CHAPTER IV
RESULTS AND DISCUSSION

Preparation and Characterization of CM-Chitin/Natural Rubber Blends

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4.1 Abstract

Carboxymethyl chitin (CM-chitin), a water-soluble derivative of chitin, has several interesting biological activities, leading to its potential use in biomedical field, such as wound dressing application. However, CM-chitin film is rigid and possesses poor mechanical properties. In order to improve flexibility of CM-chitin film, it was blended with natural rubber latex obtained from Hevea brasiliensis since natural rubber has high flexibility and elasticity. In this present study, CM-chitin/deproteinized natural rubber blend was prepared before being fabricated into film. The effect of the blend compositions on the chemical structure, morphology, thermal stability and mechanical properties of the blend films was determined by FT-IR, scanning electron microscope (SEM), thermogravimetric analyser (TGA) and universal testing machine, respective. In addition to the blend compositions, the effects of crosslinking time with glutaraldehyde on the mechanical properties, degree of swelling and weight loss of crosslinked blend films were also investigated.

Keywords:

CM-chitin, Natural rubber, Blend film, Wound dressing, Biomaterial

4.2 Introduction

Blends of polymeric materials obtained from renewable resources are of great interest in the production of new green products. Chitin, or poly β-(1-4)-N-acetyl-D-glucosamine, is the second most abundant polysaccharide found in mature after cellulose. Chitin is a major structural component in the exoskeleton of arthropod and the cell walls of fungi and yeast (Jayakumar *et al*, 2010). However, the existence of high crystallinity of chitin limits its solubility in common solvents. Hence, the carboxymethyl chitin (CM-chitin), a water-soluble derivative of chitin, becomes much more interest. The introduction of carboxyl groups into the chitin structure interrupts both inter- and intra-hydrogen bonding, thus improving the solubility of the resulting CM-chitin. Recently, much attention has been paid to CM-chitin as a wound dressing material because it provides versatile properties, including retaining moist environment (Kumar *et al.*, 2010), enhancing wound healing properties (Jayakumar *et al*, 2011), and being biocompatible (Peesan *et al*, 2003). Since the CM-chitin-based materials are brittle, blending of CM-chitin with other flexible biomaterials is necessary in order to obtain the desirable mechanical properties as well as ease of handle. Natural rubber, cis-1,4-polyisoprene, is naturally produced by the Hevea brasiliensis trees. Due to the outstanding flexibility, natural rubber is one of the most appropriate materials for blending with CM-chitin. Nevertheless, the presence of proteins in the natural rubber latex can cause severe allergy in sensitive persons (Wagner *et al*, 1999). Thus, deproteinization step is an important process to ensure that natural rubber is safe to be used as health care products. In this present study, CM-chitin/deproteinized natural rubber blend was prepared before being fabricated into film. To evaluate the potential use of the CM-chitin/natural rubber blend film as a new polymeric biomaterial for wound dressing application, the effect of blend ratios on morphology, mechanical properties as well as thermal properties were investigated.

4.3 Experimental

4.3.1 Materials and Chemicals

Shells of Metapenaeus dobsoni shrimps were kindly provided by Surapon Foods Public Co., Ltd. (Thailand). Natural rubber latex was obtained from Hevea brasiliensis trees (Chantaburi province, Thailand). Anhydrous sodium hydroxide (NaOH) pellets (Labscan analytical science, analytical grade), 50 %(w/w) NaOH solution (Chemical enterprise, commercial grade), hydrochloric acid (HCl, Labscan analytical science, analytical grade), glacial acetic acid (CH_3COOH, J.T.Baker, analytical grade), acetone (Chemical enterprise, commercial grade), monochloroacetic acid ($\geq$97 % purity, Fluka, analytical grade), tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl, Scharlau chemie S.A. , molecular biology grade), modified Lowry protein assay kit (Thermo Scientific), 28.0-30.0 wt.% ammonium hydroxide (NH_3) solution (J.T. Baker, analytical grade), and toluene (Labscan analytical science, analytical grade) were used in this study.

4.3.2 Preparation of Chitin

To prepare chitin, shrimp shells were first dried under sunlight for a few days. Then, 1 kg of dried shrimp shells was immersed in 10 L of a 1 N HCl solution with occasional stirring at room temperature for 2 days. The acidic solution was changed daily. The demineralized shrimp shells ware subsequently neutralized by deionized water and dried at 60 °C for 48 hr. The demineralized shrimp shells were further deproteinized in a 4 % w/v NaOH solution at a ratio of NaOH (solution:shrimp shells of 10:1) with continuous stirring at 80 °C for 4 hr. The obtained chitin was filtrated, neutralized by distilled water, and dried at 60 °C for 24 hr.

4.3.3 Preparation of CM-Chitin

CM-chitin was synthesized via alkaline chitin solution route. Firstly, 20 g of chitin was added to a 42 % (w/w) NaOH solution. The mixture was kept under reduced pressure in a vacuum oven at room temperature for 30 min. Then, 640 g of crushed ice was added to the solution in order to get a 4 %(w/w) alkaline chitin solution

in a 14 %(w/w) NaOH solution. The solution was stirred for 30 min at low temperature in ice bath. After that, 388 g of a 25 %(w/w) monochloroacetic acid in a 14 %(w/w) NaOH solution was slowly added to the chitin mixture with stirring for 30 min. The mixture solution was settled at room temperature overnight and neutralized by glacial CH_3COOH. The mixture was subsequently dialyzed with running water for 2 days and with deionized water for 2 days. Then, the mixture was centrifuged at 12000 rpm for 10 min at room temperature to remove insoluble materials. The supernatant was added to acetone and centrifuged at 12000 rpm for 10 min at 10 °C to remove the precipitates. The obtained CM-chitin product was reprecipitated in ethanol. Finally, CM-chitin was removed from ethanol by centrifugation and dried at 60 °C for 24 hr.

4.3.4 Preparation of Deproteinized Natural Rubber Latex

The commercial natural rubber latex was centrifuged at 12000 rpm for 5 min at room temperature to remove proteinaceous materials in serum. Then, a 4 %(v/v) NH_4OH solution was added to the supernatant. To further remove the protein residues, a 0.04 %(w/v) protease was also added. The solution was shaken in a shaking incubator at 100 rpm for 48 hr at 37 °C. After that, the solution was centrifuged at 12000 rpm for 5 min to remove protease and the remaining proteins. The deproteinized natural rubber latex was resuspended and preserved in a 4 % (v/v) NH_4OH solution.

4.3.5 Preparation of CM-Chitin/Natural Rubber Blend Films

The CM-chitin/natural rubber blend films were prepared by solution-casting method. Firstly, a desired amount of CM-chitin was dissolved in a 4 %(v/v) NH_4OH solution to get a 2 %(w/v) CM-chitin solution. The obtained CM-chitin solution was mixed with deproteinized natural rubber latex at five different CM-chitin:natural rubber ratios (100:0, 90:10, 80:20, 70:30, and 60:40). These mixtures were casted on the Teflon mold at room temperature before drying at 35 °C.

4.3.6 Preparation of Plasticized CM-Chitin/Natural Rubber Blend Films

The plasticized CM-chitin/natural rubber blend films were prepared by starting with dissolve CM-chitin in 4 % w/w NH_4OH to get 2 %w/v CM-chitin solution. And CM-chitin solution was mixed with glycerol to get homogeneous with different

weight ratio. (CM-chitin/natural rubber/glycerol, 80/20/10, 80/20/20, 80/20/30). After that the mixture was blended with deproteinized natural rubber latex. Then these mixtures were casted on the Teflon mold at room temperature before drying at 35 °C.

4.3.7 Crosslinking of Blend Films

The blend films were crosslinked in a glutaraldehyde vapor system in a seal desiccator. A 10 ml of 25 % glutaraldehyde solution was added into a sealed desiccator. The blend films were placed on a holed shelf in the desiccator at room temperature and the desiccator was closed by the cover. The blend film was crosslinked in glutaraldehyde vapor with different time: 15 min, 30 min, 45 min, and 60 min.

4.3.8 Characterization and Analytical Methods

An Ultraviolet-Visible (UV-VIS) Spectrophotometer (Tecan, Infinite M200) was used to quantify the protein content of raw natural rubber latex and deproteinized natural rubber latex based on the modified Lowry method at a wavenumber of 750 nm.

The morphological structure of film specimens was examined under a scanning electron microscope (SEM, Hitachi, S-4800). The samples were put on the holder with an adhesive tape and coated with a thin layer of platinum. The SEM images were taken by using an acceleration voltage of 2 kV with a magnification of 3000 times.

The mechanical properties of the CM-chitin/natural rubber blend films were investigated by universal testing machine (Lloyd) using yield setup mode with preload of 0.1 N, a speed of 50 mm/min, and a gage length of 4 cm. The values of stress at maximum load and elongation at break were reported.

The thermogravimetric analyzer (TGA, Perkin Elmer, TGA7) was used to determine the thermal behavior of CM-chitin/natural rubber blend films in the temperature range of 30 °C to 750 °C at a heating rate 20 °C/min under nitrogen flow.

The degree of substitution of the CM-chitin was evaluated by way of an elemental analysis using a CHNS/O analyzer (Perkin Elmer PE2400 Series II) at combustion and reduction temperatures of 950 °C and 500 °C, respectively. Each sample

was analyzed in air (60 psi), using oxygen as a combustion gas (15 psi) and helium as a carrier gas (20 psi).

The viscosity was measured with an Ubbelode type viscometer at 25 °C. The ionic strength was adjusted by the dialysis of CM-chitin with NaCl solution.

4.4 Results and Discussion

4.4.1 Characterization of chitin

4.4.1.1 Degree of deacetylation of chitin

The degree of deacetylation (DD) of chitin which was prepared by deminerization and deproteinization of shrimp shells (Rinaudo et al., 2006) was estimated by formula from previous report (Sannan et al., 1977). DD value of chitin was estimated from IR spectra of chitin (figure 4.1). It was equal to 0.32. This value indicated that synthesized chitin consisted of 32% chitosan units and 68% chitin units.
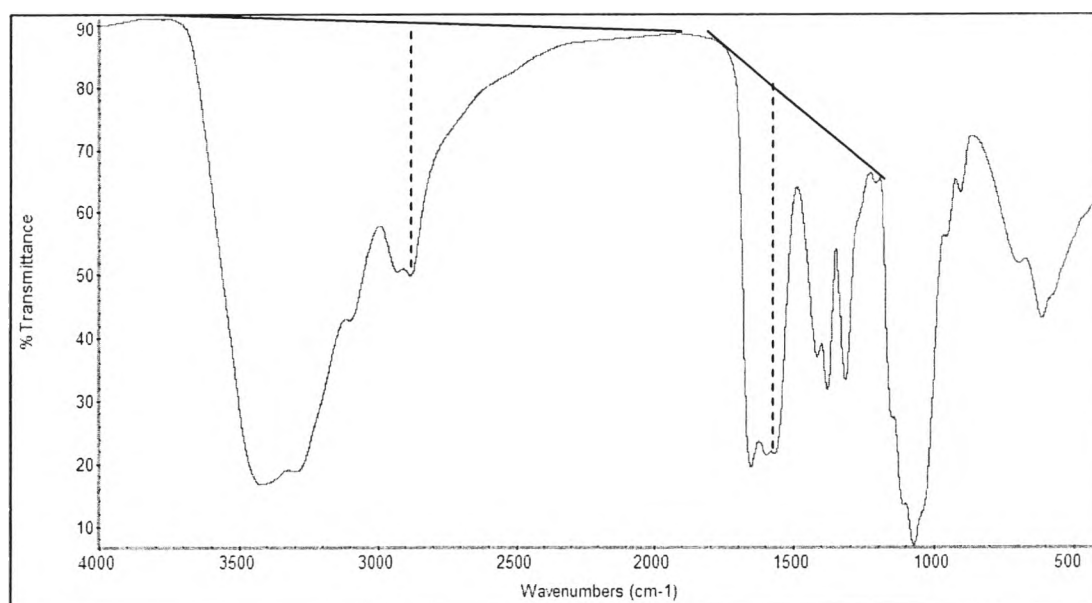

Figure 4.1 FT-IR spectrum of chitin.

4.4.1.2 Degree of substitution of CM-chitin

The degree of substitution (DS) of CM-chitin prepared by carboxymethylation of chitin (Hjerde, *et al.*, 1997) was estimated by formula from previous report (Huang, *et al.*, 2010). DS value of chitin was equal to 0.45.

4.4.1.3 Molecular weight of CM-chitin

Molecular weight of CM-chitin could be investigated from the measurement of viscosity average molecular weight (M_v) following the formula from previous report (Tokura, *et al.*, 1983), $[\eta]=KM^{\alpha}$ where K is7.92 x 10^{-5} and α is 1.00. The estimated M_v was found to be 66000.

4.4.1.4 FT-IR spectra of the CM-chitin film and chitin film
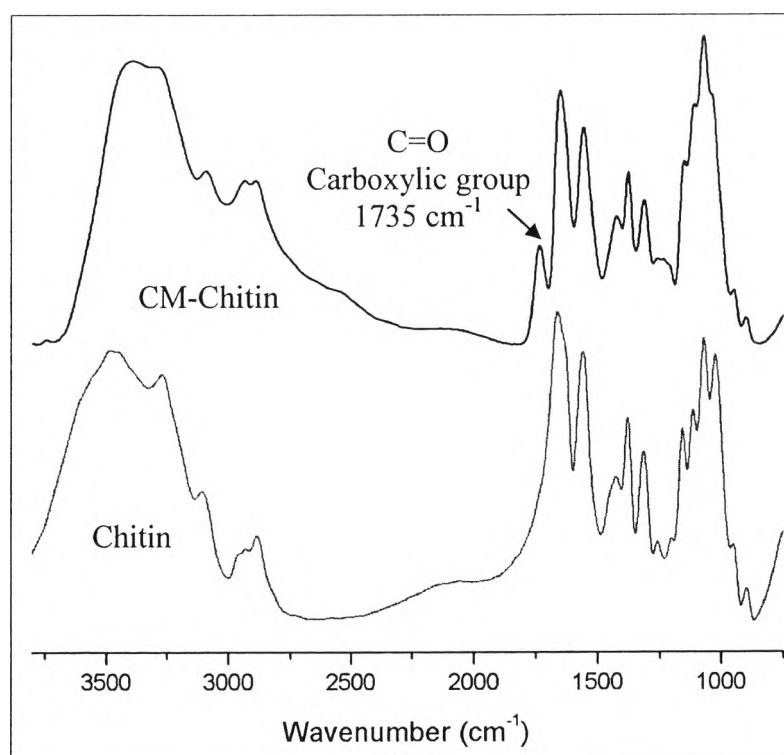
Figure 4.2 FT-IR spectra of CM-chitin film and chitin film.

The prepared CM-chitin from alkaline chitin was in Na-salt form which did not show the peak corresponded to carboxylic group of CM-chitin (Wongpanit, *et al.*, 2005). Therefore, synthesized CM-chitin was dropped by 0.05M HCl in order to change Na-form to be H-form. After that the characteristic peak at 1735 cm^{-1} assigned to C=O stretching of carboxylic group was observed which shown in figure 4.2.

4.4.1.5 Protein content analysis of natural rubber latex

The absorbance values of the deproteinized natural rubber latex and commercial natural rubber latex were compared with the standard curve of protein content from the modified Lowry method.

Table 4.1 The percentage of protein removal

Type of natural rubber	Protein Content (μg/ml)	Protein Removal (%w)
Untreated natural rubber	1315.582	0
Protease treated natural rubber	0.375	99.97

The result from table 4.1 showed that the protein content of the deproteinized natural rubber latex was equal to 0.375 ug/ml and the protein content of commercial natural rubber latex was equal to 1315.582 ug/ml. Then, the enzymatic treatment (Perella, *et al.*, 2002) can effectively remove protein from the natural rubber latex as a level of 99.97%.
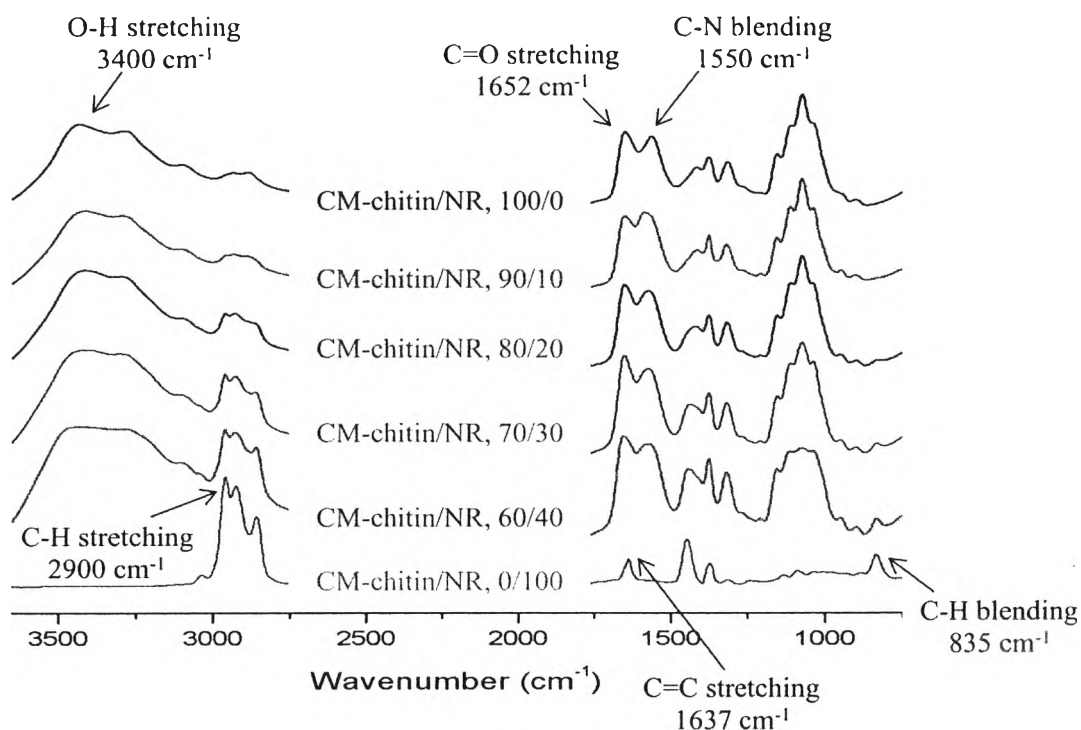
4.4.2 Characterizations of CM-chitin/natural rubber blend films

4.4.2.1 FT-IR spectra of the CM-chitin/natural rubber blend films

The FT-IR spectra of CM-chitin/natural rubber blend films in different ratios were shown in figure 4.3 The result showed that the characteristic peak at 2900 cm^{-1} assigned to C-H stretching of alkane groups, 1637 cm^{-1} assigned to C=C

stretching and 835 cm^{-1} assigned to O-H OOP blending were also detected in natural rubber spectrum which shown in figure 4.3 (CM-chitin/natural rubber: 0/100). Moreover, the characteristic peak at 3400 cm^{-1} assigned to O-H stretching, 1652 cm^{-1} assigned to C=O stretching, and 1550 cm^{-1} assigned to C-N blending of amide group in CM-chitin were detected in pure CM-chitin spectrum which shown in figure 4.3 (CM-chitin/natural rubber: 100/0). The FT-IR spectra of blend films of all composition exhibited the presence of both of CM-chitin and natural rubber. The peak intensity at 2900 cm^{-1} of alkene group increased and at 1652 cm^{-1} of amide group decreased when natural rubber content varied from 0 % to 40 %.


Figure 4.3 FT-IR spectra of CM-chitin/natural rubber blend films in different ratio: 100/0, 90/10, 80/20, 70/30, 60/40, and 0/100.

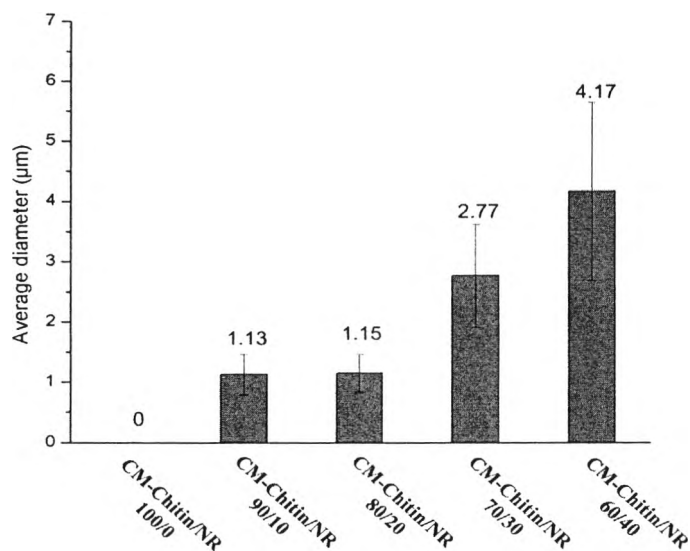*4.4.2.2 The morphology of the CM-chitin/natural rubber blend films*

The morphology of CM-chitin/natural rubber blend films at magnification of 3000x was shown in figure 4.4. The smooth surface was obtained from the pure CM-chitin and pure natural rubber, whereas, the rough surface was obtained the blend films at each compositions. This can be described that the incorporation of natural rubber into CM-chitin could resulted in the phase separation and the higher natural rubber content led to the higher phase separation. The morphology of CM-chitin/natural rubber blend films after natural rubber removal by toluene was shown in figure 4.5. According to a good solvent for natural rubber, toluene can leach some of rubber particles out then, it can produce some amount of voids on the surface of CM-chitin matrix. The SEM micrographs of all compositions at magnification of 3000x showed that the phase separation between rubber phase and CM-chitin matrix was observed in the blend films. This rubber agglomeration was due to the separation between polar material phase of CM-chitin and non-polar material phase of natural rubber (Carvalho, *et al.*, 2003). However, these voids indicated the good dispersion of rubber agglomeration on the CM-chitin matrix. In addition, the result showed that void sizes increased when natural rubber content increased. The average diameter of void sizes of CM-chitin/natural rubber blend films after natural rubber removal by toluene was shown in figure 4.6. CM-chitin/natural rubber at ratio of 90/10 exhibited the average diameter of void sizes equal to 1.13 μm which similar to the average diameter of void sizes of CM-chitin/natural rubber at the ratio of 80/20 at 1.15 μm. However, the addition of higher natural rubber content into CM-chitin at the ratio of 70/30 and 60/40 revealed the larger void sizes. The average diameter of void sizes increased significantly to 2.77 μm and 4.17 μm respectively.
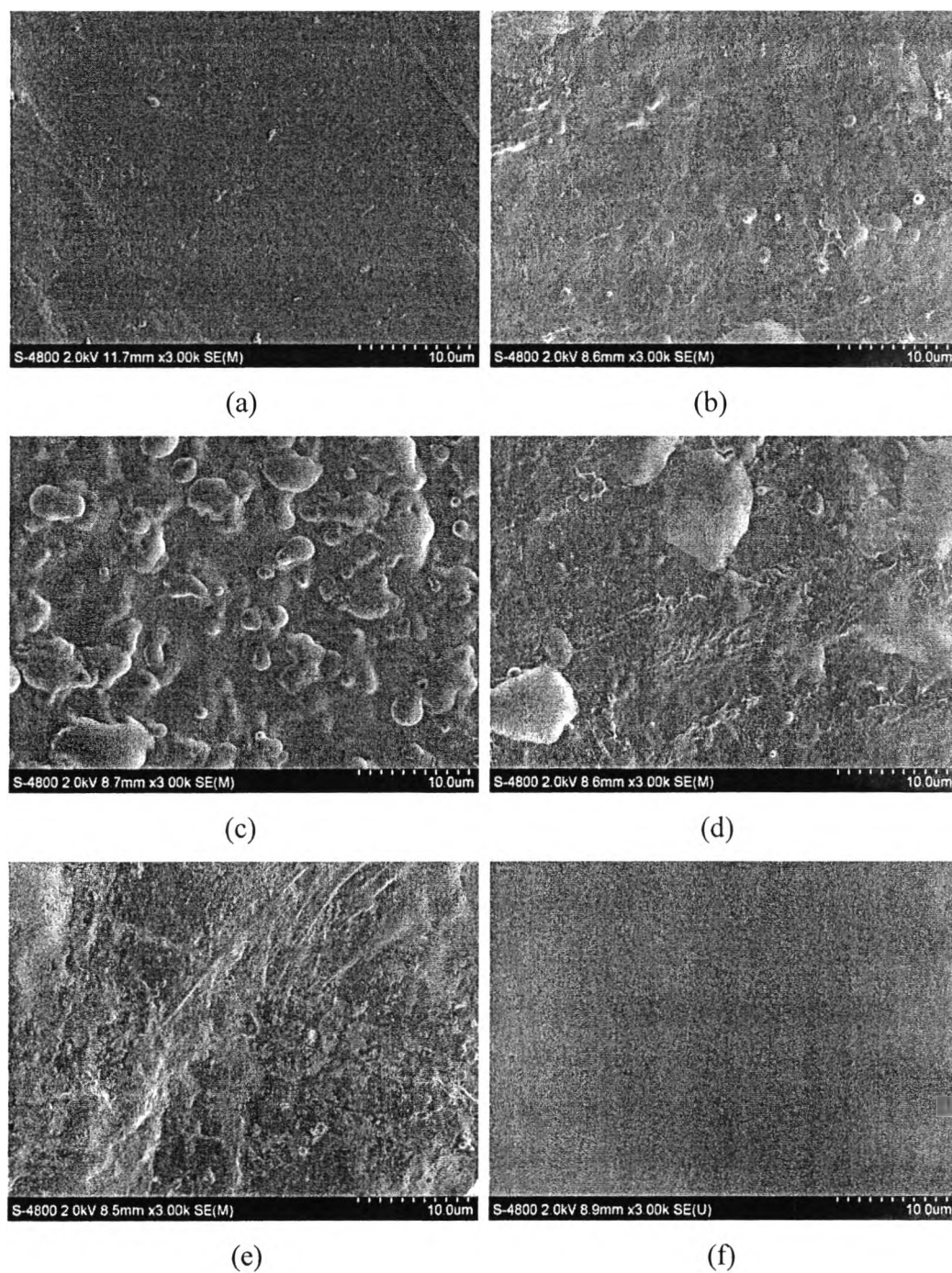
Figure 4.4 SEM micrographs of surface of CM-chitin/natural rubber blend films having different ratio: (a) 100/0, (b) 90/10, (c) 80/20, (d) 70/30, (e) 60/40, and (f) 0/100.
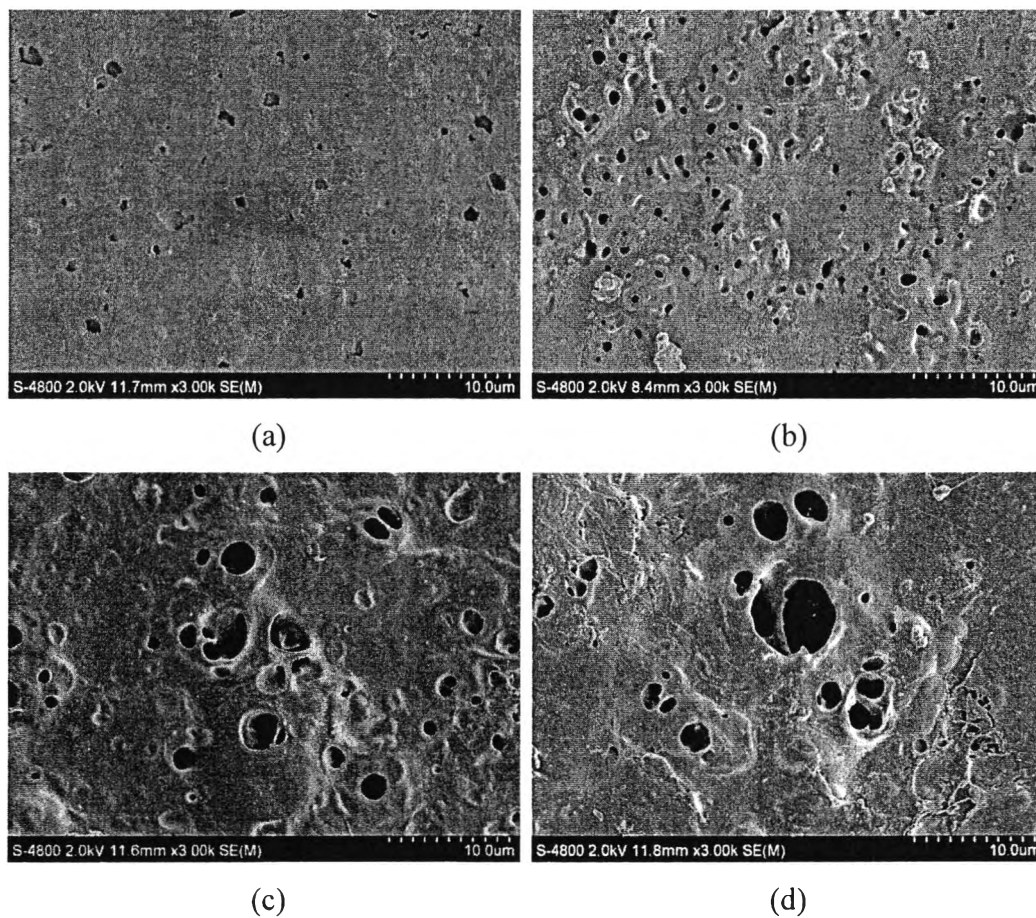
Figure 4.5 SEM micrographs of surface of toluene leaching CM-chitin/natural rubber blend films having different ratio: (a) 90/10, (b) 80/20, (c) 70/30, and (d) 60/40.

Figure 4.6 Average diameter of natural rubber phase in blend films.

The morphology of crossectional area of blend films at magnification of 1000x was shown in figure 4.7. The SEM micrograph indicated that rubber phases were good dispersed in overall crossectional area. It was demonstrated that the phase separation was occurred uniformly throughout of film thickness.
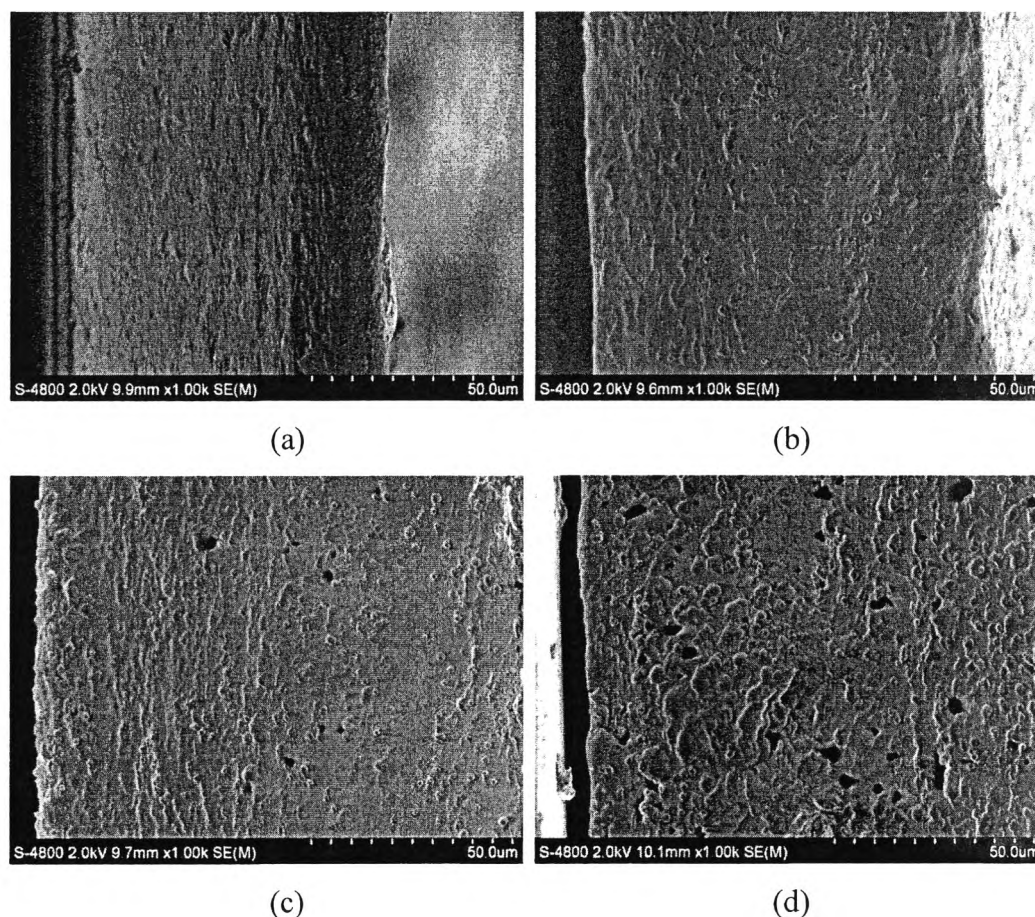
Figure 4.7 SEM micrograph of crossectional area of CM-chitin/natural rubber blend film having different ratio: (a) 90/10, (b) 80/20, (c) 70/30, and (d) 60/40.

4.4.2.3 The mechanical testing of the CM-chitin/natural rubber blend films

The effect of natural rubber content on the mechanical properties of CM-chitin/natural rubber blends was shown in figure 4.8. When the natural rubber content varied from 0 % to 40 %, the tensile strength decreased from 68.48 MPa to 30.04 MPa. However, the elongation at break increased from 5.98 % to 10.60 % in the blend films consisting of natural rubber content from 0 % to 20 % while the elongation at break tend to decreased from 10.60% to 6.98 % when the natural rubber content increased from 20 % to 40 %. This can be described that there was a good disperse of

natural rubber phase within CM-chitin matrix only less than 20 % natural rubber content which related with the average diameter of void size of natural rubber phase within CM-chitin matrix (figure 4.6). The average diameters of void size of the blend films consisting of 10% and 20% natural rubber were almost similar together at 1.13µm and 1.15 µm. This good dispersion of natural rubber phase could enhance the elongation at break to CM-chitin/natural rubber blend films. Furthermore, when the natural rubber content was more than 20 %, the natural rubber phase agglomerated together and the average diameter of void sizes increased to 2.77 µm and 4.17 µm. This agglomeration of natural rubber in the blend films suggested to cause the defect in the blend films and led to the poor mechanical properties.
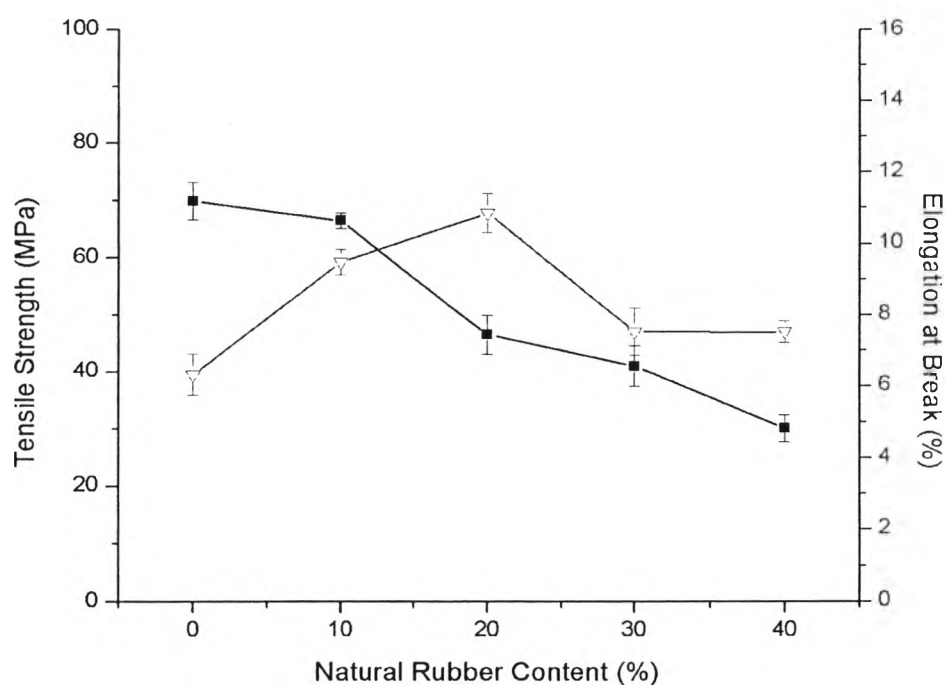
Figure 4.8 Change in tensile strength and the percentage of elongation at break of the CM-chitin/natural rubber blends as a function of natural rubber content.

4.4.2.4 The thermogravimetric analysis of the CM-chitin/natural rubber blend films

The thermal degradation behavior of the CM-chitin was shown in figure 4.9 (a). The TGA thermograms of CM-chitin showed the weight loss in two stages. The initial stage ranged between 30 °C and 250 °C that might correspond to the loss of the decomposition of adsorbed water. The second stage of the weight loss started at 250 °C to 350 °C due to the degradation of CM-chitin. While the thermal degradation behavior of the natural rubber was shown in figure 4.9 (f), the TGA thermograms of natural rubber showed that the degradation of natural rubber ranged between 300 °C and 450 °C (Johns *et al.*, 2008). From the TGA thermograms of CM-chitin/natural rubber blend films (figure 4.9 (a), (b), (c), (d), and (e)), in comparison with CM-chitin, the higher amount of natural rubber in the range of 0 % to 20 %, the better thermal stability of the blends was observed. It can be concluded that higher thermal stability and slower degradation of the blends are due to slight interaction between CM-chitin and natural rubber molecules.
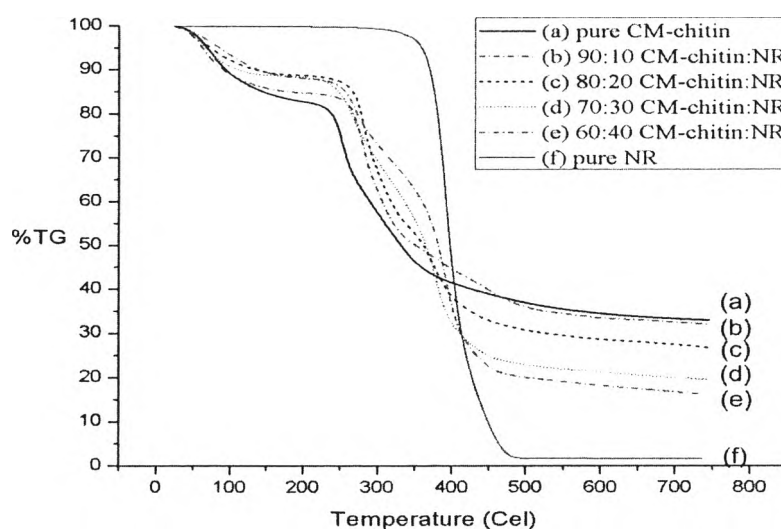

Figure 4.9 The thermogravimetric analysis of the CM-chitin/natural rubber blend films having different ratios: (a) 100/0, (b) 90/ 10, (c) 80/20, (d) 70/30, (e) 60/40, and (f) 0/100.

4.4.3 Characterizations of plasticized CM-chitin/natural rubber blend films

4.4.3.1 The morphology of the plasticized CM-chitin/natural rubber blend films

The morphology of CM-chitin/natural rubber, which blended with plasticizer which is glycerol (Follain, *et al.* 2006) and the blend films after natural rubber removal by toluene, was shown in figure 4.10. The weight ratios of glycerol were varied from 0 phr to 30 phr in CM-chitin/natural rubber: 80/20 blend films. The SEM micrographs of all composition were characterized at magnification of 3000x. The result showed that when glycerol content increased from 0 phr to 30 phr, the amount of voids of natural rubber agglomeration decreased. This can be ascribed as glycerol could interact with protein or lipid at the rubber surface which lead to the decreasing of natural rubber agglomeration (Carvalho, *et al.* 2003). According to table 4.1, the proteins in natural rubber latex were removed out about 99.97 %. It seems to be least amount of protein which can not react with glycerol. Due to the measurement of protein removal by modified Lowry method performed from the protein solution of natural rubber film. And the percentage of protein removal implied to the protein which can be dissolved into the solution. There was some protein remained on the natural rubber surface and can interact with glycerol. It is well known that NH_4OH (ammonium hydroxide) can suspend natural rubber. The suspension occurred from the impulsion of positive charge in protein on natural rubber surface and NH_4OH. Hence, the material from deproteinization of natural rubber latex can suspend in NH_4OH. Consequently, it was indicated that there still are some proteins which were not able to remove by enzymatic treatment remained in natural rubber. However, the overall proteins at natural rubber surface could not be dissolved in the material preparation, so the protein removal was 99.97 %. The low amount of remaining protein could not affect to the allergy when it was used as wound dressing.
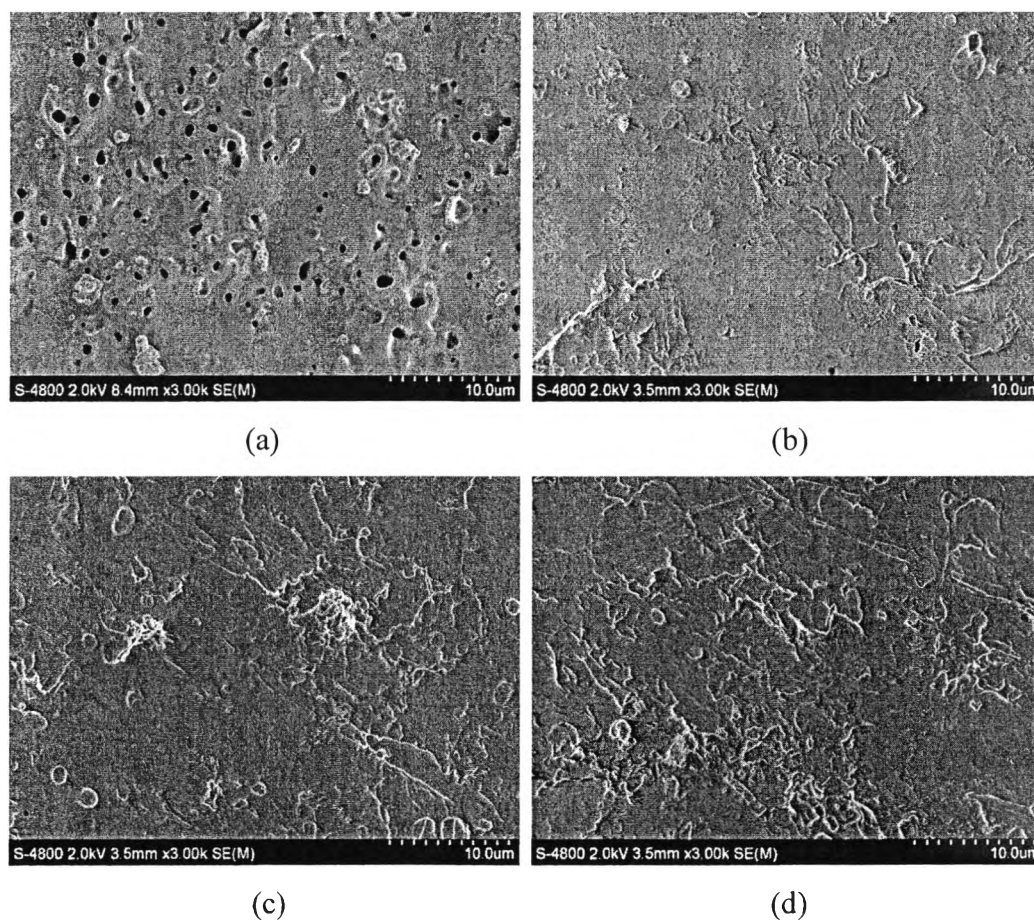
Figure 4.10 SEM micrographs of surface of toluene leaching CM-chitin/natural rubber/glycerol blend films having different ratios: (a) 80/20/0, (b) 80/20/10, (c) 80/20/20, and (d) 80/20/30.

4.4.3.2 The mechanical testing of the CM-chitin/natural rubber/glycerol blend films

The mechanical properties of CM-chitin/natural rubber/glycerol blends as a function of glycerol content in CM-chitin/natural rubber: 80/20 was exhibited in figure 4.11. The tensile strength increased from 46.47 MPa to 57.90 MPa when the glycerol content varied from 0 phr to 10 phr and the tensile strength decreased from 57.90 MPa to 34.39 MPa when the glycerol content varied from 10 phr to 30 phr. However, the addition of glycerol to the blend films from 0 phr to 30 phr improved the elongation at break from 10.85 % to be 50.10 %. This can be ascribed as when the small

amount of glycerol interacted with CM-chitin chains and link them together, the higher tensile strength was observed (Follain, *et al.* 2006). In contrast, the large amount of glycerol could extend the CM-chitin chain and enhanced mobility of the chains which resulted in the decreasing of tensile strength.
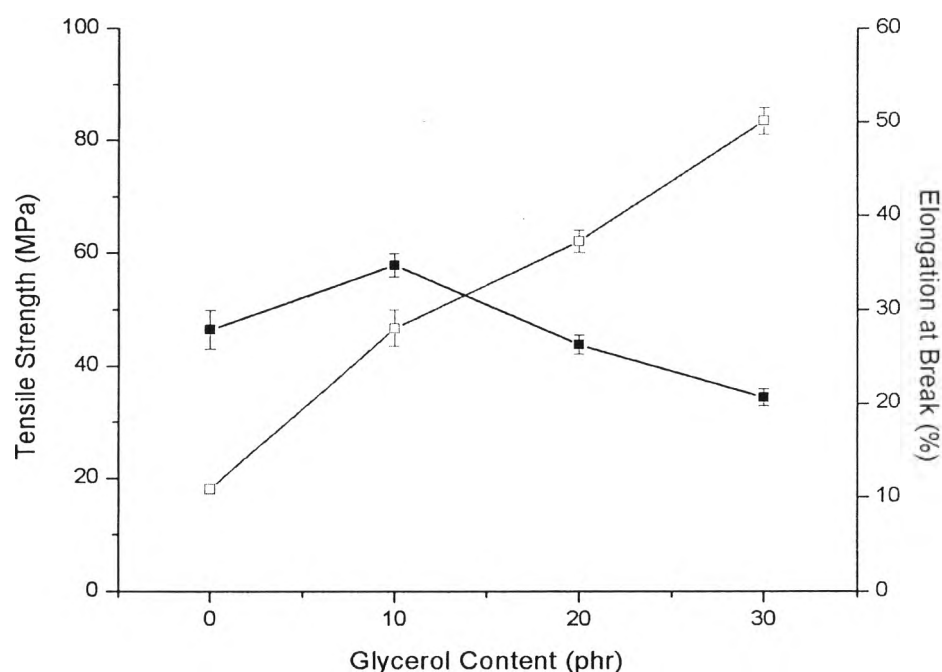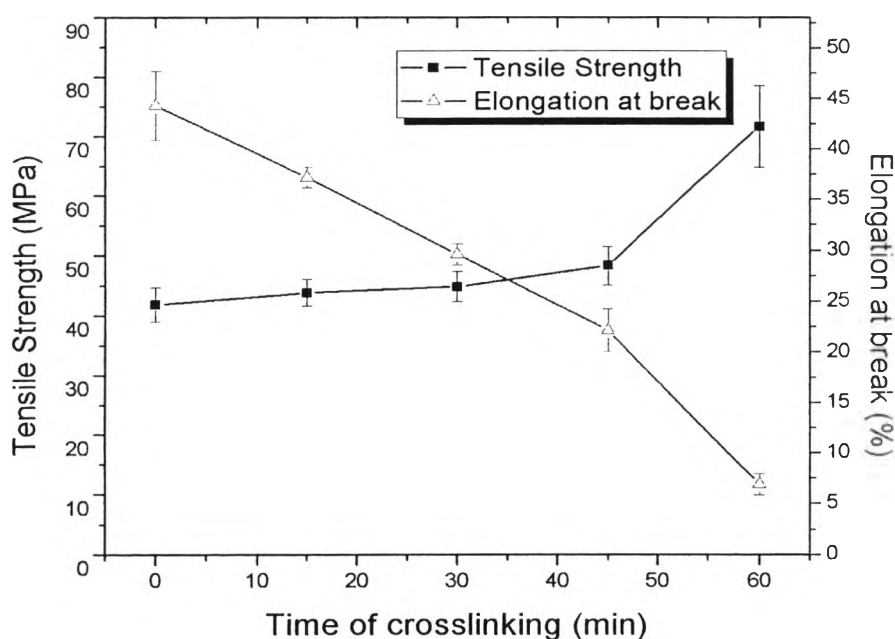

Figure 4.11 Change in tensile strength and the percentage of elongation at break of the crosslinked CM-chitin/natural rubber/glycerol blend films as a function of glycerol content.

4.4.4 Characterizations of crosslinked CM-chitin/natural rubber/glycerol blend films

4.4.4.1 The mechanical testing of the crosslinked CM-chitin/natural rubber/glycerol blend films

The mechanical properties of crosslinked CM-chitin/natural rubber/glycerol blend films at ratio 80/20/10 with glutaraldehyde as a function of crosslinking time were shown in figure 4.12. The results indicated that the longer crosslinking time enhanced the tensile strength from 41.89 MPa to 71.64 MPa but the

elongation at break decreased from 44.26 % to 6.87 %. According to glutaraldehyde can react with nitrogen in acetamide group and also hydroxyl group of CM-chitin (Zhang, Y. *et al.* 2006), it can be ascribe as when blend films were crosslinked, CM-chitin chains were more connected which led to the reduction of chain mobility. Thus, CM-chain could not move easily and then the elongation at break decreased. As the same reason, the network of CM-chitin matrix resulted in the higher tensile strength. In compare with previous study, the mechanical properties of other crosslinked biomaterials such as chitosan film (Kanh *et al.*, 2000), chitin film (Yusof *et al.* 2004), salmon gelatin film and pollock gelatin film (Chiou *et al.* 2008) were shown in table 4.2. It was found that tensile strength and elongation at break of CM-chitin/natural rubber/glycerol blend films did not show the significantly different with other crosslinked biomaterials.

Figure 4.12 Change in tensile strength and the percentage of elongation at break of the CM-chitin/natural rubber/glycerol blend films as a function of time of crosslinking.

Table 4.2 The tensile strength and the elongation at break of other biomaterials

Type	Tensile strength	Elongation at Break	References
Chitosan film	67.11±1.27	21.35±2.12	Kanh, T. A. *et al.* (2000))
Chitin film	38.34±9.54	9.87±2.10	Yusof, N. *et al.* (2004)
Salmon gelatin film	51.20±4.70	3.44±0.25	Chiou, B.S. *et al.* (2008)
Pollock gelatin film	50.10±4.90	3.57±0.32	Chiou, B.S. *et al.* (2008)

4.4.4.2   Degree of swelling and weight loss of CM-chitin/natural rubber/glycerol blend films
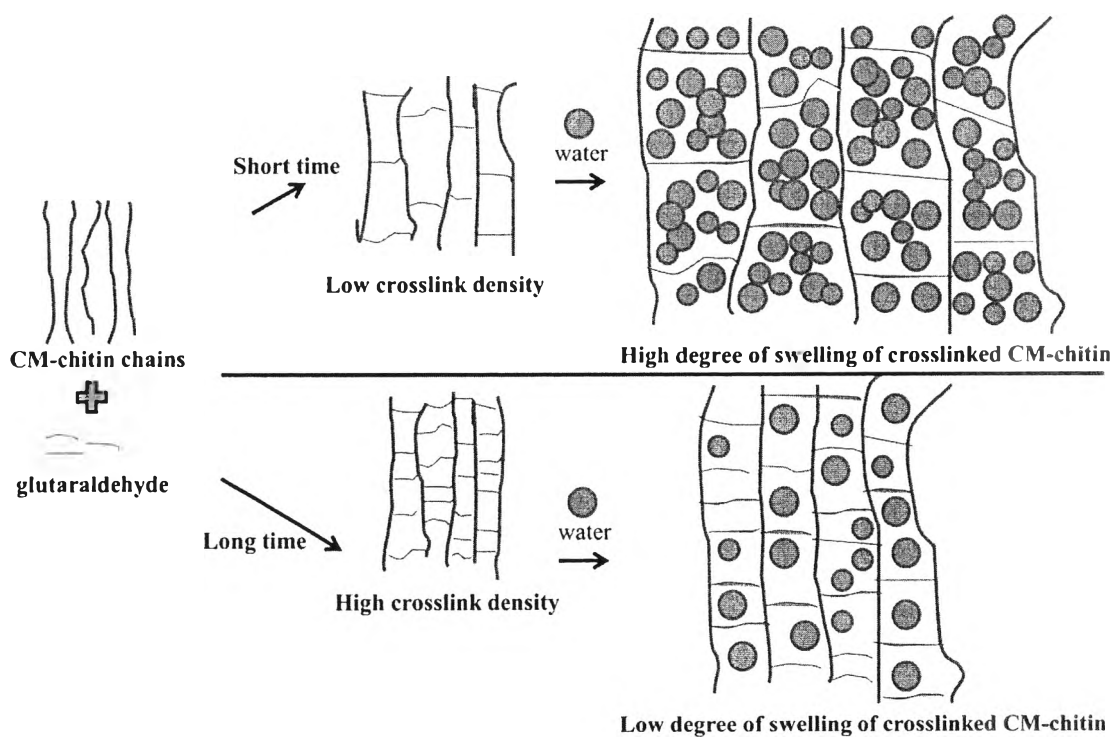
The degree of swelling and weight loss of the crosslinked CM-chitin/natural rubber/glycerol blend films at ratio 80/20/10 as a function of crosslinking time after immersion in buffer pH 5.5 at 37 °C for 24 hr were shown in table 4.3. As the results, when the time of crosslinking increased, the degree of swelling decreased from 665.46 % to 8.55 % and the weight loss decreased from 26.98 % to 19.81 %. Due to glutaraldehyde can react with nitrogen in acetamide group and hydroxyl group of CM-chitin, the network of CM-chitin was formed. The swelling model was represented in figure 4.13. When the blend film was crosslinked in a short time, the lower crosslink density and high degree of swelling after immersion were obtained. And according to the low crosslink density, some CM-chitin units which were not crosslink could be dissolved and it caused weight loss of the blend films. In contrast, when blend film was crosslinked in a long time, high crosslink density and low degree of swelling after immersion were observed. The amount of uncrosslinked CM-chitin units decreased and resulted in the decreasing of weight loss.

The results of the crosslinked CM-chitin/natural rubber/glycerol blend films at ratio 100/0/10 as a function of crosslinking time after immersion in buffer pH 5.5 at 37 °C for 24 hr were shown in table 4.4. When compare with previous results, degree of swelling and weight loss of ratio 100/0/10 was found to higher than ratio 80/20/10. It can be concluded that the addition of natural rubber reduced both degree of

swelling and weight loss of the crosslinked blend films due to the hydrophobicity of natural rubber.

Table 4.3 Degree of swelling and weight loss of CM-chitin/natural rubber/glycerol, 80/20/10 (immersion in buffer pH 5.5 at 37 °C for 24 hr)

Time of Crosslinking	Degree of Swelling (%)	Weight Loss (%)
15 min	665.46 ± 8.55	26.98 ± 0.88
30 min	541.13 ± 8.73	25.42 ± 0.77
45 min	368.63 ± 3.63	22.17 ± 1.29
60 min	253.49 ± 5.72	19.81 ± 0.82

Figure 4.13 Swelling model of crosslinking of CM-chitin chains.

Table 4.4 Degree of swelling and weight loss of CM-chitin/natural rubber/glycerol, 100/0/10 (immersion in buffer pH 5.5 at 37 °C for 24 hr)

Time of Crosslinking	Degree of Swelling (%)	Weight Loss (%)
15 min	843.60 ± 7.83	38.68 ± 1.91
30 min	614.58 ± 8.82	32.04 ± 1.60
45 min	446.57 ± 7.43	30.14 ± 0.83
60 min	376.79 ± 5.48	28.43 ± 0.62

4.5 Conclusion

In this study, the natural rubber was blended with the CM-chitin before preparing the blend films by solution-casting technique. The addition of natural rubber could improve shrinkage stability, flexibility and thermal degradation stability of the blend films. The addition of glycerol to the blend films could reduce phase separation between CM-chitin matrix and natural rubber. In addition, at 10 phr of glycerol could improve both the tensile strength and elongation at break of the blend films. Glutaraldehyde was able to effectively crosslink the blend films. As the increasing of crosslinking time, the degree of swelling and weight loss were found to decrease. Moreover, the addition of natural rubber could improve weight loss of the blend films which can be utilized for wound dressing applications.

4.6 Acknowledgements

4.7 References

Carvalho, A.J.F., Job, A.E., Alves, N., Curvelo, A.A.S., and Gandini, A. (2003) T3hermoplastic starch/natural rubber blends. Carbohydrate Polymer, 53, 95-99.

Chiou, B.S., Avena-Bustillos, R.J., Bechtel, P.J., Jafri, H., Narayan, R., Imama, S.H., Glenn, G.M., and Orts W.J. (2008) Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. European Polymer Journal, 44, 3748–3753.

Follain, N., Joly, C., Dole, P. Roge, B., and Mathlouthi, M. (2006) .Quaternary starch based blends: Influence of a fourth component addition to the starch/water/glycerol system,. Carbohydrate Polymers, 63, 400-407.

Hjerde, R.J.N., Varum, K.M., Grasdalen, H., Tokura, S., and Smidsrod, O. (1997) Chemical composition of O-(carboxymethyl)-chitins in relation to lysozyme degradation rates. Carbohydrate Polymer, 34, 131-133.

Huang, X., Wu, Y., Wei, S., Liao, C., and Chen, Q. (2010) Preparation and characterization of carboxymethylated β-chitins and their abilities of moisture absorption and retention. International Journal of Biological Macromolecules, 47, 223–227.

Jayakumar, R., Menon, D., Manzoor, K., Nair, S.V. and Tamura, H. (2010) Biomedical applications of chitin and chitosan based nanomaterial-A short review. Carbohydrate Polymer, 82, 227-232.

Jayakumar, R., Prabaharan, M., Kumar, P.T.S., Nair, S.V. and Tamura, H. (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnology Advances, 29, 322-337.

Johns, J.,and Rao, V. (2008) Characterization of natural rubber latex/chitosan blends. International Journal of Polymer Analysis and Characterization, 13, 280-291.

Khan, T.A., Peh, K.K., and Ch'ng , H.S. (2000) Mechanical, bioadhesive strength and biological evaluation of chitosan film for wound dressing. Journal of Pharmacy & Pharmaceutical Science, 3, 303-311.

Kumar, P.T.S., Abhilash, S., Manzoor, K., Nair, S.V., Tamura, H., and Jayakumar, R. (2010) Preparation and characterization of novel b-chitin/nanosilver composite scaffolds for wound dressing applications. Carbohydrate Polymer, 80, 761-767.

Peesan, M., Rujiravanit,R., and Supaphol, P. (2003) Characterisation of beta-chitin/poly(vinyl alcohol) blend films. Polymer Testing, 22, 381-387.

Perrella, F.W., and Gaspari, A.A. (2002) Natural rubber latex protein reduction with an emphasis on enzyme treatment. Methods, 27, 77-86.

.Rinaudo, M. (2006) Chitin and chitosan: Properties and application, Progress in Polymer Science, 31, 603-632.

Sannan, T., Kurita, K. Ogura, K. and Iwakura, Y. (1977) Studies on chitin: 7.Ir Spectroscopic determination of degree of deacetylation. Polymer, 19, 458-459.

Tokura, S, Nishi, N., Tsutsumi, A., and Somorin, O. (1983) Studies on chitin VIII. Some properties of water soluble chitin derivatives. Polymer Jounal, 15(6), 485-489.

Wagner, B., Krebitz, M., Buck, D., Niggemann, B., Yeang, H.Y., Han, K.H., Scheiner and Breiteneder, H. (1999) Cloning, expression, and characterization of recombinant Hev b 3, a Hevea brasiliensis protein associated with latex allergy in patients with spina bifida. Journal of Allergy and Clinical Immunology, 104, 1084-1092

Wongpanit,P., Sanchavanakit, N., Pavasant, P., Supaphol, P., Tokura, S., Rujiravanit, R. (2005) Preparation and characterization of microwave-treated carboxymethyl chitin and carboxymethyl chitosan films for potential use in wound care application. Macromolecular Bioscience, 5, 1001-1012.

Yusof, N.L. Lim, L.Y. and Khor, E. (2004) Flexible chitin films: structural studies. Carbohydrate Research, 399, 2701-2711

Zhang, Y., Venugopal, J., Huang, Z.M., Lim, C.T. and Ramakrishna, S. (2006). Crosslinking of the electrospun gelatin nanofibers. Polymers, 47, 2911-2917.