/v) chitosan, 3.0 % (w/v) sodium alginate, 0.3 % (w/v) starch solution, 0.1 % (w/v) glycerol/ sorbitol. According to mechanical properties, CAS-3, CASG-2, CASS-2 composite membranes were selected to be used for biomedical applications.

FT IR analysis, indicated that the functional groups were present of selected CAS-3, CASG-2, CASS-2 composite membranes. SEM micrographs revealed the similar pattern of pores distribution on the surface of these membranes. CAS-3 composite membranes showed more sponge like nature and cluster form which enhances sorption property of membrane and CASG-2 composite membrane showed surface nature uniformly pores and homogeneous nature and CASS-2 composite membrane showed scabrous and patchy surface.

The antimicrobial activities of selected CAS-3, CASG-2, and CASS-2 composite membranes were tested by agar well diffusion method. The selected CAS-3, CASG-2 and CASS-2 composite membranes showed antimicrobial activities. Especially, CASG-2 composite membrane showed the highest activity on six microorganisms.

According to these results, the modified CASG-2 composite membrane is more suitable to be used as burn wound healing due to its mechanical properties and antimicrobial activity.

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