



## CHAPTER IV RESULTS AND DISCUSSION

### 4.1 Preparation of Chitin

Shrimp shells compose of three major components, which are chitin, calcium carbonate, and protein. Calcium carbonate and protein can be removed by solvent extraction and chitin will be obtained as the remaining substance.

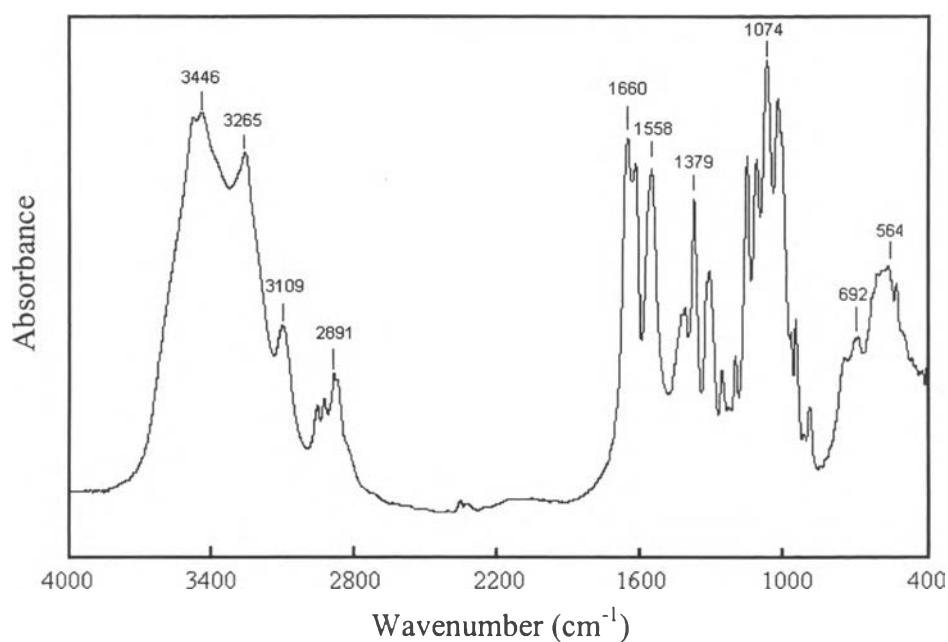
In this research, chitin was prepared from shells of *Penaeus merguensis* shrimp by demineralization with hydrochloric acid solution and deproteinization with sodium hydroxide solution in order to remove the calcium carbonate and the protein, respectively. The yield obtained during chitin production is shown in Table 4.1.

**Table 4.1.** Yield of chitin production from shrimp shell

Material	Yield* (%)
Shrimp shell	100
Product after demineralization	48.67
Product after deproteinization (chitin)	31.30

\*dry weight basis

FTIR spectrum of chitin is showed in Figure 4.1. 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 inevitably subjected to N-deacetylation during deproteinization process under alkaline condition and heating. The degree of deacetylation of chitin was 30.41 %.



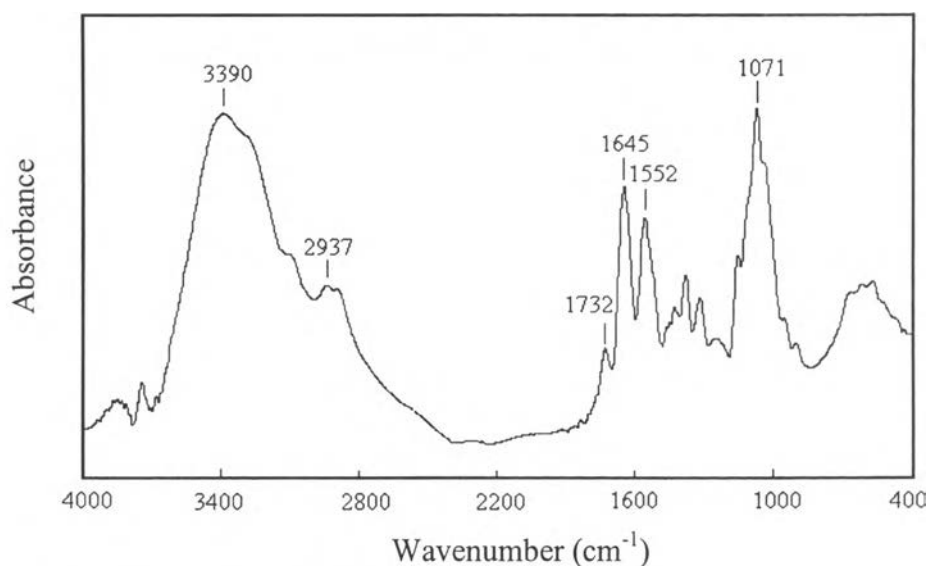
**Figure 4.1** FTIR spectrum of chitin powder.

#### 4.2 Preparation of CM-Chitin

Chitin is insoluble in common solvents. Thus the dissoluble property of chitin can be improved by chemical modification. Chitin was modified to be CM-chitin, a water-soluble derivative of chitin. The FTIR spectrum of CM-chitin is shown in Figure 4.2 and the absorption frequencies of characteristic bands of CM-chitin are summarized in Table 4.2.

**Table 4.2.** FTIR characteristic absorption bands of CM-chitin

Frequencies (cm <sup>-1</sup> )	Assignment and remarks
1732	C=O stretching of carboxylic group
1645	C=O stretching of acetamide group
1552	NH deformation
1071	C-O stretching vibration



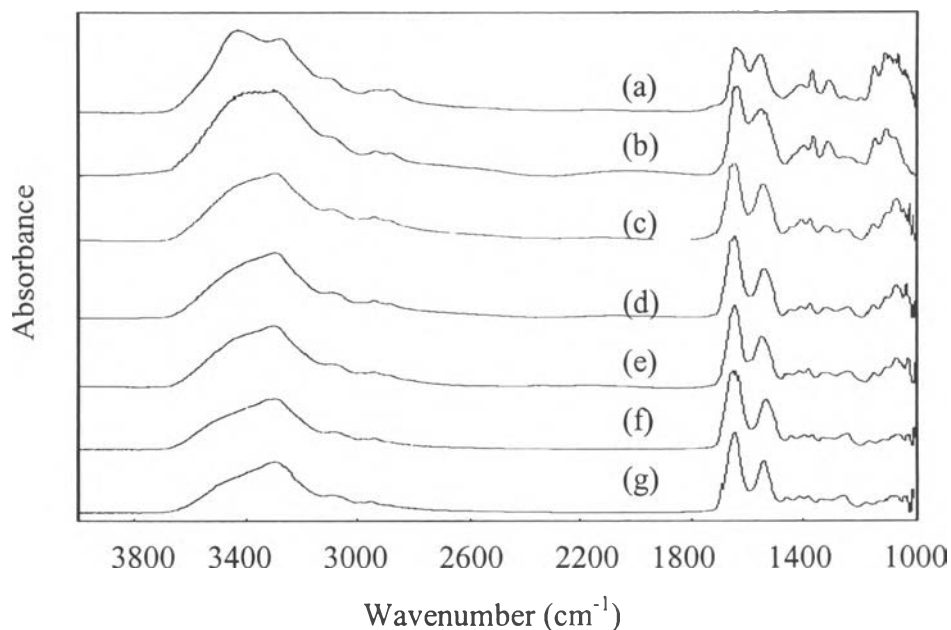
**Figure 4.2** FTIR spectrum of CM-chitin.

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 intrinsic viscosity was 6.4 (100 ml/g). The viscosity-average molecular weight of CM-chitin obtained from the calculation was  $8.08 \times 10^4$  g/mol.

### 4.3 Characterization of CM-Chitin/Silk Fibroin Blend Films

#### 4.3.1 FTIR Analysis of Blend Films

The conformation characterization of pure and blend films, as well as the study of specific interactions between CM-chitin and silk fibroin were carried out by using FTIR spectrometer. The FTIR spectrum of silk fibroin [Figure 4.3(g)] shows the characteristic absorption bands at  $1656 \text{ cm}^{-1}$  (amide I),  $1541 \text{ cm}^{-1}$  (amide II), and  $1243 \text{ cm}^{-1}$  (amide III), assigned to random coil form. The FTIR spectrum of CM-chitin [Figure 4.3(a)] shows the characteristic absorption bands at  $1647 \text{ cm}^{-1}$  (amide I),  $1561 \text{ cm}^{-1}$  (amide II). The FTIR spectra of CM-chitin/silk fibroin blend films [Figure 4.3(b-f)] are characterized by the presence of absorption bands of the pure components, of which intensities are roughly related to the blending ratio.

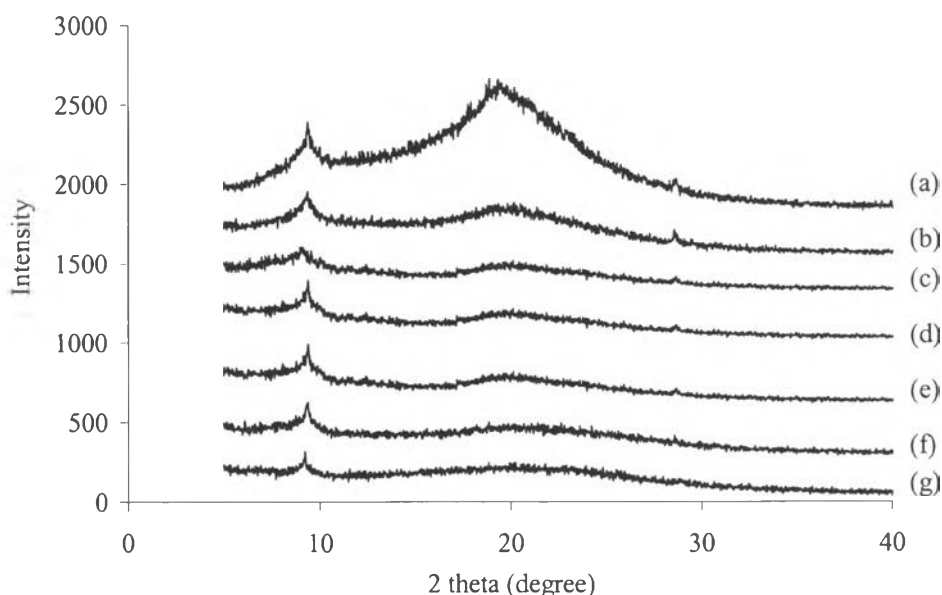


**Figure 4.3** FTIR spectra of pure and blend films at various compositions of CM-chitin to silk fibroin, CM-chitin/silk fibroin composition: (a) 100/0 (CM-chitin); (b) 80/20; (c) 60/40; (d) 50/50; (e) 40/60; (f) 20/80; and (g) 0/100 (silk fibroin).

#### 4.3.2 X-ray Diffraction Patterns

Wide-angle X-ray diffraction (WAXD) patterns of the films are shown in Figure 4.4. CM-chitin films exhibited crystalline peaks at  $2\theta = 9.4^\circ$  and  $2\theta = 19.3^\circ$  [Figure 4.4(a)], whereas silk fibroin films showed a non-crystalline structure [Figure 4.4 (g)]. According to Freddi *et al.* (1995), the dissolution of silk fibroin was caused by reagent penetrating into the adjacent chains, resulting in breaking hydrogen bonds between polymer chains. This led to the decrease in crystallinity of silk fibroin films as compared to the original silk fibroin fiber. The pattern of the blend films exhibited a gradual transformation from characteristic crystalline peaks of CM-chitin to the completely amorphous pattern of silk fibroin with the increasing of silk fibroin content in the blend films. Chen *et al.* (1997) studied the crystallinity of pure silk fibroin membrane and found that the diffraction pattern of pure silk fibroin membrane showed no clear  $2\theta$  peak. Our result revealed that the diffraction patterns of pure and blend films did not give clear information about the crystallinity because the crystalline structures of CM-chitin and silk fibroin were remarkably

during dissolution process. Therefore, the CM-chitin/silk fibroin blend films were mainly amorphous.

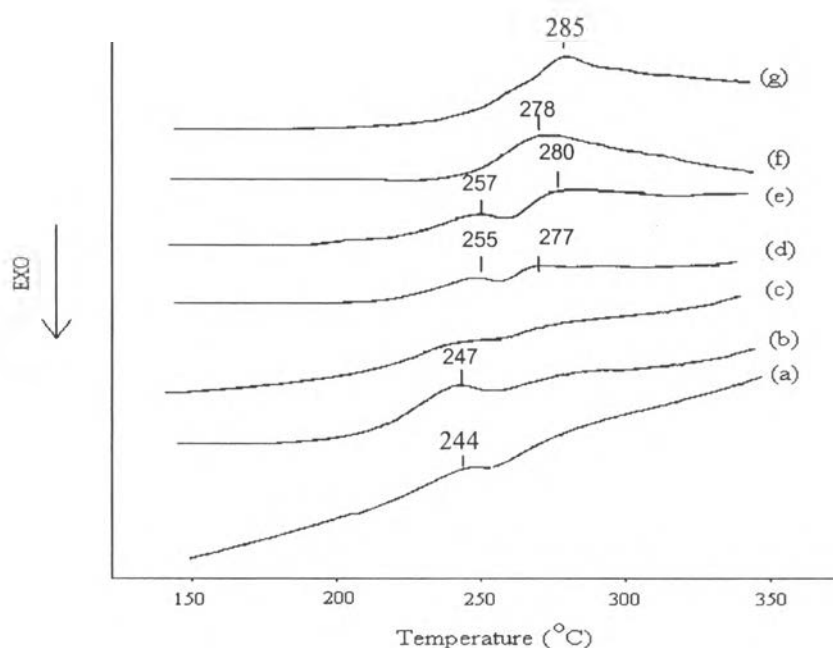


**Figure 4.4** Wide-angle X-ray diffraction patterns of CM-chitin/silk fibroin blend films, CM-chitin/silk fibroin composition: (a) 100/0 (CM-chitin); (b) 80/20; (c) 60/40; (d) 50/50; (e) 40/60; (f) 20/80; (g) 0/100 (silk fibroin).

#### 4.3.3 Thermal Property

Thermal property of pure and blend films were characterized by differential scanning calorimeter (DSC). The DSC thermograms of pure and blend films are shown in Figure 4.5. The pure silk fibroin film showed an endothermic peak at 284.86°C [Figure 4.5(g)]. According to Tsukada *et al.* (1994), who studied thermal property of silk fibroin/PVA blend films, it was demonstrated that silk fibroin with low degree of crystallinity thermally decomposed at around 280-290°C. Therefore, it suggested that the endotherms observed at 284.86°C should be attributed to the thermal decomposition of silk fibroin film. The pure CM-chitin film showed an endothermic peak at 244.70°C [Figure 4.5(a)]. The thermograms of blend films are showed in Figure 4.5(b-f). The DSC thermograms of the blend films showed the broad peak of decomposition endotherm in lower temperature range than

the decomposition endotherm of silk fibroin and observed an upward shift of endothermic peak of CM-chitin. It might be suggested that there was some intermolecular interactions occurred between CM-chitin and silk fibroin. Accordingly to Freddi *et al.* (1999), who studied structure and physical properties of silk fibroin/polyacrylamide blend films, it was found that there was some changes appearing in the DSC pattern of blend films with low polyacrylamide content ( $\leq 25\%$ ) which a slight broadening of the decomposition endotherm in the lower temperature range, and a downwards shift of the crystallization peak were observed. It might be suggested that a certain degree of interaction was established between silk fibroin and polyacrylamide.



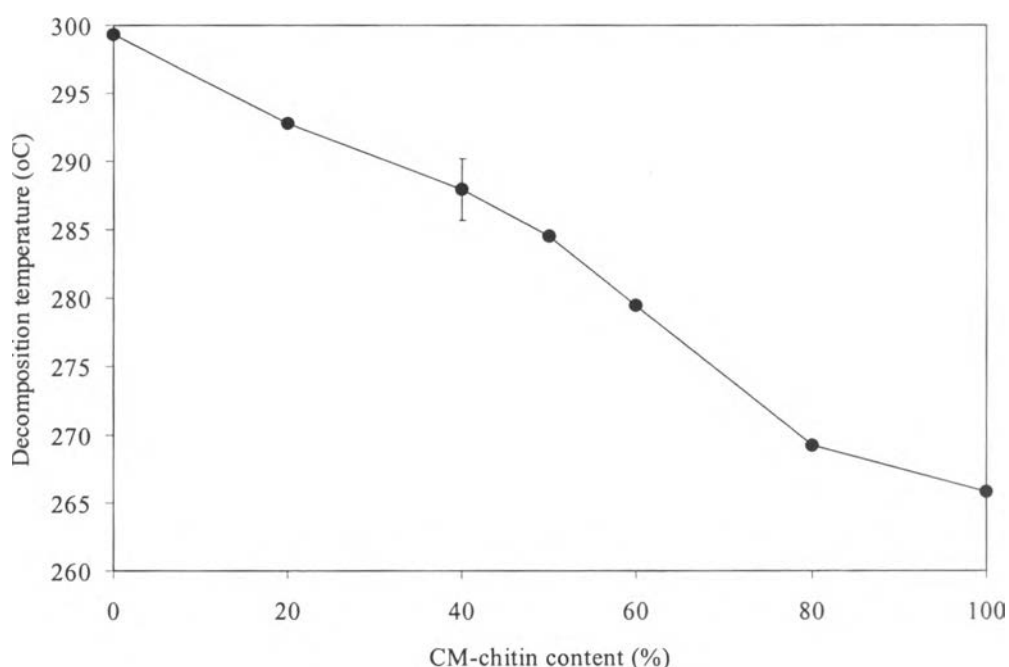
**Figure 4.5** DSC thermograms of CM-chitin/silk fibroin blend films, CM-chitin/silk fibroin composition: (a) 100/0 (CM-chitin); (b) 80/20; (c) 60/40; (d) 50/50; (e) 40/60; (f) 20/80; (g) 0/100 (silk fibroin).

#### 4.3.4 Thermal stability

The decomposition temperatures of pure and blend films (Figure 4.6) were characterized by thermogravimetric analysis (TGA). Pure silk fibroin and CM-

chitin films showed the decomposition temperatures at 299.34°C and 265.70°C, respectively. The decomposition temperatures of the blend films were in the range of the decomposition temperatures of pure silk fibroin and pure CM-chitin. The result showed that the decomposition temperatures of the blend films decreased with increasing CM-chitin content. This suggests that there was some intermolecular interaction between CM-chitin and silk fibroin. If there was no intermolecular interaction, the result of decomposition temperature would be expected to occur at two temperature. The first temperature occurred at decomposition temperature of CM-chitin and the second temperature occurred at decomposition temperature of silk fibroin.

By blending with silk fibroin, the thermal stability of the blend film could be improved as compared to pure CM-chitin blend film.



**Figure 4.6** Decomposition temperature of CM-chitin/silk fibroin blend films as a function of CM-chitin content.

These results agree with the work of Freddi *et al.* (1999) who carried out thermogravimetric analysis of silk fibroin/Polyacrylamide blend films. The thermal

behavior of silk fibroin/polyacrylamide blend films were between the two pure components.

#### 4.4 Swelling Study

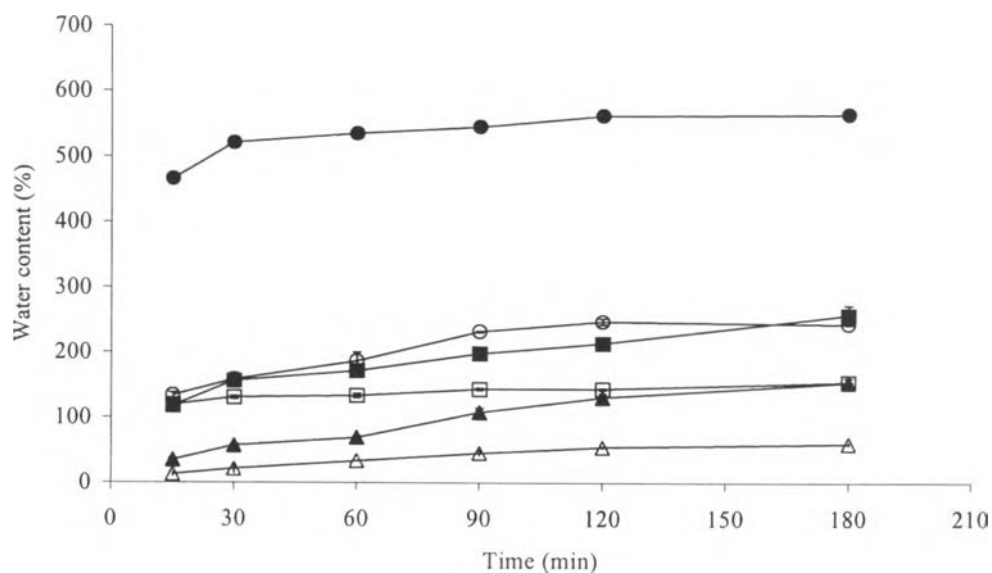
In this research, pure silk fibroin film could not be studied because the film was very brittle and cracked after water was blotted out from the film surface.

##### 4.4.1 Equilibrium Water Content (EWC)

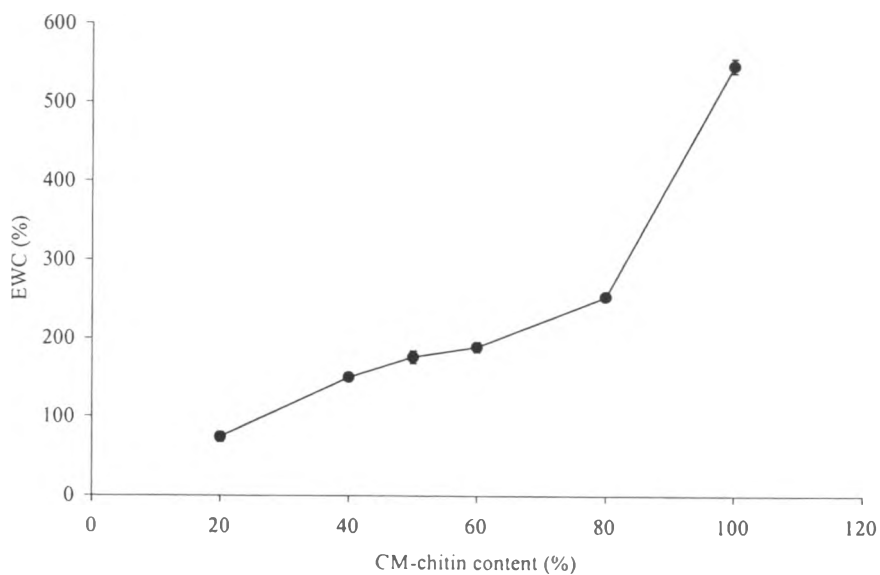
The effect of immersion time on the water content of the blend films is shown in Figure 4.7. The blend films were immersed in distilled water for 24 h. The water absorption of the films were remarkably increased within 15 min after immersed films in distilled water. This lead to the rapidly increasing water contents in the films. After that water contents of the films were gradually increased when the immersion time increased and became rather constant within 3 h. It was found that the most films reached to equilibrium state within 3 h. While some blend films ratio; 60 and 40% CM-chitin content, it still showed a slightly increased.

Figure 4.8 shows EWC of the films as a function of CM-chitin content. The EWC of pure CM-chitin film was approximately 545%. The EWC of pure silk fibroin films could not be determined because it was very brittle and cracked after water was blotted out from the film surface. The EWC of blend films increased with increasing CM-chitin content. Khor *et al.* (1996) suggested that the ability to absorb water of CM-chitin films is attributed to the introduction of carboxymethyl groups on the glucosamine residues. The presence of carboxymethyl groups distributing along chitin chains disrupts the H-bonding interactions between adjacent chitin chains.





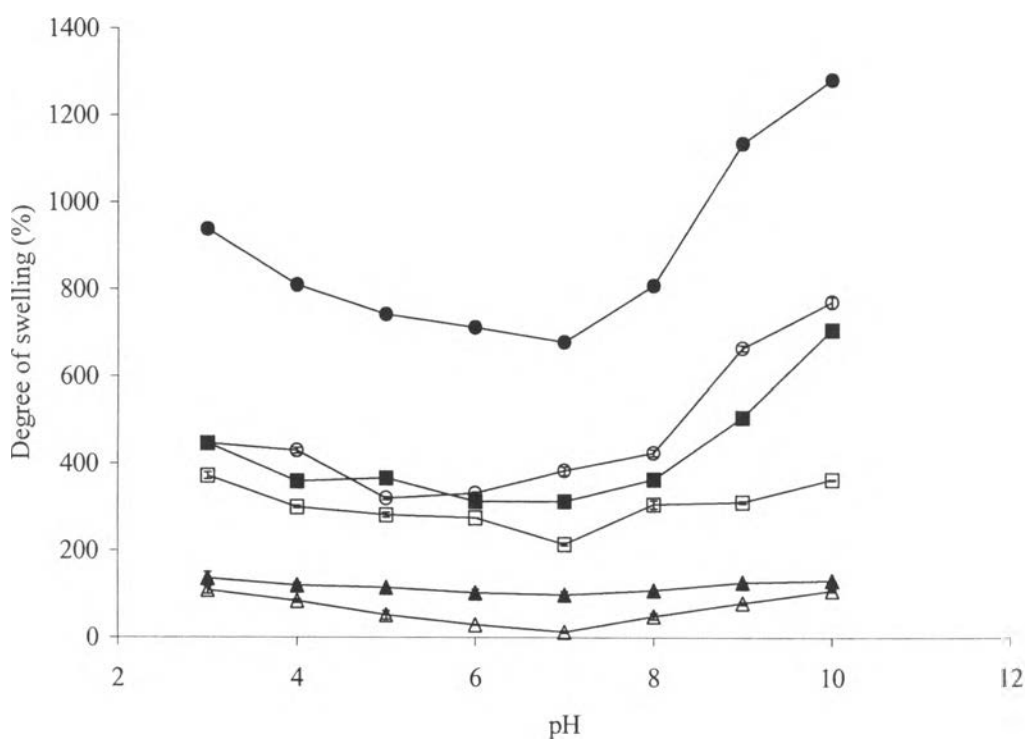
**Figure 4.7** Effect of immersion time on equilibrium water content of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde. CM-chitin/silk fibroin composition: ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; and △ 20/80.



**Figure 4.8** Equilibrium water content of CM-chitin/silk fibroin blend films with the addition of 0.01% glutaraldehyde.

#### 4.4.2 Effect of pH

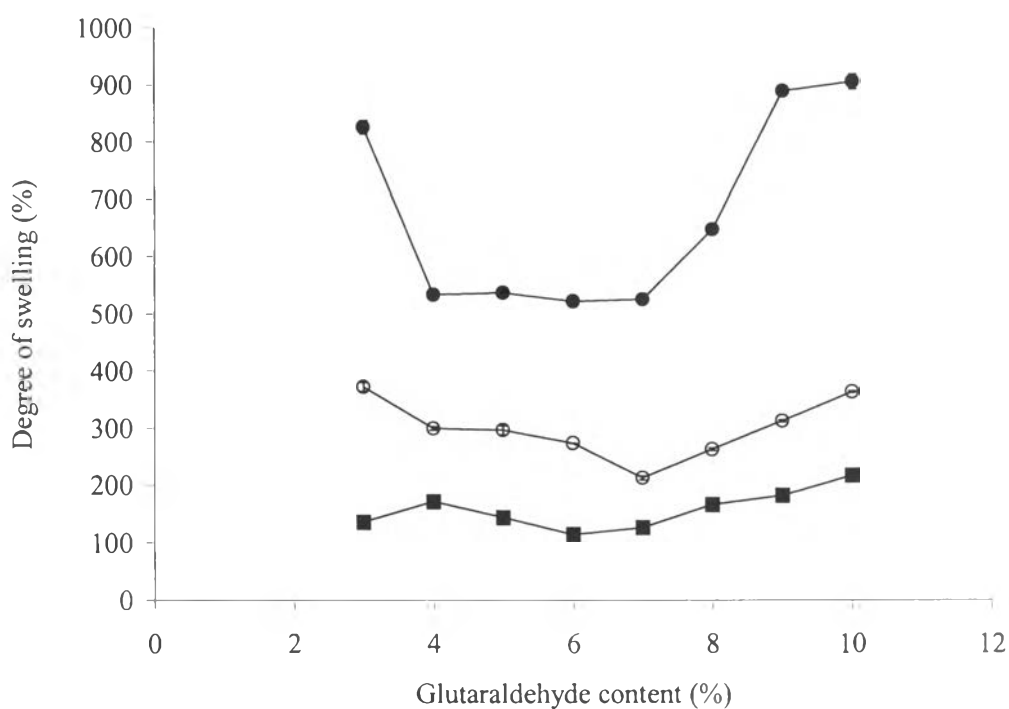
The effect of CM-chitin and the blend films of pH on the degree of swelling are shown in Figure 4.9. It was found that pure CM-chitin and blend films could swell in both acidic and alkaline pH. Tokura *et al.* (1983a) reported that the  $pK_a$  values of the carboxyl group and amino group of CM-chitin are 3.40 and 6.40, respectively. At pH less than 7 (acidic pH solution), the degrees of swelling of CM-chitin and blend films slightly increased, suggesting that the amino groups of CM-chitin molecules protonized, leading to the dissociation of the adjacent chains. At pH higher than 7 (alkaline pH solution), the degrees of swelling of CM-chitin and blend films increased. This could be explained that the carboxymethyl groups ionized, leading to the dissociation of the adjacent chains.



**Figure 4.9** Degree of swelling of CM-chitin/silk fibroin blend films with the addition of 0.01% glutaraldehyde as a function of pH. CM-chitin/silk fibroin composition: ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; △ 20/80.

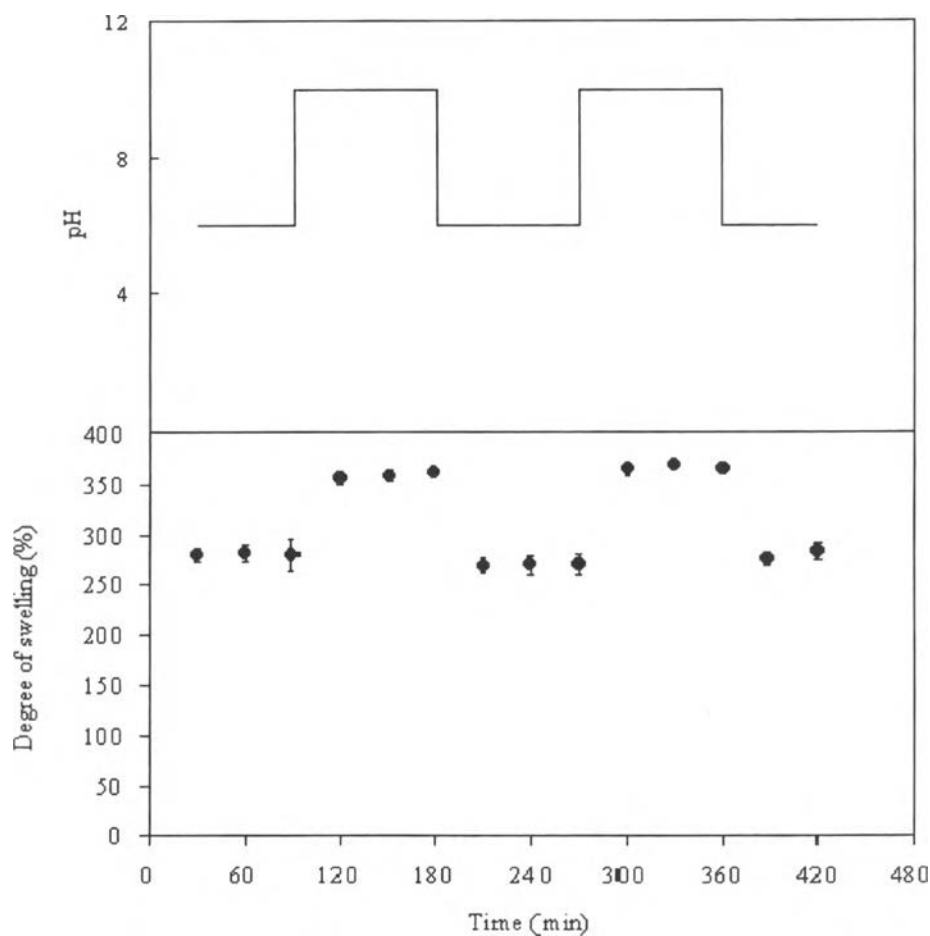
The effect of glutaraldehyde concentration on the swelling property of the blend film at 50/50 blend ratio as a function of pH is shown in Figure 4.10. The

degree of swelling decreased with increasing of glutaraldehyde concentration in the films. When the concentrations of cross-linking agent increased more hydroxyl groups in CM-chitin was consumed due to the cross-linking reaction.



**Figure 4.10** Effect of glutaraldehyde concentration on degree of swelling of CM-chitin/silk fibroin blends film as a function of pH. ● 0.005% glutaraldehyde; ○ 0.01% glutaraldehyde; ■ 0.05% glutaraldehyde.

The response of the blend films with 50% CM-chitin content to a step change in pH is shown in Figure 4.11. In this experiment, the films were brought into a buffer solution at pH 6 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. The result showed that swelling behavior of the blend films was reversible when the environmental pH changed. The blend films showed a pH-sensitive swelling characteristic that may be applicable to a controlled-release system.

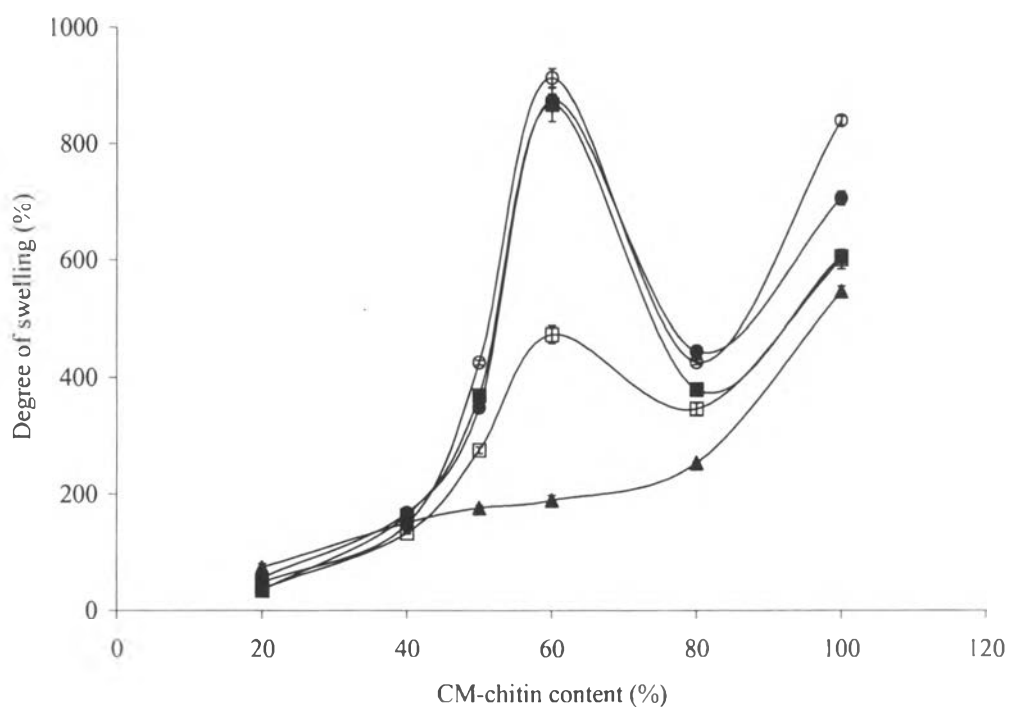


**Figure 4.11** Degree of swelling of CM-chitin/silk fibroin blend films with 50/50 blend ratio containing 0.01% glutaraldehyde on a step change in pH.

#### 4.4.3 Effect of Salt Type

The degree of swelling of CM-chitin/silk fibroin blend films in various types of salt solutions is shown in Figure 4.12. The salt solutions used in this study were NaCl, LiCl, CaCl<sub>2</sub> and FeCl<sub>3</sub> solutions. The concentration of salt solution was 0.25M. It was found that the blend film with 60% CM-chitin content showed the highest degree of swelling in NaCl, LiCl, and CaCl<sub>2</sub> solution. In other word, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solutions (NaCl and LiCl) and divalent salt solution (CaCl<sub>2</sub>). For

pure CM-chitin films, the degrees of swelling in salt solutions ranging from the highest to the lowest were in the following order: NaCl > LiCl > CaCl<sub>2</sub>, FeCl<sub>3</sub>. Tokura *et al.* (1983b) studied the specific binding site of calcium ions on CM-chitin (water-insoluble one with, DS=0.28). It was found that CM-chitin could bind with calcium ions even in the presence of monovalent cations such as sodium or potassium. The IR spectrum between the CM-chitin and Ca<sup>2+</sup>-loaded CM-chitin appeared that the tetrahedral chelation of CM-chitin toward Ca<sup>2+</sup> is assisted by the acetamide and hydroxyl groups in addition to carboxyl groups.

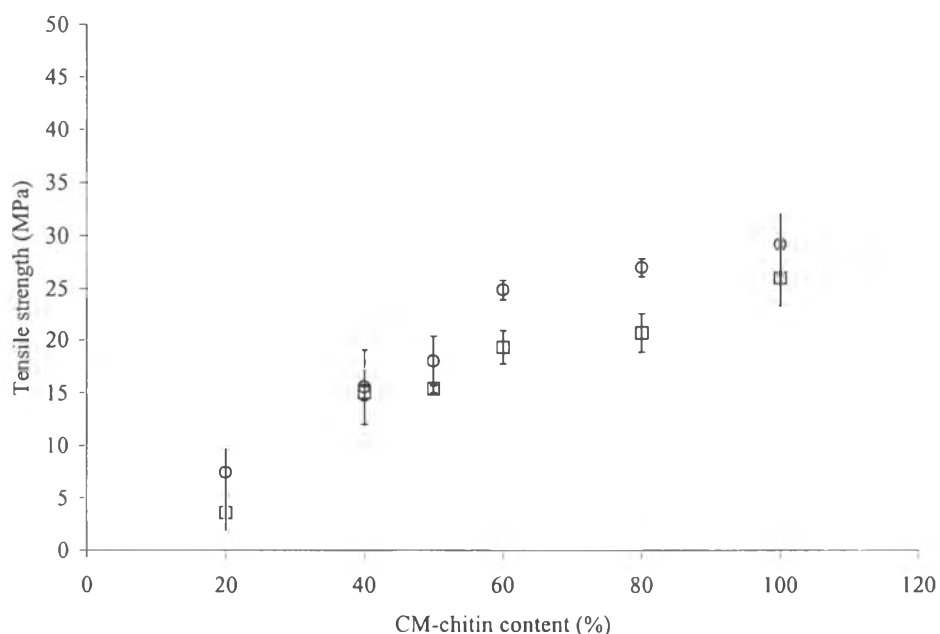


**Figure 4.12** Effect of salt types on degree of swelling of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde as a function of CM-chitin content. ● LiCl 100/0; ○ NaCl; ■ CaCl<sub>2</sub>; □ FeCl<sub>3</sub>; ▲ H<sub>2</sub>O.

## 4.5 Mechanical Properties

### 4.5.1 Tensile Strength

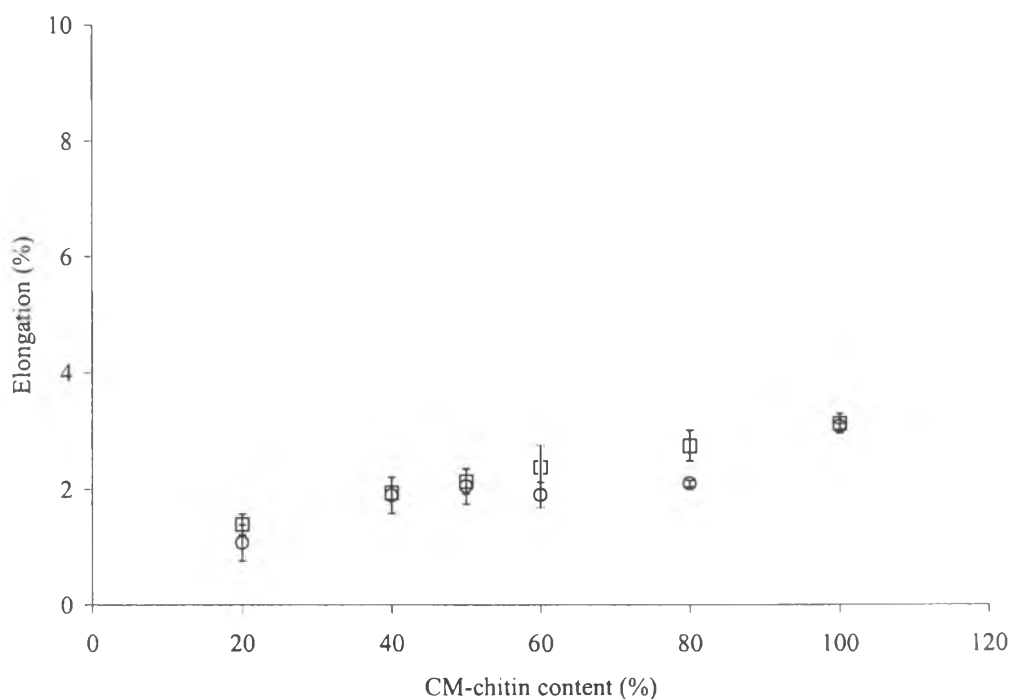
The tensile strength of the films as a function of CM-chitin content is shown in Figure 4.13. The tensile strength of pure silk fibroin could not be monitored by Lloyd tensile tester because of their brittleness. The tensile strength of the blend films increased with increasing CM-chitin content in the blend films. The cross-linked blend films showed a slightly higher tensile strength than non-cross-linked 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 film stronger, and hence the films exhibited a slightly higher tensile strength (Kim *et al.*, 1992).



**Figure 4.13** Tensile strength of CM-chitin/silk fibroin blend films as a function of CM-chitin content, □: films without glutaraldehyde; ○: films with 0.01% glutaraldehyde.

#### 4.5.2 Elongation at Break

Elongation at break is another mechanical property that is important for determining the application of polymeric films. Figure 4.14 shows the elongation at break of pure and the blend films with and without cross-linking in the dry state. This figure does not show the values of pure silk fibroin film because of the brittleness of the films. The elongation at break of the blend films increased with increasing CM-chitin content in the blend film. The cross-linked blend films showed a slightly lower elongation at break than non-cross-linked blend films. Because the presence of cross-links limited the extensibility of CM-chitin and silk fibroin chains resulting in decrease of elongation at break.



**Figure 4.14** Elongation at break of CM-chitin/silk fibroin blend films as a function of CM-chitin content, □: films without glutaraldehyde; ○: films with 0.01% glutaraldehyde.