

CHAPTER 4

Results and Discussion

4.1. Degree of Crosslinking of Chitosan Films

The degree of crosslinking of the chitosan films was determined by the imine and crosslinker concentration. The crosslinker was the non polar $=\text{CH}-(\text{CH}_2)_3-\text{CH}=\text{}$ link between chitosan chains of crosslinked chitosan films. The thickness of crosslinked chitosan films was calculated from the f value as shown in equation 2.5, or measured with a micrometer. The f value was obtained by the subtraction of the IR spectrum of uncrosslinked chitosan film on the computer. The thickness of the crosslinked chitosan films were shown in Table 4.1.

Table 4.1 The factor and thickness of the crosslinked chitosan films.

Glutaraldehyde Conc. (%w/w)	Crosslinking Time (min.)	Factor	Thickness (cm.)
0.02	20	1.04169	0.00529
0.02	40	0.83359	0.00424
0.04	20	0.98634	0.00501
0.04	40	0.74113	0.00377
0.06	20	0.96270	0.00489
0.06	40	1.00000	0.00508

The absorbance of imine groups was measured in each crosslinking condition: 0.02%, 0.04%, 0.06% w/w glutaraldehyde concentration for 20 and 40 minutes of

crosslinking time by subtraction of the IR spectrum of uncrosslinked chitosan film. The IR spectrum of uncrosslinked chitosan film was shown in Figure 4.1. The spectral subtractions of crosslinked chitosan films were shown in Figure. 4.2-4.7. The imine concentration and crosslinker concentration could be calculated from equation 2.6 and 2.7 sequentially as shown in Table 4.2.

Table 4.2 Determination the imine and crosslinker concentration of the crosslinked chitosan films.

Glutaraldehyde conc. (%w/w)	Crosslinking Time (min.)	Thickness (cm.)	Absorbance at 1665 cm ⁻¹	[C=N] (equiv/L)	[crosslinker] (equiv/L)
0	0	0.00508	0	0	0
0.02	20	0.00529	0.40411	1.55848	0.77924
0.02	40	0.00424	0.84798	4.08636	2.04318
0.04	20	0.00501	0.56937	2.31904	1.15952
0.04	40	0.00377	0.79929	4.33255	2.166275
0.06	20	0.00489	0.68641	2.8641	1.43205
0.06	40	0.00508	1.9129	7.6848	3.8424

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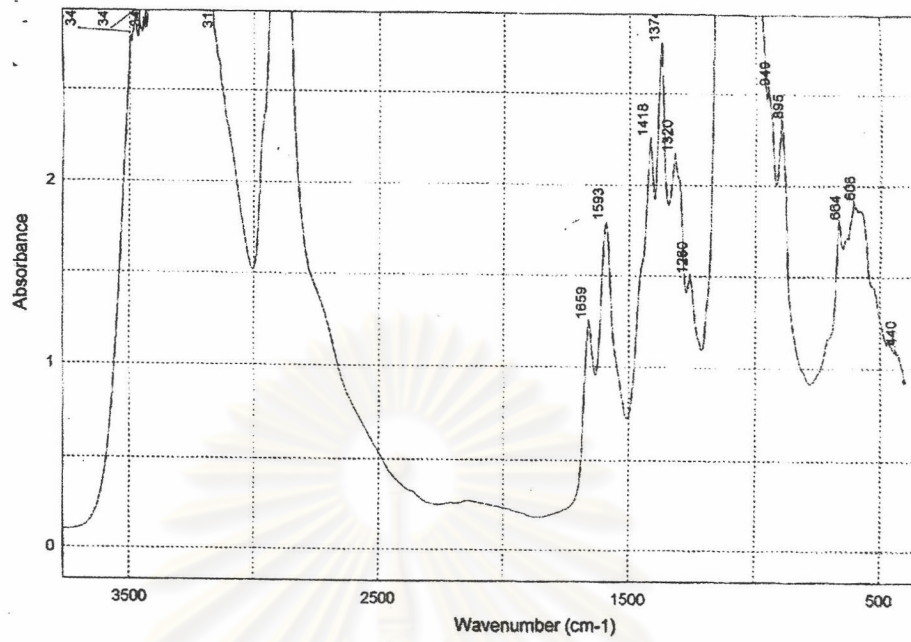


Figure 4.1 The IR spectrum of uncrosslinked chitosan film

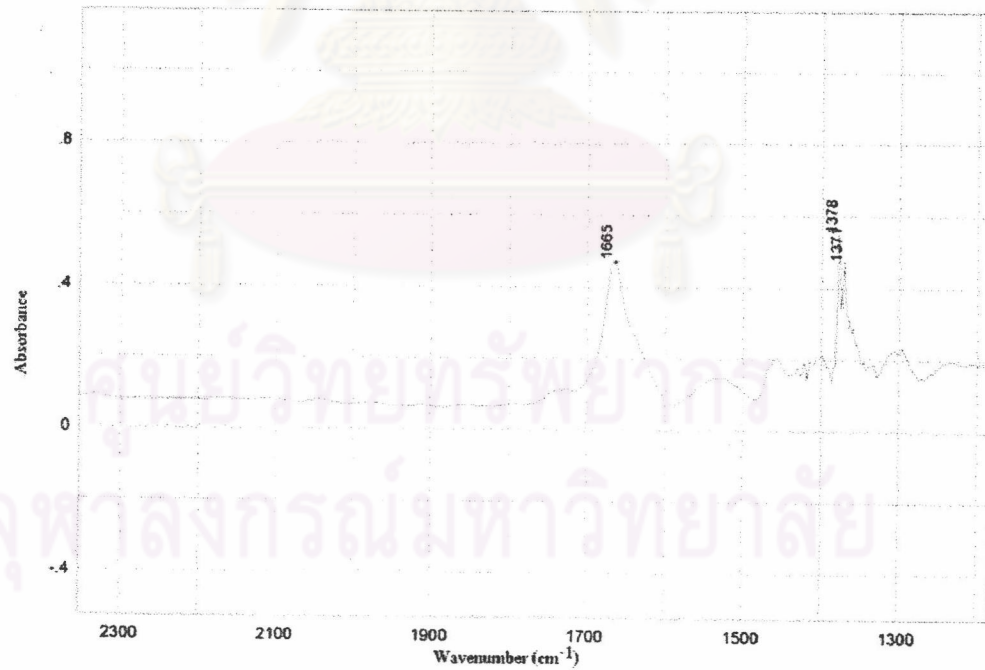


Figure 4.2 The spectral subtraction of 0.02% crosslinked chitosan film, 20 minutes by IR spectrum of uncrosslinked chitosan film.

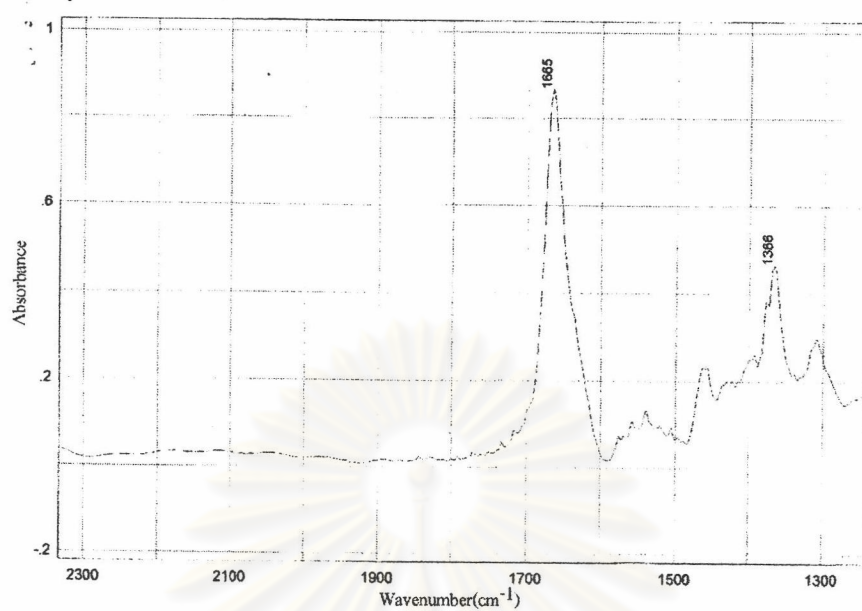


Figure 4.3 The spectral subtraction of 0.02% crosslinked chitosan film, 40 minutes by IR spectrum of uncrosslinked chitosan film.

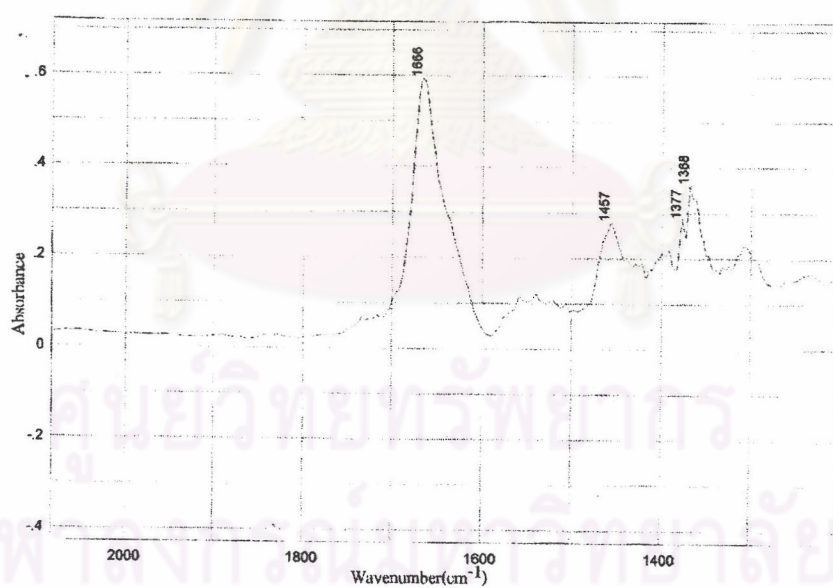


Figure 4.4 The spectral subtraction of 0.04% crosslinked chitosan film, 20 minutes by IR spectrum of uncrosslinked chitosan film

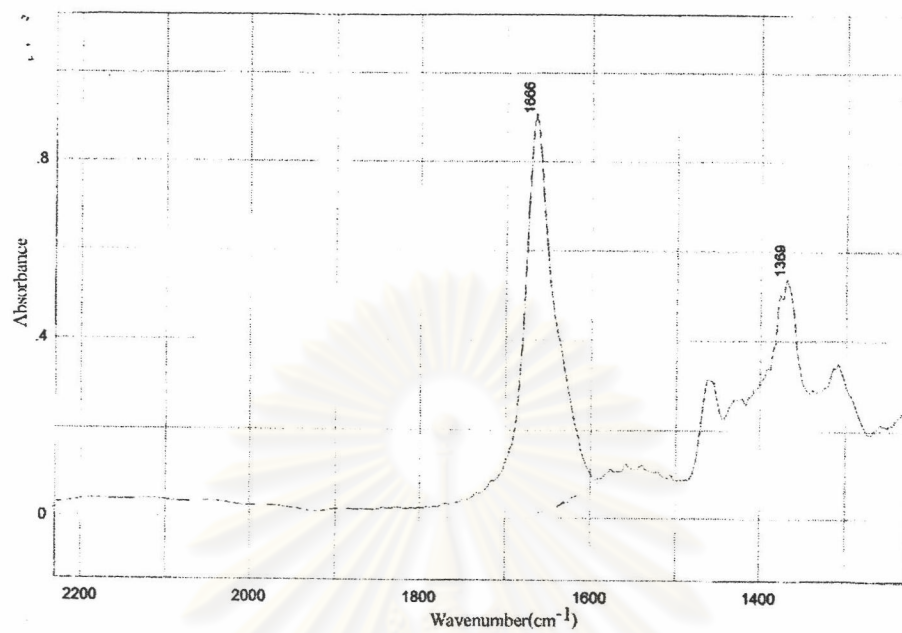


Figure 4.5 The spectral subtraction of 0.04% crosslinked chitosan film, 40 minutes by IR spectrum of uncrosslinked chitosan film

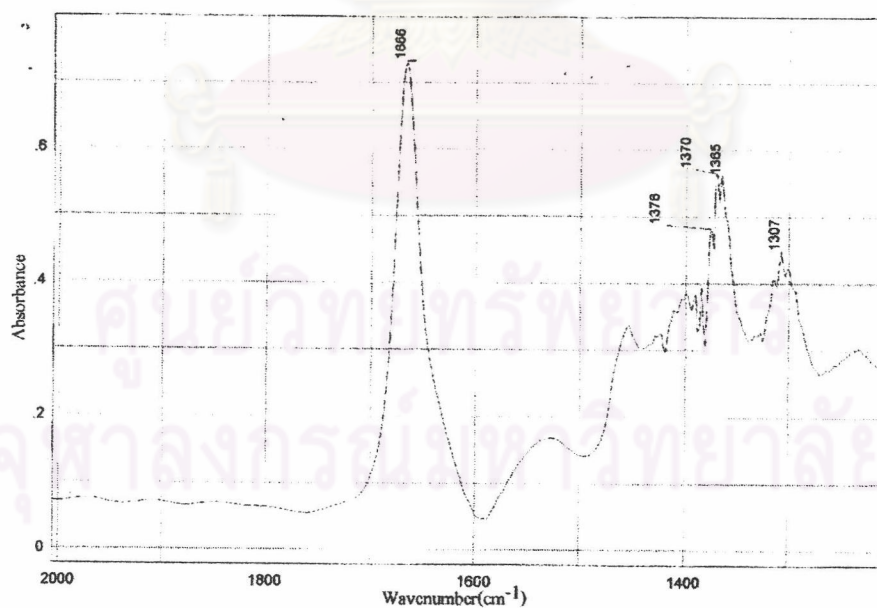


Figure 4.6 The spectral subtraction of 0.06% crosslinked chitosan film, 20 minutes by IR spectrum of uncrosslinked chitosan film

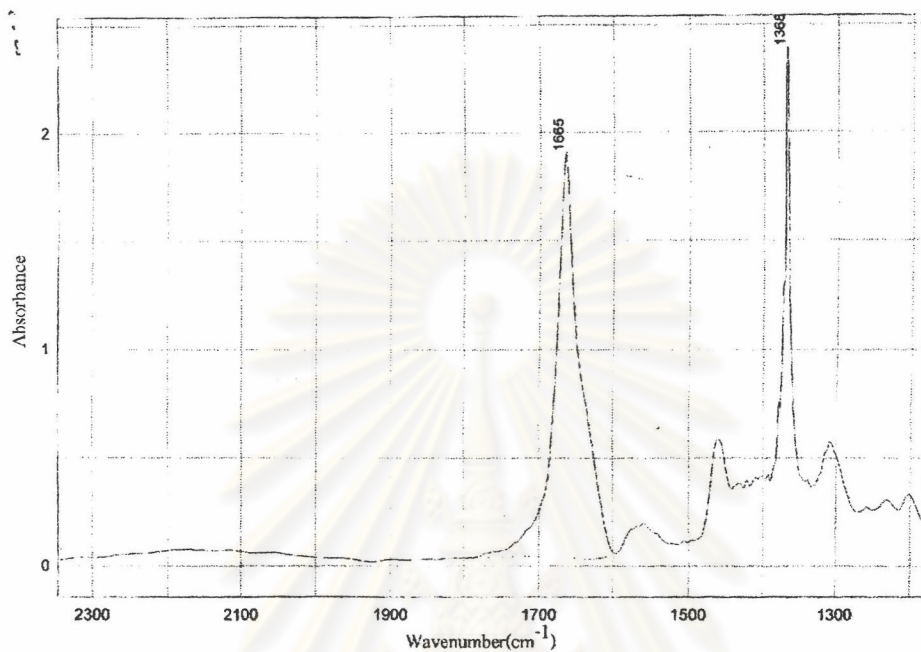


Figure 4.7 The spectral subtraction of 0.06% crosslinked chitosan film, 40 minutes by IR spectrum of uncrosslinked chitosan film

In Figure 4.8 and 4.9, An increase of glutaraldehyde concentration and crosslinking time resulted in higher imine and crosslinker concentrations. An increase of hydrophobic content might result in more hydrophobic interaction between non-polar solvent and membrane. This interaction might increase the permeation rate and affinity for non-polar solvent of membranes.

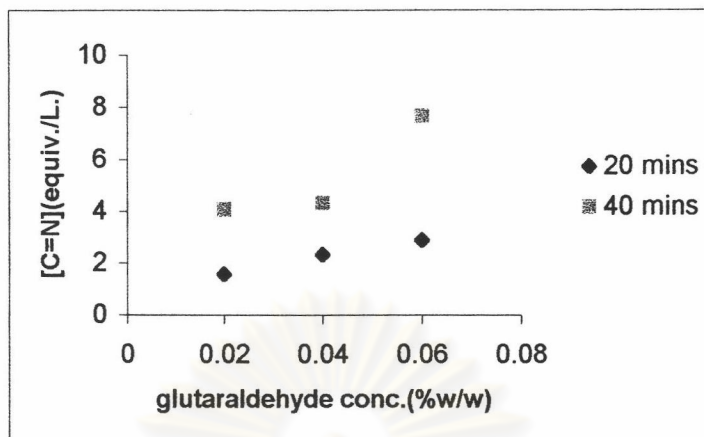


Figure 4.8 Effects of glutaraldehyde concentration and crosslinking time on the imine concentration (2% w/w chitosan solution)

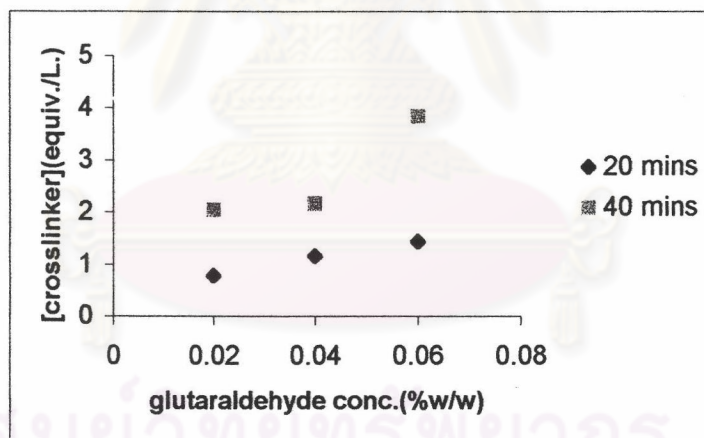


Figure 4.9 Effects of glutaraldehyde concentration and crosslinking time on the crosslinker concentration (2%w/w chitosan solution)

The second method for the measurement of the film thickness was by the micrometer and a second series of membrane was analyzed on this measurement. The results were shown in Table 4.3. The trends were similar to the first calculated by factor shown in Table 4.2.

Table 4.3 Determination the imine and crosslinker concentration of the crosslinked chitosan films

Glutaraldehyde conc. (%w/w)	Crosslinking Time (min.)	Thickness (cm.)	Absorbance at 1665 cm ⁻¹	[C=N] (equiv/L)	[crosslinker] (equiv/L)
0	0	0.0057	0	0	0
0.02	20	0.0059	0.42608	1.4738	0.7369
0.04	20	0.0058	0.48182	1.6950	0.8475
0.06	20	0.0057	0.61341	2.1962	1.09812

4.2. Swelling in Water.

The swelling in water could determine the degree of crosslinking in chitosan films. The percentage of swelling in water of crosslinked chitosan films with 0.02%, 0.04%, 0.06% w/w glutaraldehyde concentration, 20 minutes of crosslinking time was compared to uncrosslinked chitosan film as shown in Table 4.4.

Table 4.4 The percentage of swelling in water of the uncrosslinked and crosslinked chitosan films.

% Glutaraldehyde Conc. (%w/w)	Dry Weight (g.)	Wet Weight (g.)	% Swelling in Water
0	0.0970	0.1896	95.46
0.02	0.0598	0.1121	87.45
0.04	0.0640	0.1151	79.84
0.06	0.0332	0.0590	77.71

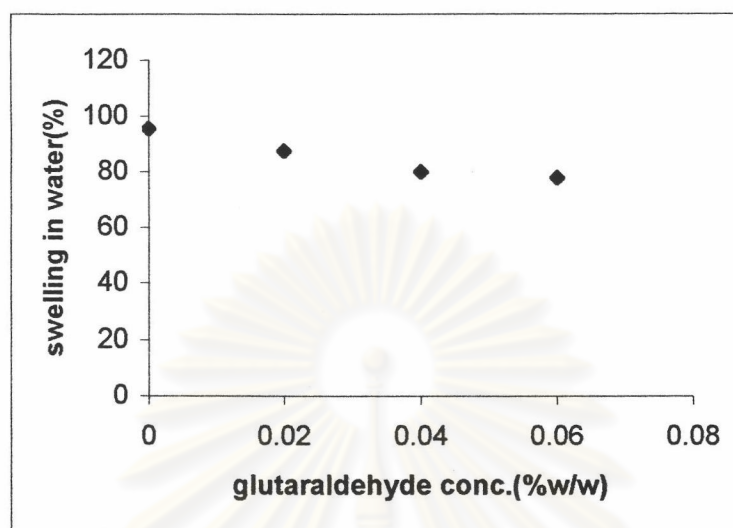


Figure 4.10 Effect of glutaraldehyde concentration on the percentage of swelling in water of crosslinked chitosan film

From Figure 4.10, the percentage of swelling in water decreased with increasing glutaraldehyde concentration. This result might be concluded that an increase of glutaraldehyde concentration resulted in the more hydrophobic in films. The more hydrophobic part was $=\text{CH}-(\text{CH}_2)_3-\text{CH}=\text{}$ links between chitosan chains that meant the degree of crosslinking increased.

4.3 Effect of Glutaraldehyde Concentration on the Tensile Strength of Films

The chitosan films were crosslinked by 0.05%, 0.1%, 0.2%(w/w) glutaraldehyde solution using crosslinking time of 40 and 80 minutes, the wet films became too brittle. This result was assumed that the films became so brittle because the glutaraldehyde concentration was too much, or the crosslinking time was too long. The crosslinking conditions were changed to 0.02%, 0.04%, 0.06% (w/w) glutaraldehyde solution for 20, 40 minutes of crosslinking time. At 40 minutes of crosslinking time, the dry films were still brittle, so it was too hard to measure the tensile strength. The crosslinking time might

be too long that resulted in the brittle films. At 20 minutes of crosslinking time for 0.02%, 0.04%, 0.06%(w/w) glutaraldehyde solution, the crosslinked chitosan films showed the tensile strength as shown in Table 4.5 and Figure 4.11 compared to uncrosslinked chitosan film.

Table 4.5 The tensile strength of chitosan films at various glutaraldehyde concentration and 20 minutes of crosslinking time

Glutaraldehyde conc. (%w/w)	Thickness (mm.)	Elongation (mm.)	% Elongation	Max. Load (N)	Load at Break (N)	Load at Yield (N)
0	0.058	7.44	7.63	10.28	10.27	1.03
0.01	0.057	1.82	20.3	8.26	7.66	0.32
0.02	0.057	1.96	20.1	9.16	9.16	0.7
0.04	0.056	2.16	22.04	8.67	8.48	0.89
0.06	0.057	1.89	18.99	8.91	8.89	0.79

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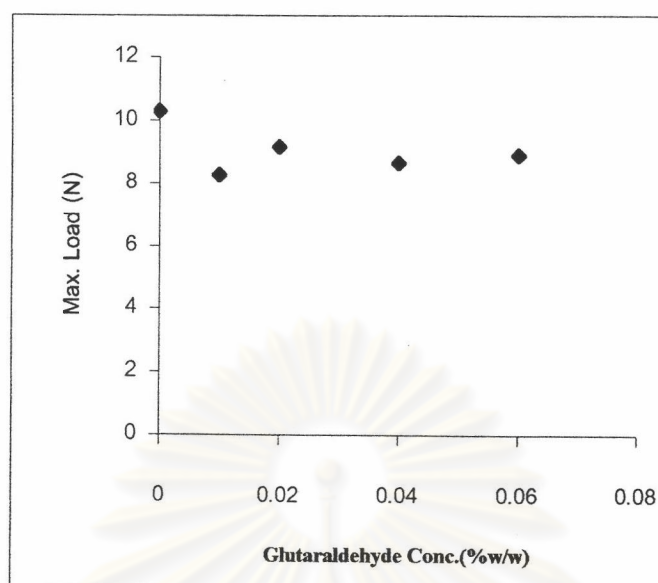


Figure 4.11 Effect of glutaraldehyde concentration on the tensile strength

In Figure 4.11, the tensile strength of the crosslinked chitosan films decreased with increasing glutaraldehyde concentration. The crosslinked chitosan with 0.02%w/w glutaraldehyde concentration showed the highest tensile strength. The crosslinking occurred under heterogeneous condition, so crosslinking reaction occurred only within the amorphous region (Tual and Espouche, 2000). The cause of weaker crosslinked chitosan than uncrosslinked chitosan films, it might be caused by the high chitosan concentration that made the casting solutions produce high degree of crystallinity and small amorphous region. Since the polymer molecules were parallel to each other in an orderly manner, crystalline regions were set up; the polymer were held together by hydrogen bonds and Van der Waals force to form a more dense network (Yang and Zall, 1957).

4.4 Solvent Resistance of Uncrosslinked and Crosslinked Chitosan Films.

From the results of 4.3, the crosslinked chitosan films with 0.02%w/w glutaraldehyde concentration and 20 minutes of crosslinking time that showed the highest

tensile strength were determined the solvent resistance by the tensile measurement compared to uncrosslinked chitosan films as shown in Table 4.6 and 4.7.

Table 4.6 The tensile strength of the uncrosslinked chitosan films after soaked films in various solvents

Solvent Type	Thickness (mm.)	Elongation (mm.)	% Elongation	Max. Load (N)	Load at Break (N)	Load at Yield (N)
Blank	0.057	6.8	68.28	10.95	10.91	0.27
Ethyl acetate	0.057	3.57	35.7	13.46	13.31	0.47
MIBK	0.057	3.4	34.03	10.36	10.27	0.51
Hexane	0.057	4.73	46.87	10.12	10.04	0.44
Ethanol	0.057	3.47	35.03	6.27	6.26	0.46

Table 4.7 The tensile strength of the crosslinked chitosan films after soaked films in various solvents

Solvent Type	Thickness (mm.)	Elongation (mm.)	% Elongation	Max. Load (N)	Load at Break (N)	Load at Yield (N)
Blank	0.057	1.47	14.97	10.22	10.03	0.81
Ethyl acetate	0.057	1.73	17.43	14.88	14.13	0.48
MIBK	0.057	1.8	18.23	13.77	13.74	0.6
Hexane	0.057	4.03	40.43	11.69	11.69	1.09
Ethanol	0.057	1.37	13.63	6.16	5.92	0.32

Table 4.8 The physical properties of solvents (Smallwood, 1993)

Solvent Type	Molecular Weight (g/mole)	Hilbrand Solubility Parameter (δ) (cal/cm^3) ^{1/2}	Dielectric Constant (ϵ) at 20°C
Hexane	86	7.3	1.9
Ethanol	46	13.4	24.3
MIBK	100	8.4	2.8
EtOAc	88	9.1	6.02

Table 4.9 Difference ($\Delta\delta$) in solubility parameters of chitosan with various solvents ($\delta_{\text{chitosan}} = 10.16$, Appendix)

Solvent Type	$\Delta\delta$ ($\delta_s - \delta_{\text{chitosan}}$)
Ethanol	3.2
MIBK	-1.7
Ethylacetate	-1.1
Hexane	-2.9

As the results were shown in Table 4.6,4.7 and Figure 4.12, 4.13. After soaked films in each solvents, the tensile strength decreased in order: EtOAc > MIBK > hexane > EtOH in case of both uncrosslinked and crosslinked chitosan films. After uncrosslinked chitosan films were soaked in EtOAc, the tensile strength was higher. This might be due to the dielectric constant ($\epsilon = 6.02$) that showed the polarity of ethyl acetate. As the chitosan's structure was composed of hydroxyl group and primary amine group, therefore

the polar interaction occurred between chitosan and solvent molecules that resulted in the higher tensile strength.

In case of ethanol, the film- solvent polar interaction was too severe because the dielectric constant of ethanol was very high ($\epsilon = 24.3$). This result might be caused by the solvation effect that resulted in the rigidity of chitosan molecule as well as cellulose acetate in a highly polar solvent said by Kamide and Saito (1992)

In case of MIBK and hexane, these solvents are non polar or hydrophobic solvents because of low the dielectric constant. The tensile strength of chitosan films after soaked films in MIBK was higher than in hexane because the dielectric constant of MIBK was higher than hexane. The tensile strength of uncrosslinked chitosan films after soaked films in both solvents was lower because of the small $\Delta\delta$, therefore the swelling of chitosan films increased that affected more space for solvent molecules between chitosan chains. The more swelling of chitosan films resulted in a decrease of the tensile strength.

These results might be concluded that the tensile strength of chitosan films depended on the dielectric constant of solvent and the different of solubility parameter between chitosan and solvent.

The crosslinked chitosan films with 0.02% w/w glutaraldehyde solution and 20 minutes of crosslinking time showed the higher tensile strength than uncrosslinked chitosan films after soaked films in various solvents. This result was clearly shown in case of MIBK and hexane due to the hydrophobic of crosslinked chitosan caused by more insertion of non polar $=\text{CH}-(\text{CH}_2)_3-\text{CH}=\text{}$ links between chitosan chains. The hydrophobic of crosslinked chitosan films resulted in the hydrophobic interaction between non-polar solvent and hydrophobic link of films, therefore the tensile strength was higher than uncrosslinked chitosan films. In case of EtOAc, the solvent was both polar and non-polar therefore, the hydrophobic interaction resulted in the higher tensile strength. In case of ethanol, the crosslinked chitosan films became weaker after soaked in ethanol.

In case of uncrosslinked compared to crosslinked CTS films after soaked in each solvents, it showed the same ordering tensile strength in each solvents. This might be due to a little bit increase of hydrophobic of crosslinked chitosan films.

Since the crosslinked chitosan film was the selective layer of composite membrane; therefore, the result corresponded to Musale and Kumar (2000b) mentioned that swelling of the crosslinked chitosan/polyacrylonitrile composite nanofiltration membrane in solvent. The swelling of membrane in non polar solvent was less than in polar solvent.

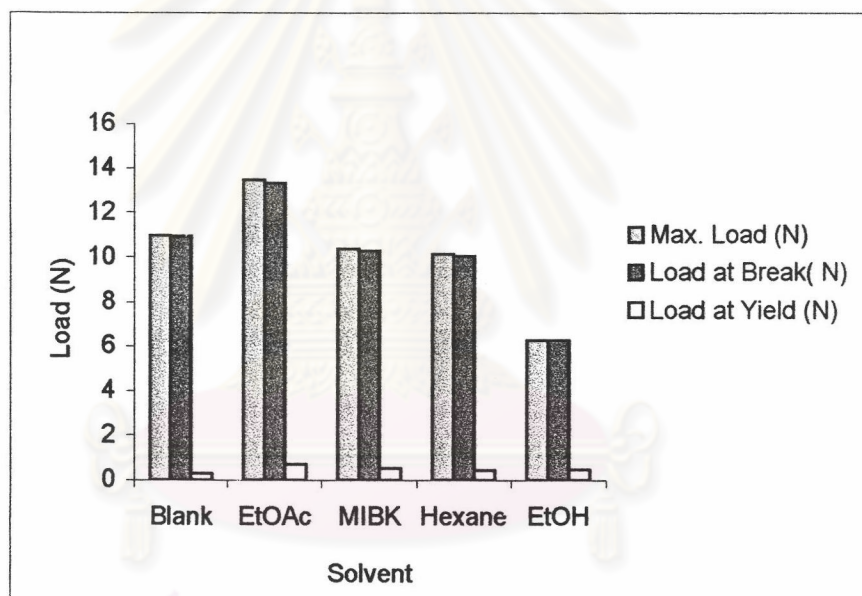


Figure 4.12 Solvent resistance of uncrosslinked chitosan films.

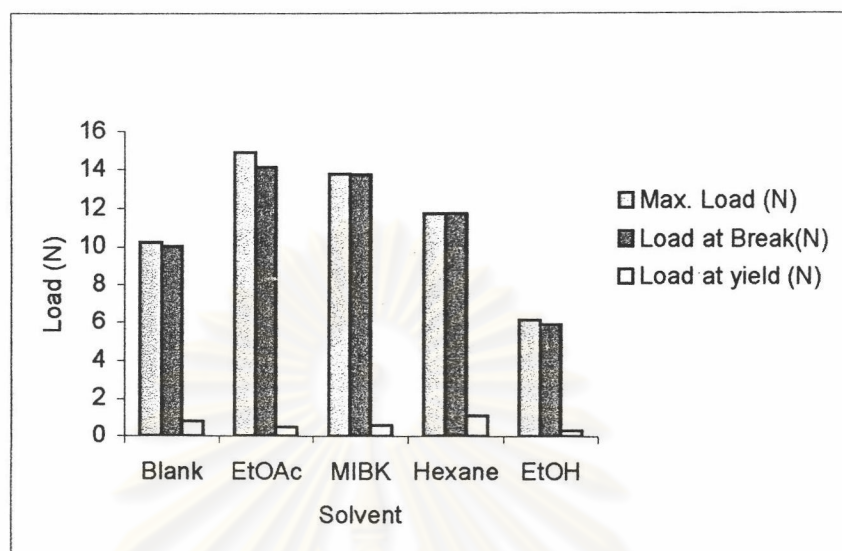


Figure 4.13 Solvent resistance of crosslinked chitosan films with 0.02% w/w glutaraldehyde solution, 20 minutes of crosslinking time.

4.5 Morphology of Membrane

Scanning electron micrographs of surfaces and cross-sections of 14% w/w polyacrylonitrile ultrafiltration membrane, polyacrylonitrile/chitosan membranes were shown in Figure 4.14-4.16. Figure 4.15 showed a finger-typed substructure that supported the membrane with dense skin at surface and large pore penetrating the entire membrane cross-section. The pores increased in diameter from the top to the bottom side. After coating with chitosan, the 14% PAN membrane cross-section from Figure 4.16 showed the denser surface. The results could be concluded that chitosan solution penetrated in the surface area. The change of pore size on the surface could not be concluded from the SEM micrographs. The pore size was too small to see the change of pore size by SEM micrographs.

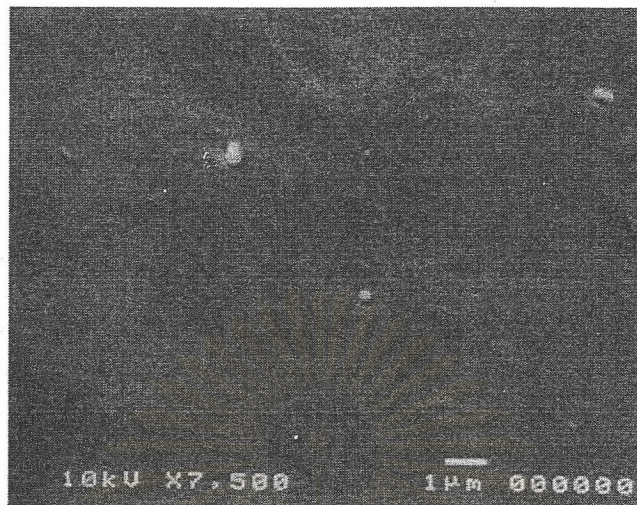


Figure 4.14 Scanning electron micrographs at the surface of 14% w/w PAN membrane

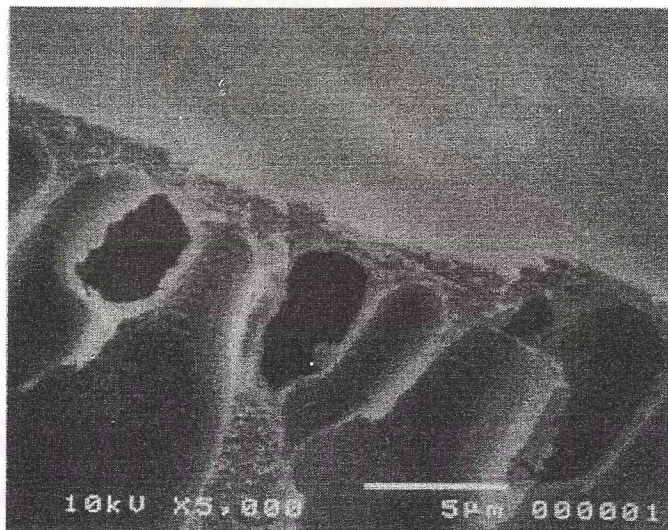


Figure 4.15 Scanning electron micrographs of cross-section of 14% w/w PAN membrane



Figure 4.16 Scanning electron micrographs of cross-section of 14% w/w PAN membrane after coating with chitosan solution (2% w/w)

4.6 Molecular Weight Cut Off (MWCO) of Membranes.

The percentage of separation of each membranes could be analyzed by TOC (Total Organic Carbon Analyzer). the MWCO , the solute molecular weight for which the membrane shows 90% rejection, 14% w/w PAN membranes showed MWCO about 600,000 Dalton shown in Figure 4.17. After coating the chitosan solution on 14% PAN membrane, the membrane showed more selectivity because the MWCO of membrane was about 90,000-100,000 Dalton. This result might be because the chitosan layer covered the pores at the surface, and the pores became smaller. After crosslinking, PAN/CTS composite membranes had no separation. The crosslinked chitosan layer might be weaker after crosslinked because the thickness of chitosan layer was too thin. However the molecular weight cut-off of all membranes was in the ultrafiltration range.

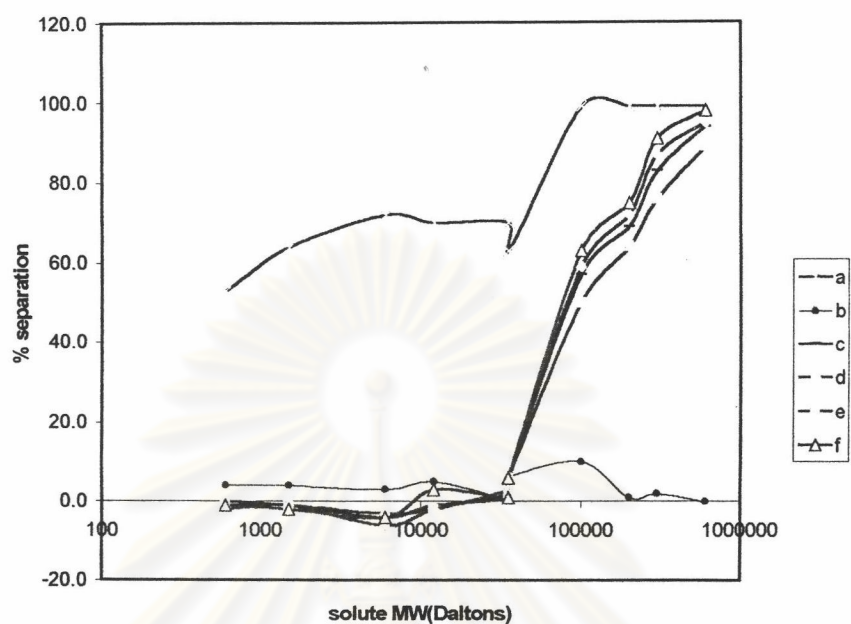


Figure 4.17 The separation of membranes; a: 14% PAN/CTS membrane; b: 14%w/w PAN/CTS membrane crosslinked with 0.02%w/w glutaraldehyde solution for 20 minutes of crosslinking time; c,d,e,f: 14%w/w PAN membrane

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