

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preparation of Chitin

Shrimp shells generally compose of three major components, which are chitin, calcium carbonate, and protein. After calcium carbonate and protein are removed by solvent extraction, chitin will be obtained as the remaining portion. In this research, chitin was prepared from shrimp shells of *Penaeus merguensis* by demineralization with hydrochloric acid solution and deproteinization with aqueous sodium hydroxide solution in order to remove calcium carbonate and protein, respectively. The yield obtained during the process of chitin production is shown in Table 1. Chitin was obtained as a white flaky material.

Table 4.1 Yield of chitin production from shrimp shells.

Material	Yield* (%)
Shrimp shell	100
Product after demineralization	46.31
Product after deproteinization (chitin)	27.73

* dry weight basis

Chitin has some extent of amino groups other than acetamide groups at C2 position of N-acetyl glucosamine repeating units. The degree of deacetylation of chitin depends on the nature of chitin resources and the conditions used during deproteinization. The chitin used in this study was inevitable subjected to N-deacetylation during deproteinization process under alkaline condition and heating. According to the method of Sannan *et al.*,

1978, the degree of deacetylation of chitin calculated from FTIR (Figure 4.1) was 21.54%.

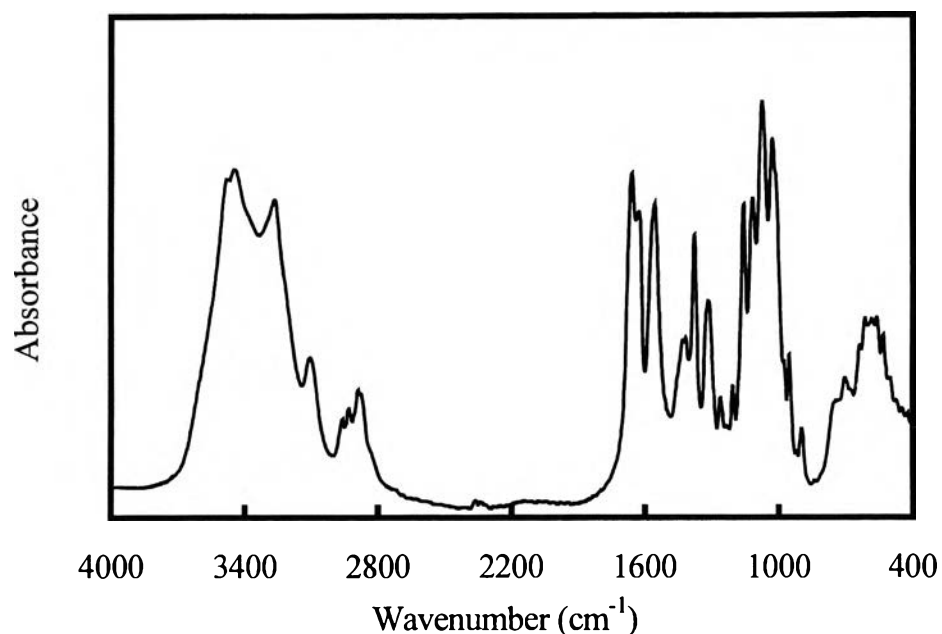


Figure 4.1 FTIR spectrum of chitin powder.

The method used to determine the molecular weight of chitin was based on viscosity measurement by the method of Lee, 1974. The viscosity-average molecular weight of chitin was determined based on Mark-Houwink (eqn. 3.6).

The intrinsic viscosity was determined from the Y-intercept of the plot between (η_{sp}/C) versus chitin concentration (g/100 ml) and $[\ln(\eta_{rel}/C)]$ versus concentration of chitin (g/100 ml) (Figure 4.2). The intrinsic viscosity was obtained at 16.67 (100 ml/g). The viscosity-average molecular weight of chitin was calculated to be 1.04×10^6 g/mol.

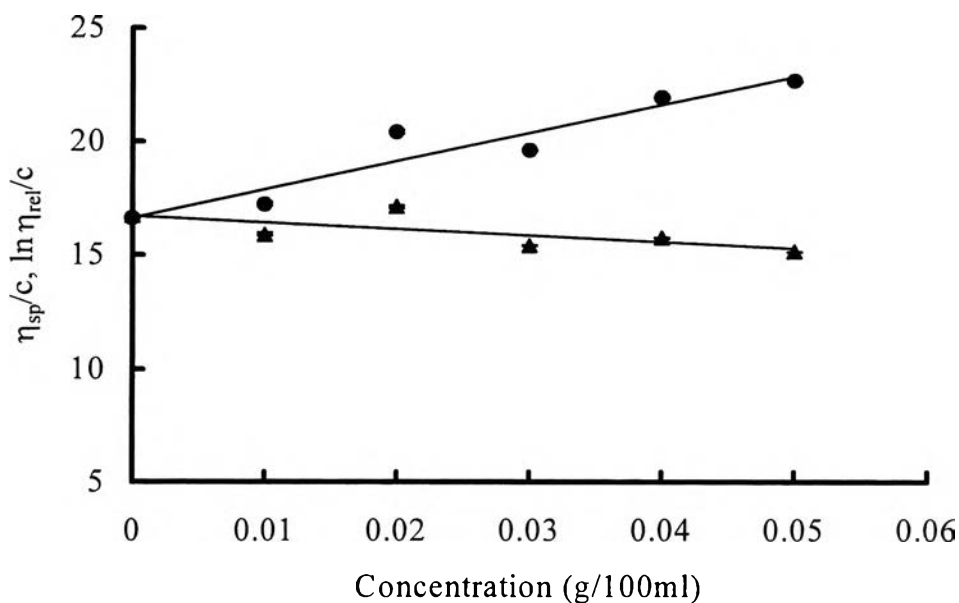


Figure 4.2 Reduced viscosity (η_{sp}/C) and ($\ln(\eta_{rel})/C$) versus concentration of chitin solution, ● : η_{sp}/C , ▲ : $\ln(\eta_{rel})/C$.

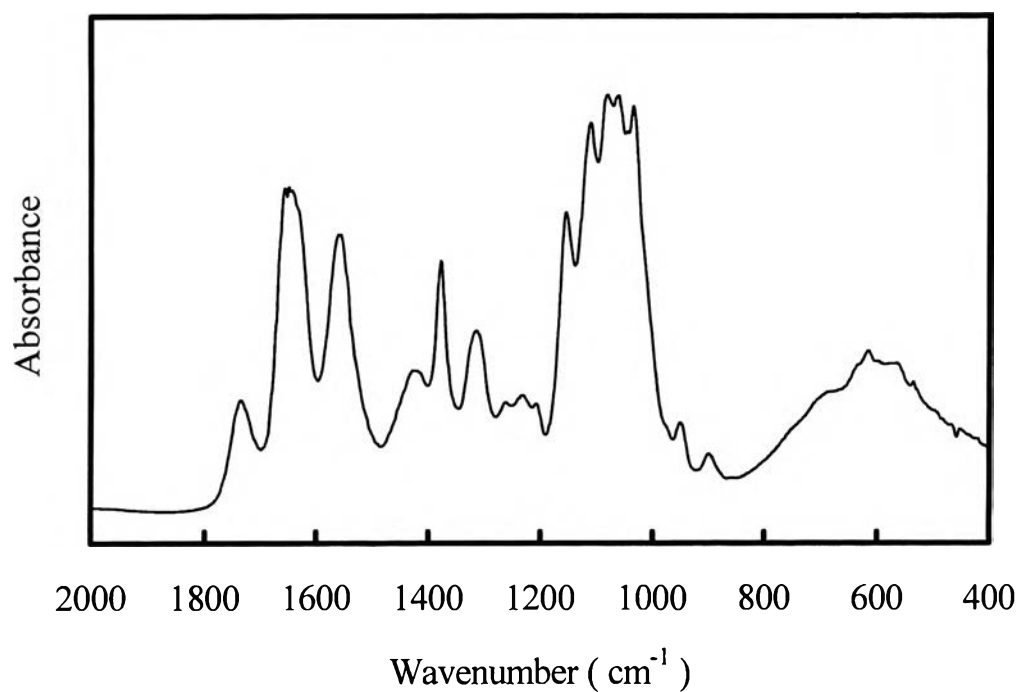
4.2 Preparation of CM-chitin

Due to the difficulty to dissolve chitin in common solvents, chitin is chemically modified to CM-chitin in order to achieve the ease of dissolution. The FTIR spectrum of CM-chitin is shown in Figure 4.3 and the absorption frequencies of characteristic bands of CM-chitin are summarized in Table 4.2.

The absorption due to carbonyl stretching of carboxylic group appears at 1735 cm^{-1} and primary hydroxyl groups at 1070 cm^{-1} decreases on carboxymethylation (Figure 4.3). The degree of carboxymethylation was estimated to be 0.45 under the reaction condition from elemental analysis.

Table 4.2 FTIR characteristic absorption bands of CM-chitin.

Frequencies (cm^{-1})	Assignment and remarks
1735	C=O stretching of carboxylic group
1659	C=O stretching of acetamide group
1561	NH deformation
1317	CN band and CH_2 wagging
1070 and 1031	C-O stretching vibration

**Figure 4.3** FTIR spectrum of CM-chitin.

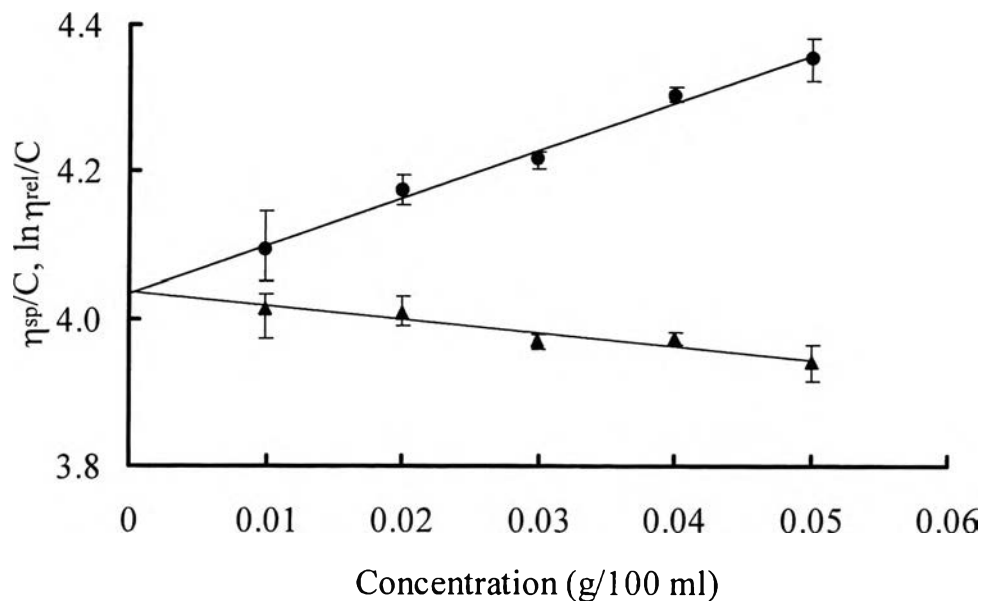


Figure 4.4 Reduced viscosity (η_{sp}/C) and $\ln(\eta_{rel})/C$ versus concentration of CM-chitin solution, ●: η_{sp}/C , ▲: $\ln(\eta_{rel})/C$.

The molecular weight of CM-chitin was determined by viscometric method. According to the method of Kaneko (1982), the molecular weight of CM-chitin was derived from its intrinsic viscosity. The plot of reduced viscosity (η_{sp}/C) and $[\ln(\eta_{rel})/C]$ versus concentration of CM-chitin solution is shown in Figure 4.4. This plot shows the extrapolated value of each line reaches the same position and this value was referred to intrinsic viscosity of CM-chitin. The average-molecular weight of CM-chitin was determined based on Mark-Houwink equation (eqn. 3.7).

The viscosity-average molecular weight of CM-chitin obtained from the calculation was 5.09×10^4 .

The molecular weight of CM-chitin was also determined by GPC. Number-average molecular weight and weight-average molecular weight of CM-chitin are 3.02×10^5 and 9.89×10^5 , respectively.

4.3 Characterization of CM-chitin/PVA Blend Films

4.3.1 FTIR Analysis of the Blend Films

FTIR spectroscopy is one of the most powerful techniques for investigation of multi-component system because it provides information on the blend composition (Lee *et al.*, 1996). Figure 4.5 shows the FTIR spectra of CM-chitin, PVA, and the blend films measured at wavenumber ranging from 2000 to 400 cm^{-1} . The FTIR spectrum of pure PVA film is shown in Figure 4.5 (a). The characteristic peaks of PVA at 1419 cm^{-1} , 1091 cm^{-1} , and 854 cm^{-1} are attributed to OH deformation (in plane), C-O stretching, OH deformation (out of plane) (Miya *et al.*, 1984). While the characteristic absorption bands of CM-chitin at 1610 cm^{-1} belongs to C=O stretching of acid salt (Tokura *et al.*, 1983a), 1659 cm^{-1} and 1561 cm^{-1} belongs to amide I and amide II bands, and the others occurred at 1157 cm^{-1} and 1050 cm^{-1} are the characteristic peaks of pyranose ring (Kurita *et al.*, 1991). The FTIR spectra of CM-chitin/PVA blend films with various blend ratios (Figure 4.5 (b-f)) were characterized by the presence of absorption bands of the pure components, whose intensities were roughly related to the blending ratio.

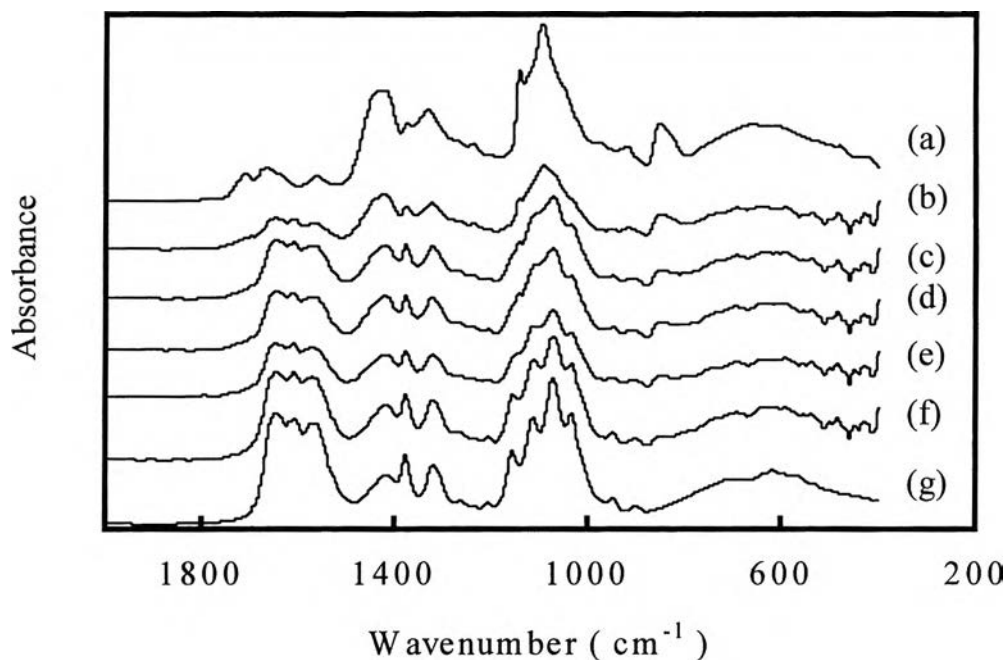


Figure 4.5 FTIR spectra of pure and blend films at various compositions of CM-chitin to PVA, CM-chitin/PVA composition: (a) 0/100 (PVA); (b) 20/80; (c) 40/60; (d) 50/50; (e) 60/40; (f) 20/80; (g) 100/0 (CM-chitin).

4.3.2 X-ray Diffraction Patterns

Wide-angle x-ray diffraction (WAXD) patterns of the films were measured to compare the crystalline structure of CM-chitin film and PVA film with the blend films. The X-ray diffraction pattern of the films is shown in Figure 4.6. The crystalline structure of CM-chitin and PVA films appeared to be nearly the same positions of 2θ degree. Figure 4.6 (g) shows the CM-chitin exhibited crystalline peaks at $2\theta = 9.4^\circ$ and $2\theta = 19.3^\circ$. PVA showed the crystalline peaks at $2\theta = 9.4^\circ$ and $2\theta = 20^\circ$. In the X-ray diffraction pattern of the blend films, no peak other than those of CM-chitin and PVA was observed. For the blends, their reflection patterns are similar to these of CM-chitin and PVA with small reducing in their intensity. These results are similar to the work of Kim *et al.* (1992a) who studied the properties of PVA/chitosan blend membrane. Kim *et al.* (1992a) found that the intensity

of peak of chitosan drops upon blending so blending of chitosan with PVA decreases the crystallinity of chitosan.

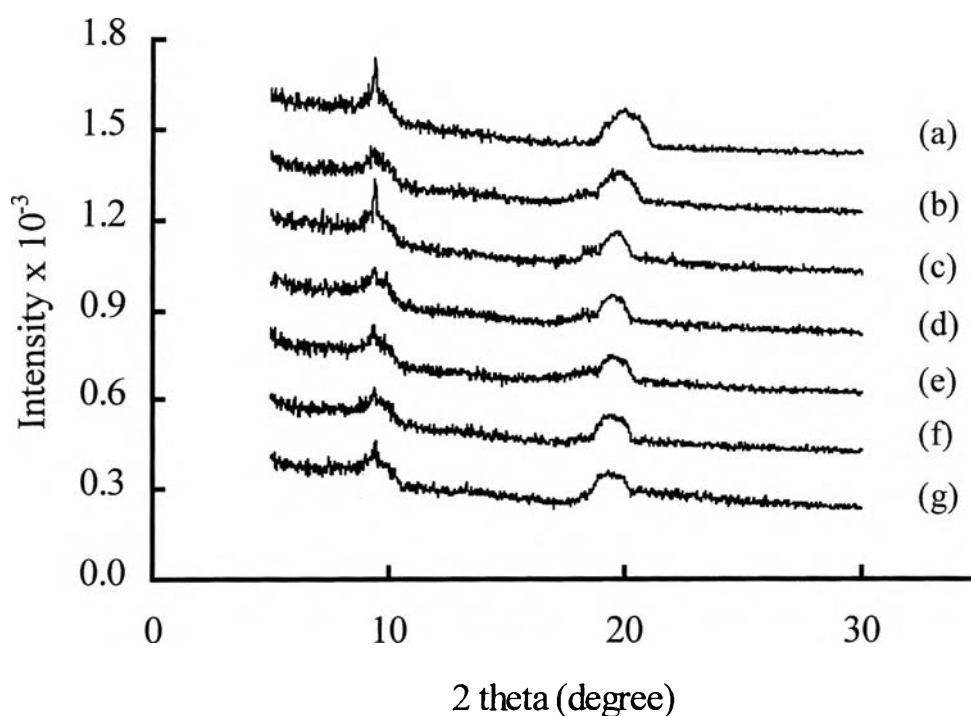


Figure 4.6 Wide-angle X-ray diffraction patterns of CM-chitin/PVA blend films, CM-chitin/PVA composition: (a) 0/100 (PVA); (b) 20/80; (c) 40/60; (d) 50/50; (e) 60/40; (f) 80/20; (g) 100/0 (CM-chitin).

4.3.3 Thermal Property

Thermal property of the films was examined by differential scanning calorimeter (DSC). It was of particular interest to estimate how the thermal transition of PVA varied with blending, since the CM-chitin homopolymer studied in this study did not show any significant transitions in

the temperature range of the DSC scan. CM-chitin undergoes the thermal degradation at high temperature in air. The results of the measurements are shown in Figure 4.7. The homogeneous PVA (Figure 4.7 (a)) gives a relatively large, sharp melting endotherm with a peak (T_m) at around 220°C. The endothermic peak of PVA tends to be somewhat smaller by blending with CM-chitin.

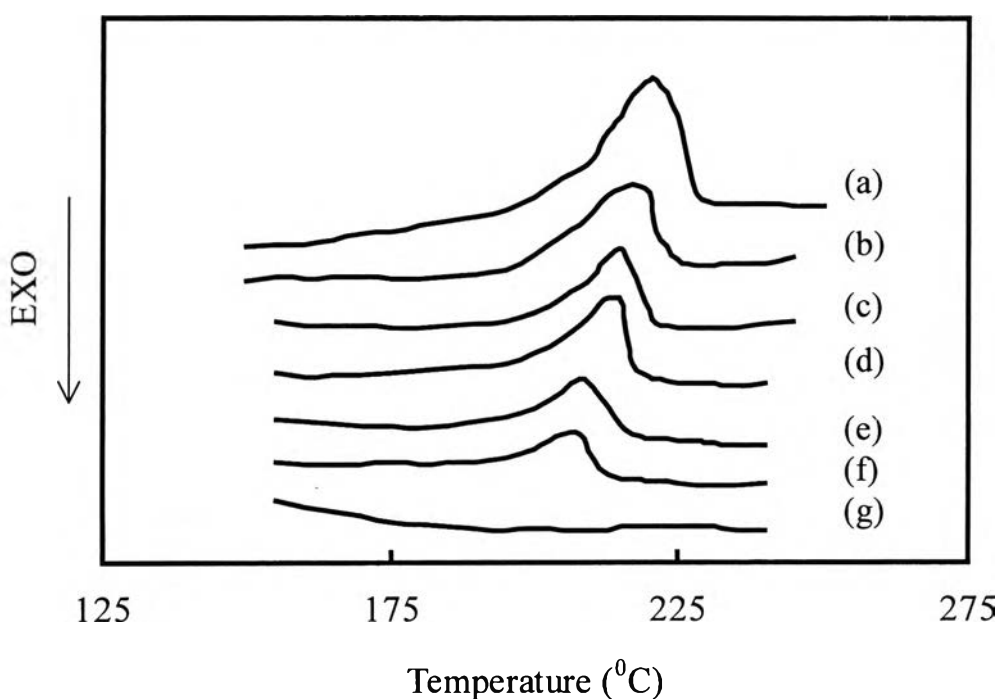


Figure 4.7 DSC thermograms of CM-chitin/PVA blend films, CM-chitin/PVA composition: (a) 0/100 (PVA); (b) 20/80; (c) 40/60; (d) 50/50; (e) 60/40; (f) 80/20; (g) 100/0 (CM-chitin).

This agrees with the study of Lee *et al.*, (1996) who determined the properties of β -chitin and PVA blends. Lee *et al.*, (1996) found that as PVA was blended with β -chitin up to 50%, the intensity of the peak of PVA was reduced rapidly, with a shift to lower temperature of melting point.

4.3.4 Thermal Stability

The thermal stability is an important property, which can definitively determine the performance of a material for practical use. The degradation temperature (T_d) of pure and the blend films as a function of CM-chitin content is shown in Figure 4.9. Pure CM-chitin and pure PVA films were stable until temperature at 265.9°C and 280.98°C, respectively (Figure 4.8). The thermal stability of the blend films was in the range of the T_d of pure CM-chitin and pure PVA films. It was found that the decomposition temperature of the blend films increased with increasing of PVA content.

From the thermal analysis, the degradation temperature of PVA film was higher than that of CM-chitin film. By blending of CM-chitin with PVA, the thermal stability of CM-chitin/PVA blend films could be improved as compared to that of CM-chitin film. Furthermore, the T_d of the blend films occurred at one temperature over a temperature range between the T_d of CM-chitin and T_d of PVA films. This suggested that there was good intermolecular interaction between CM-chitin and PVA. If there was no intermolecular interaction, the resulting T_d would be expected to occur at two T_d s. The first temperature occurred at T_d of CM-chitin and the second temperature occurred at T_d of PVA.

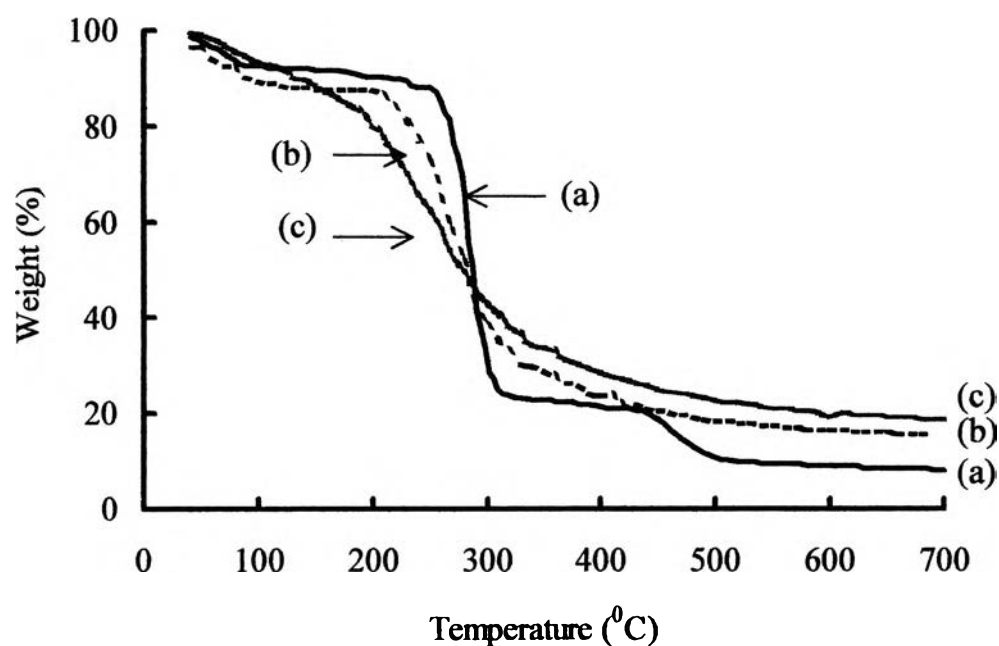


Figure 4.8 TGA thermograms of CM-chitin/PVA blend films, CM-chitin/PVA composition: (a) 0/100 (PVA); (b) 50/50; (c) 100/0 (CM-chitin).

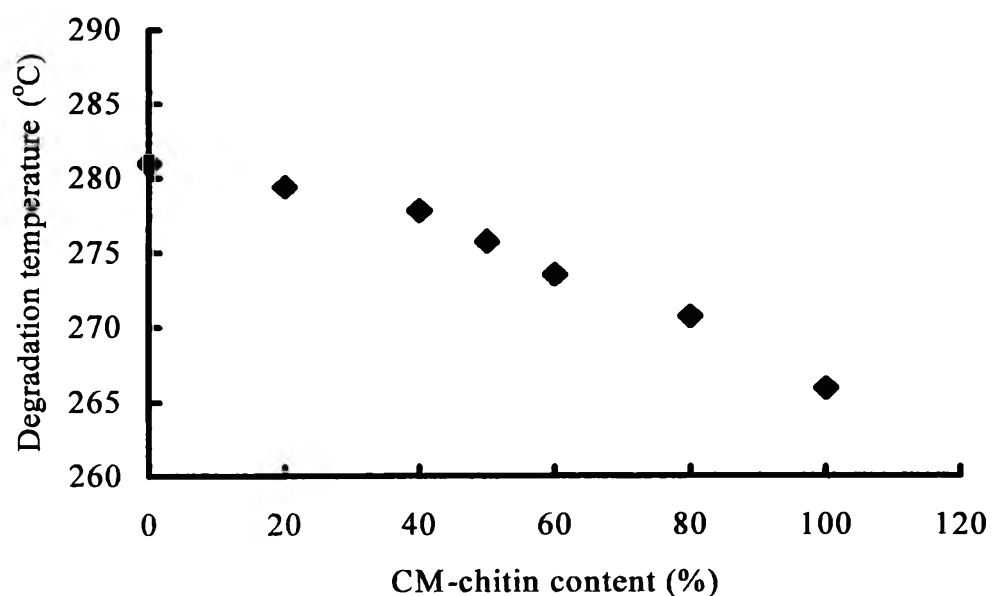


Figure 4.9 Thermal decomposition temperature of CM-chitin/PVA blend films as a function of CM-chitin content.

The results agree with the work of Williamson *et al.* (1998) who studied the properties of semi-interpenetrating network (SIPN) of poly(N,N-dimethylacrylamide) (DMAM) with cellulose or chitin. The degradation of DMAM-SIPN blend occurred in one stage over a temperature range between DMAM and the polysaccharide. This suggested good intermolecular interaction between DMAM matrix and the polysaccharides.

4.4 Swelling Study

4.4.1 Equilibrium Water Content

The effect of immersion time on the equilibrium water content (EWC) of the blend films is shown in Figure 4.10. All samples in water reached an equilibrium state within 1.30 h. Kim *et al.* (1992a) studied swelling characteristics of cross-linked PVA/chitosan blend membrane and found that the samples reached an equilibrium state within 1 h.

Figure 4.11 shows EWC of the films as a function of CM-chitin content. The EWC of CM-chitin films was approximately 360% while the EWC of PVA films was around 150%. It was found that the EWC of the blend films increased as CM-chitin content increased. Khor *et al.* (1996) suggested that the ability to absorb water of CM-chitin films is attributed to the introduction of carboxymethyl group on the C-6 of the glucose residue. The presence of carboxymethyl groups distributing along chitin chains disrupts the H-bonding interactions between adjacent chitin chains. This facilitated the diffusion of water into the matrix of CM-chitin films (Khor *et al.*, 1996).

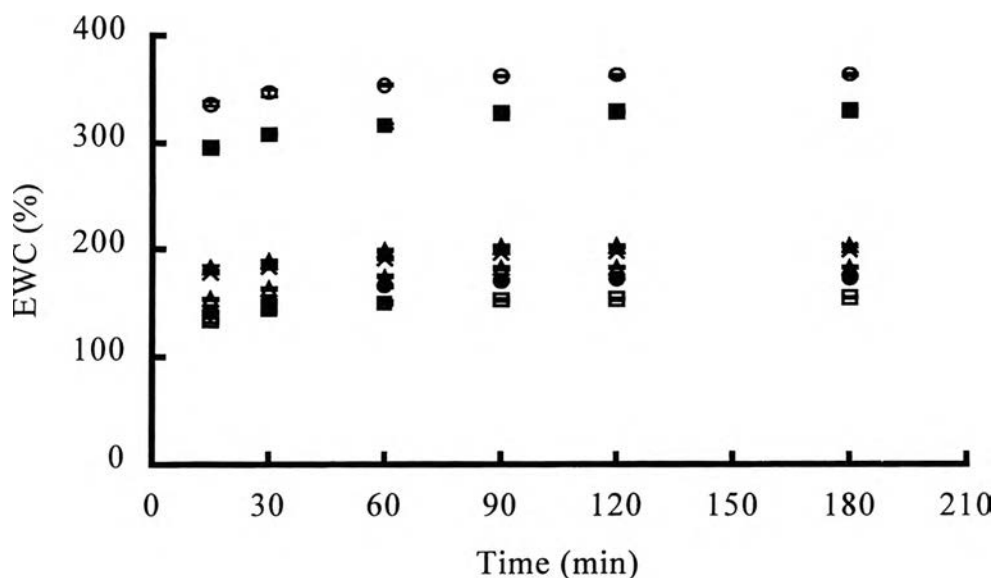


Figure 4.10 Effect of immersion time on equilibrium water content of CM-chitin/PVA blend films with the addition of 0.01% glutaraldehyde, CM-chitin/PVA composition: ●: 100/0 (CM-chitin); ■: 80/20; △: 60/40; x: 50/50; □: 40/60; ○: 20/80; ▲: 0/100 (PVA).

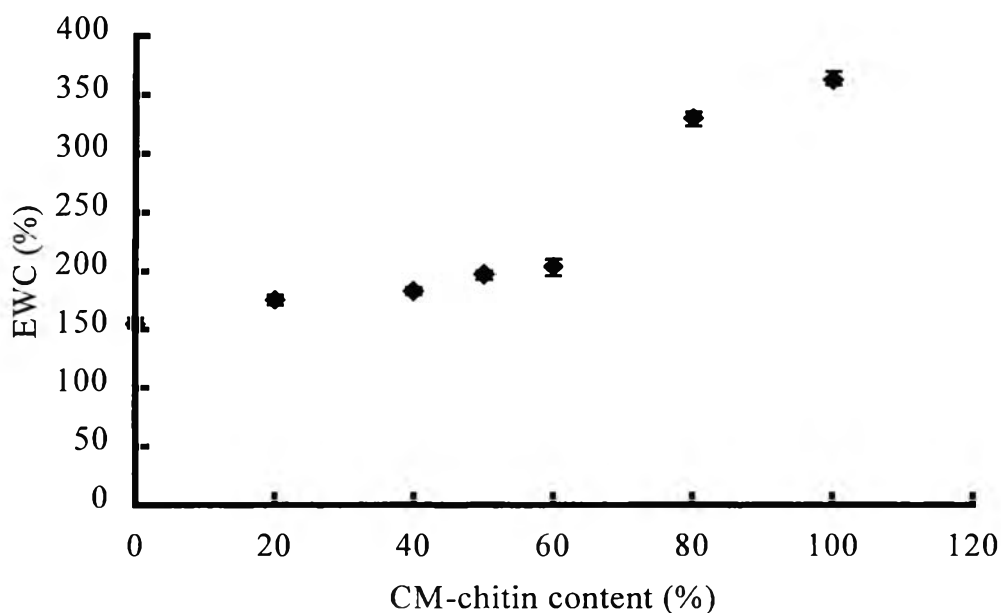


Figure 4.11 Equilibrium water content of CM-chitin/PVA blend films with the addition of 0.01% glutaraldehyde.

4.4.2 Effect of pH

The effect of pH on the degree of swelling of CM-chitin/PVA blend films with various blend compositions is shown in Figure 4.12. The degrees of swelling of PVA films were constant for the whole pH range from pH 3 to 11 due to pH stability of PVA (Gudeman *et al.*, 1995). On the other hand, the degree of swelling of CM-chitin films and the blend films increased substantially in the range of pH less than 5 and in the range of pH higher than 7. The pK_a of the carboxymethyl groups is about 3.4 and the amino group having a pK_a of about 6.4 (Tokura *et al.*, 1983a). The reason to explain the effect of pH on the degree of swelling of CM-chitin films and the blend films is that in acidic pH (pH<5) solutions, the amine groups of CM-chitin molecules are ionized leading to the dissociation of the adjacent chains (Kim

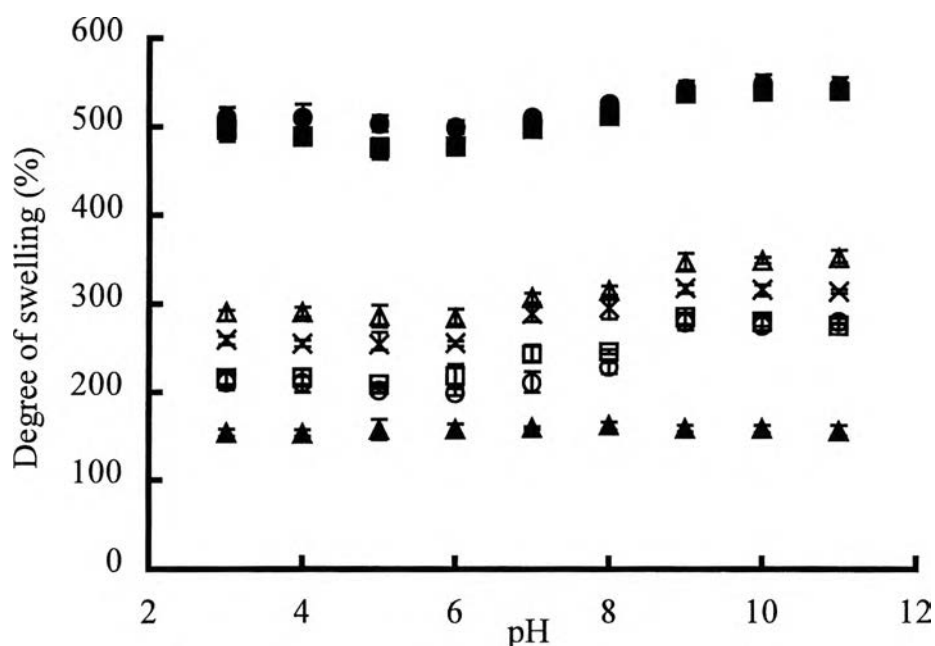


Figure 4.12 Degree of swelling of CM-chitin/PVA blend films with the addition of 0.01% glutaraldehyde as a function of CM-chitin content, CM-chitin/PVA composition: ●: 100/0 (CM-chitin); ■: 80/20; △: 60/40; x: 50/50; □: 40/60; ○: 20/80; ▲: 0/100 (PVA).

et al., 1992a). For alkaline pH ($\text{pH} > 7$) solutions, the effect of pH on the degree of swelling of CM-chitin and the blend films increased because of the presence of the carboxymethyl groups that are ionizable functional groups of CM-chitin (Gudeman *et al.*, 1995). It could say that the CM-chitin/PVA blend films showed the pH sensitivity.

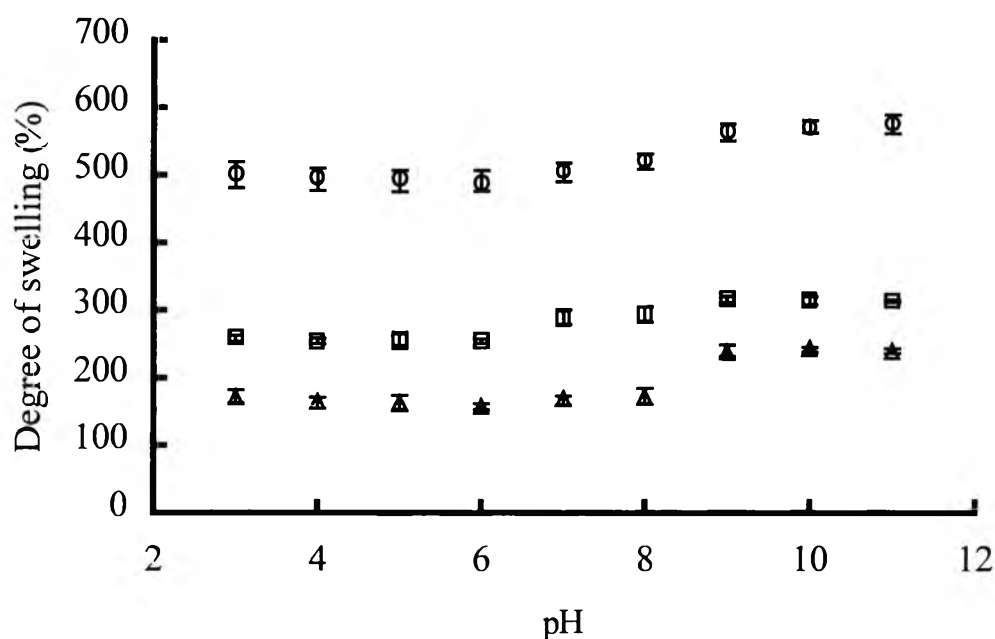


Figure 4.13 Effect of glutaraldehyde concentration on degree of swelling of CM-chitin/PVA blend films as a function of pH, ○ : 0.005% glutaraldehyde; □ : 0.01% glutaraldehyde; △ : 0.05% glutaraldehyde.

The effect of cross-linking on equilibrium degree of swelling for CM-chitin/PVA blend film with 50/50 blend ratio is shown in Figure 4.13. The equilibrium degree of swelling decreases with the increasing of the concentration of the cross-linking agent in the films. It is mainly due to the cross-linking density of the polymer chains. Moreover, as the concentration of the cross-linking agent increases, more hydroxyl groups in PVA and more hydroxyl and amino groups in CM-chitin were consumed due to the cross-linking reaction. It has been well known that hydroxyls react with aldehyde to

form acetals, and amino groups form a Schiff base (Kim *et al.*, 1992a). Therefore, as PVA and CM-chitin react with glutaraldehyde, the cross-linked blends become less capable for hydrogen bonding formation with water molecules because the intermolecular and intramolecular cross-linking caused both by acetalization and the formation of the Schiff base, resulting in the decreasing of the degree of swelling at equilibrium.

Kim *et al.*, (1992a) studied the effect of cross-linking agent on swelling property of PVA and chitosan blend membrane. They found that the cross-linking reduces the swelling ability of the membrane due to the cross-linking density and also to the discounted ability of hydrogen bonding between water molecules and hydroxyls and amino groups in the PVA and chitosan blend.

The response of the blend films with 50% CM-chitin content to a step change in pH is shown in Figure 4.14. In this experiment, the films were brought into a swelling equilibrium at pH 6 for 1.30 h and then transferred to a buffer solution at pH 10 so that an abrupt swelling was ensured. Later, the films were placed back into a buffer solution at pH 6, and shrank again. The results showed that swelling behavior of the blend films is reversible when the environmental pH is changed. The blend films show a pH-sensitive swelling characteristic that may be applicable to a controlled-release system because the blend films could change degree of swelling when pH was altered.

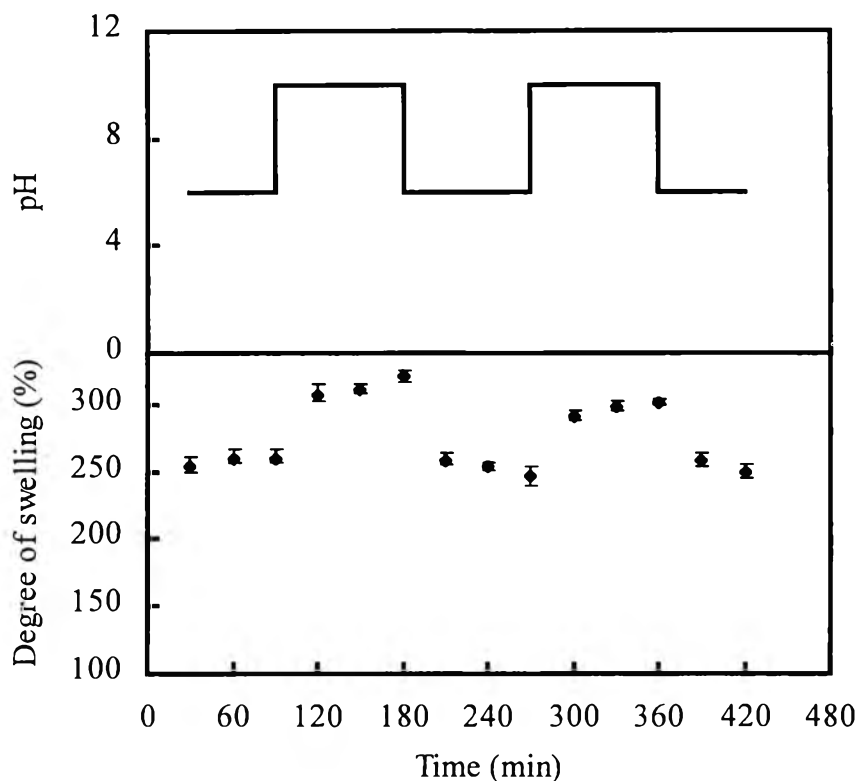


Figure 4.14 Degree of swelling of CM-chitin/PVA blend films with 50/50 blend ratio containing 0.01% glutaraldehyde on a step change in pH.

4.4.3 Effect of Salt Type

The degree of swelling of pure and the blend films in various types of salt solutions is shown in Figure 4.15. The salt solutions used in this study were NaCl, LiCl, CaCl₂, and FeCl₃ solutions. The concentration of the salt solutions was 0.25 M. It was found that, for all salt solutions, the degree of swelling of the blend films increased as CM-chitin content increased. It can be seen that the films exhibited a significant increase in the degree of swelling in salt solutions as CM-chitin content increased from 40% to 100%. However, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solutions (NaCl and LiCl). For CaCl₂ solution, Tokura *et al.*, (1983b) found that CM-chitin can bind calcium ions even in the presence of monovalent cations. The tetrahedral chelation of CM-

chitin toward Ca^{2+} occurred by the assistance of the acetamide and hydroxyl groups in addition to carboxyl groups. Watanabe *et al.*, (1992) reported that the addition of iron(III) chloride into CM-chitin solution induced gel formation so this meant CM-chitin can also bind Fe^{3+} .

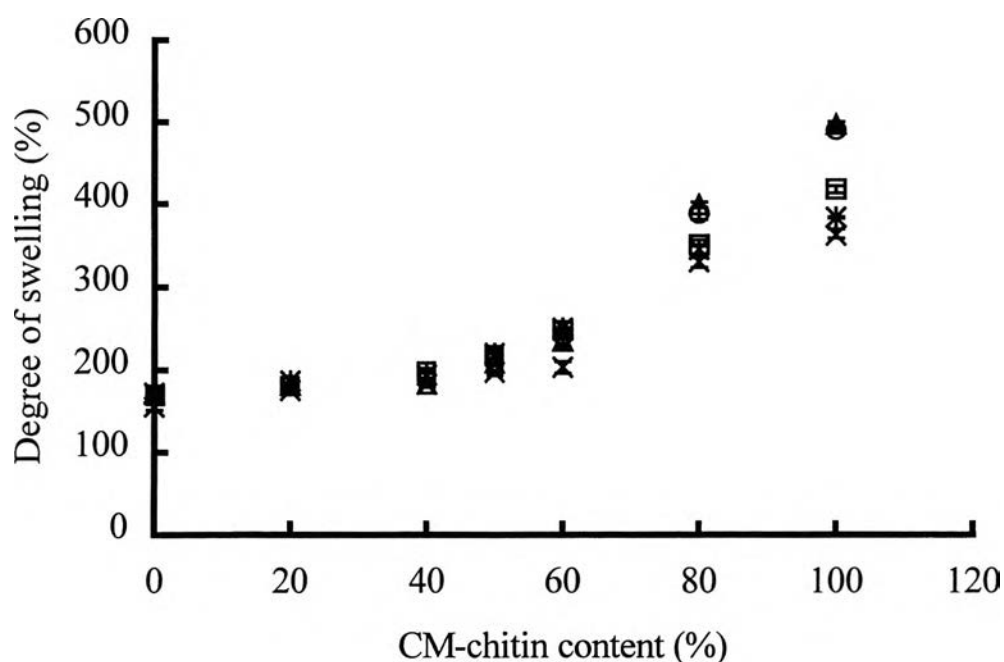


Figure 4.15 Effect of salt type on degree of swelling of CM-chitin/PVA blend films with the addition of 0.01% glutaraldehyde as a function of CM-chitin content, x: H_2O ; Δ : NaCl ; \circ : LiCl ; \square : CaCl_2 ; $*$: FeCl_3 .

Swelling behavior of CM-chitin and PVA blend films in various types of salt solution is different from that obtained from chitosan and poly (acrylic acid) semi-interpenetrating polymer network (semi-IPN). Wang *et al.* (1996) studied the effect of salt type on the degree of swelling of chitosan and poly(acrylic acid) semi-IPN and found that the degree of swelling of semi-IPN increased substantially when ionic valences of salts increased. Semi-IPN exhibited the maximum degree of swelling in the solutions of trivalent salts (Al^{3+}) and the minimum degree of swelling was obtained in the solutions of monovalent salts (K^+ , Na^+). The reason is that chitosan does not bind or bind

weakly to alkali and alkali earth metal ions resulting in lower degree of swelling (Wang *et al.*, 1996)

For PVA films and the blend films with CM-chitin content less than 40%, the effect of different salt types on the change in degree of swelling of the blend films was very small. From Figure 4.15, it was observed that pure PVA films had the lowest degree of swelling. By the addition of CM-chitin to PVA films, the degree of swelling of PVA in salt solutions could be enhanced.

4.5 Mechanical Properties

Because polymeric materials, such as films, may be subjected to various kinds of stress during use, determination of mechanical properties will be useful for practical use. Figure 4.16 shows tensile strength of CM-chitin/PVA blend films as a function of CM-chitin content with and without cross-linking agent. The maximum tensile strength was obtained for the blend films with 50% CM-chitin content for both with and without cross-linking agent. The cross-linked blend films showed a slightly higher tensile strength than non-crosslinked blend films. This may be explained that the crosslinks played a key role as a bridge that links polymer chain together to form the network structure that made the films stronger, and hence the films exhibited a slightly higher tensile strength (Kim *et al.*, 1992a). However, the increase in tensile strength also depended on the amount of cross-linking agent added that is shown in Figure 4.17. The tensile strength increased when the amount of cross-linking agent increased.

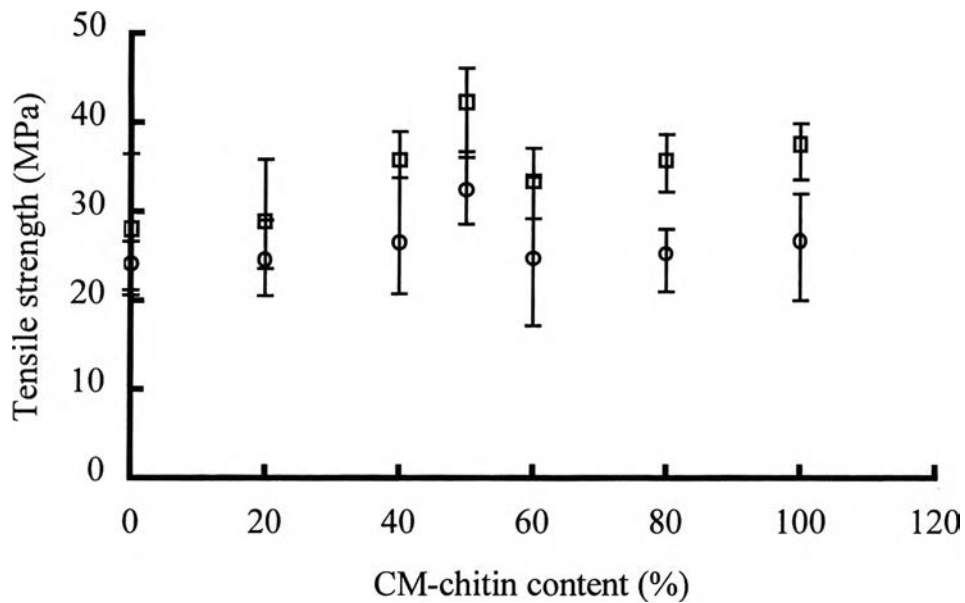


Figure 4.16 Tensile strength of CM-chitin/PVA blend films as a function of CM-chitin content, ○ : films without glutaraldehyde; □ : films with 0.01% glutaraldehyde.

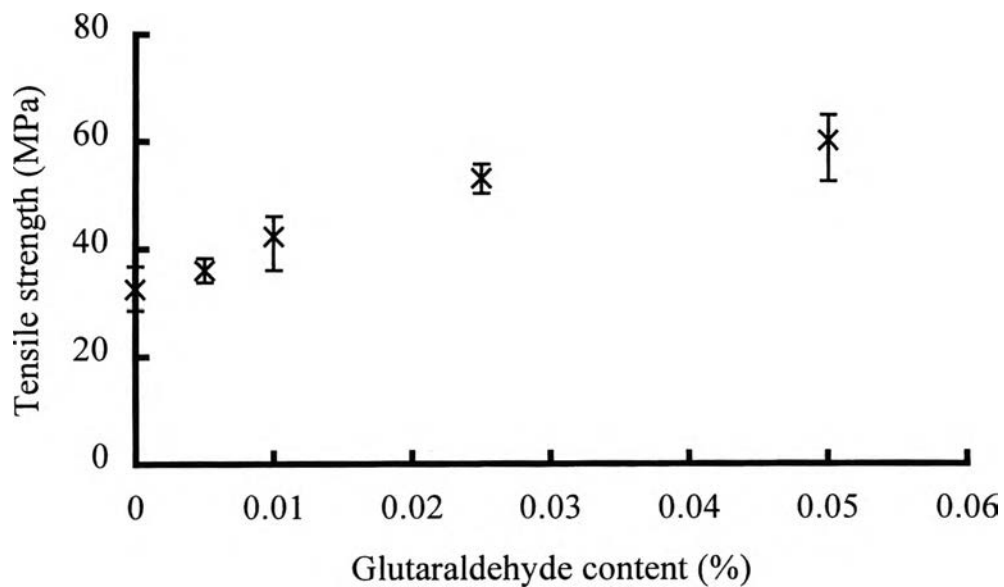


Figure 4.17 Tensile strength of CM-chitin/PVA blend films with 50/50 blend ratio as a function of percent of glutaraldehyde.

The reason for the improvement of tensile strength of CM-chitin/PVA blend films might be due to the occurrence of some specific interactions between CM-chitin and PVA molecules. These interactions are supposed to be hydrogen bonding formations between the hydroxyl groups of PVA and the hydroxyl and carboxymethyl groups of CM-chitin.

Elongation at break is another mechanical property that is important for determining the application of polymeric films. Figure 4.18 shows the elongation at break of the blend films with and without cross-linking agent as a function of CM-chitin content. It was found that the elongation at break of the blend films depended on the CM-chitin content. When CM-chitin content increased, elongation at break decreased.

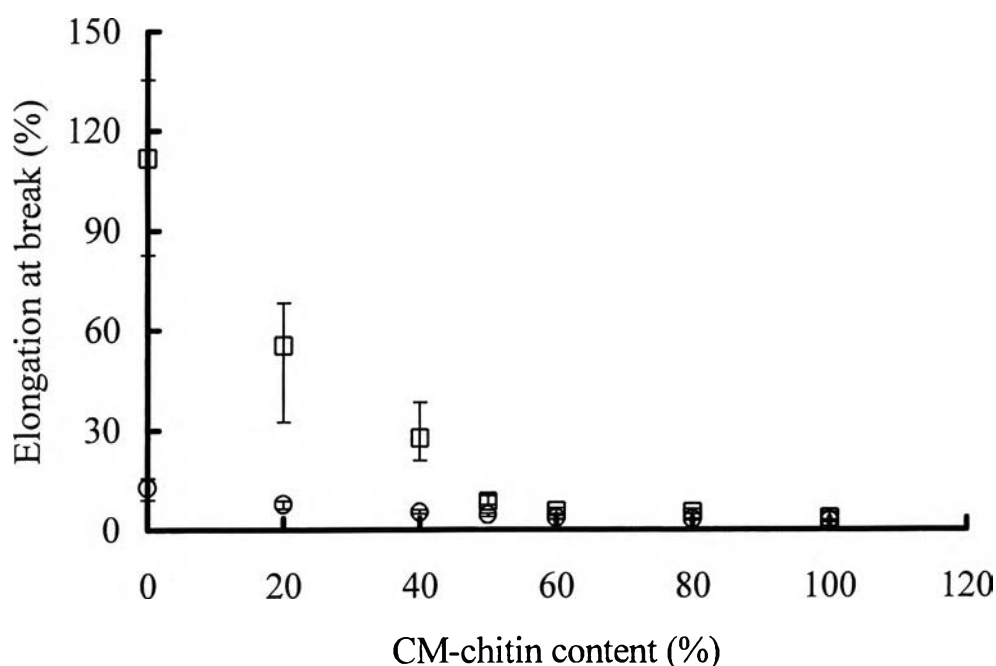


Figure 4.18 Elongation at break of CM-chitin/PVA blend films as a function of CM-chitin content, □ : films without glutaraldehyde; ○ : films with 0.01% glutaraldehyde.

For both with and without cross-linking agent, the elongation at break of the blend films was constant when CM-chitin content was over 50%. It

may be due to the structure of CM-chitin was more rigid than PVA. These amounts of CM-chitin restricted the motion of movement of the chains resulted in dramatically decreasing of the elongation at break of the blend films.

It was considered that the crosslinked blend films had a lower elongation at break than the non-cross-linked blend films since CM-chitin and PVA chains were held together by cross-linking agent. In addition, the presence of crosslinks limited the extensibility of CM-chitin and PVA chains resulting in decrease of elongation at break. Moreover, higher amount of glutaraldehyde caused lower elongation at break as shown in Figure 4.19.

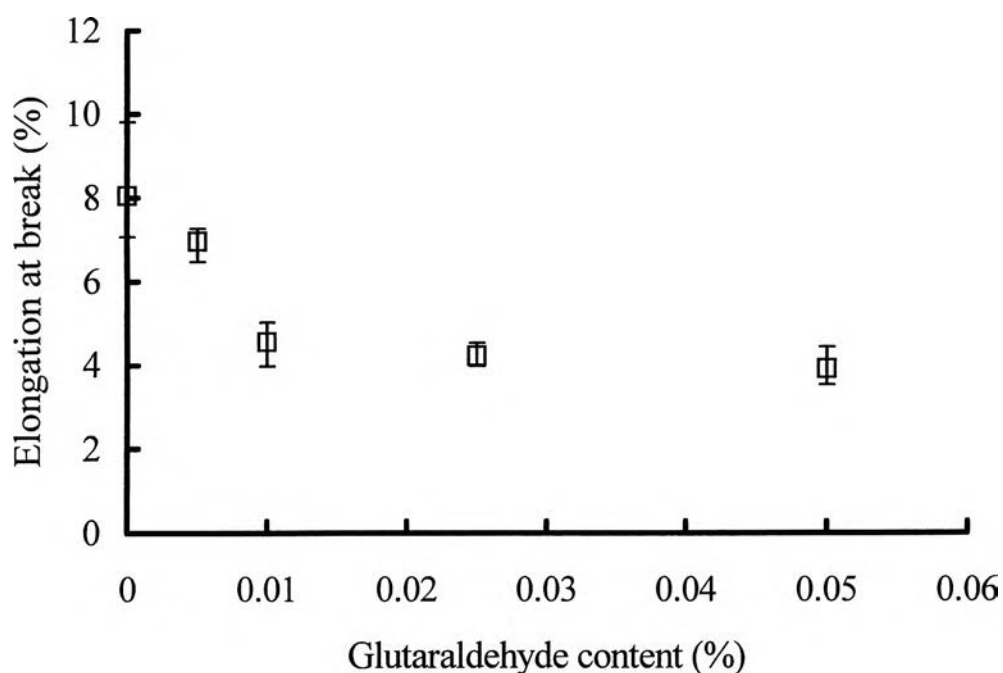


Figure 4.19 Elongation at break of CM-chitin/PVA blend films with 50/50 blend ratio as a function of glutaraldehyde.

When glutaraldehyde content was over 0.01%, the elongation at break was constant. This means 0.01% of glutaraldehyde was a proper content to bind almost all functional groups CM-chitin and PVA together.

Lee *et al.*, (1996) prepared the blend films of β -chitin and PVA from the solution of formic acid solvent system. They found that as PVA content increased, the tensile strength decreased and elongation at break increased. The blend films had the maximum tensile strength at 70% β -chitin content. They reported that the enhancement of tensile strength resulted from the existence of specific interaction between β -chitin and PVA.

Kim *et al.*, (1992a) prepared the blend films of PVA and chitosan from the solvent-casting technique. They found that the blend films that composed PVA 1.5 g, chitosan 1 g and glutaraldehyde 6×10^{-6} mol/g polymer showed the higher tensile strength than pure PVA and chitosan films. This effect can be explained from the fact that blending of chitosan with PVA leads to an intermolecular interaction between them within a reasonable range of composition. For the cross-linked blend, the tensile strength increased with the amount of cross-linking agent.

4.6 Oxygen Permeability

The oxygen permeability rates of CM-chitin/PVA blend films were determined as a function of CM-chitin content. The result is shown in Figure 4.20. The oxygen permeability rate of PVA film was $1,251,384 \text{ cm}^3/\text{m}^2 \text{ d bar}$ and that of CM-chitin film was $564,362 \text{ cm}^3/\text{m}^2 \text{ d bar}$.

The blend films of all compositions had a remarkable reducing in oxygen permeability rates as compared to pure PVA film. The minimum oxygen permeability rate was obtained for the blend film with 20% CM-chitin content. The dramatically decreasing of oxygen permeability rate of blend films as compared to pure PVA films is possibly due to the presence of CM-chitin content, which has oxygen permeability rate lower than PVA films, in the blend films. It may be due to the packing structure of CM-chitin. As a

result, the oxygen permeation through the films was restricted. (Yoshimizu and Asakara, 1990).

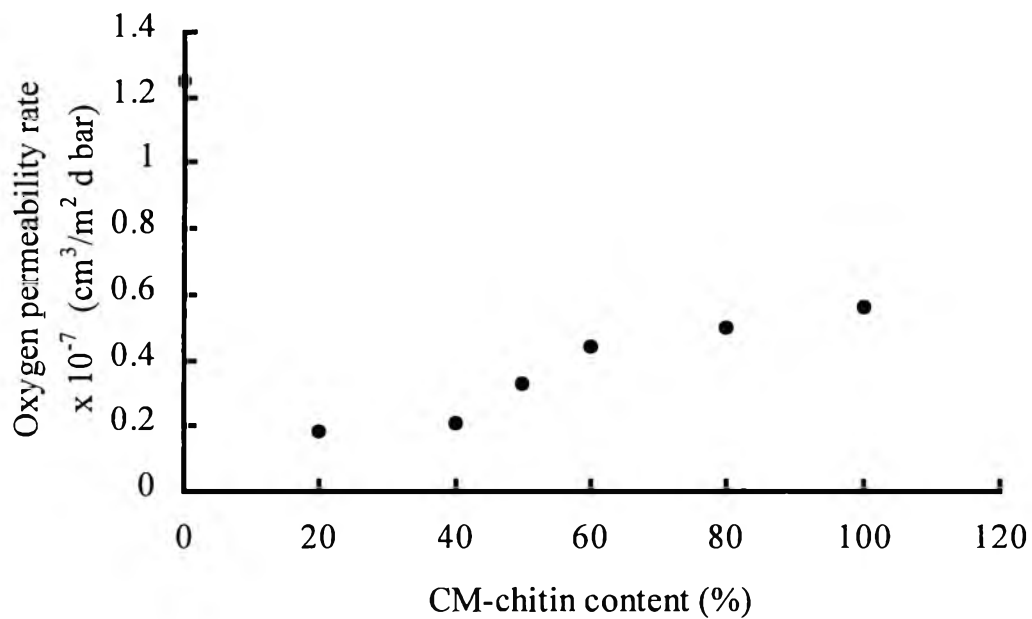


Figure 4.20 Oxygen permeability rate of CM-chitin/PVA blend films as a function of CM-chitin content.