CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization of Chitin

4.1.1 Chitin production

Shrimp shells consist of three major components which are (1) chitin, (2) calcium carbonate and (3) protein. Since the chitin does not dissolve in common solvents. Calcium carbonate and protein can be removed by solvent extraction and chitin will be obtained as the remaining portion.

In this research, chitin was prepared from shells of *Penaeus merguiensis* shrimp by demineralization with hydrochloric acid solution and deproteinization with aqueous sodium hydroxide solution in order to remove the calcium carbonate and the protein, respectively. The yield obtained during chitin production is shown in Table 4.1.1. 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	53
Production after deproteinization (chitin)	30

*dry weight basis

4.1.2 Determination of the Degree of Deacetylation (%DD) of Chitin

The method used to determine degree of deacetylation of chitin was based on infrared spectroscopic measurement by Sannan *et al.* (1978). The degree of deacetylation (%DD) was calculated from equation 3.1.

$$DD\ (\%) = 98.03 - 34.68(A_{1550}/A_{2878}) \tag{3.1}$$

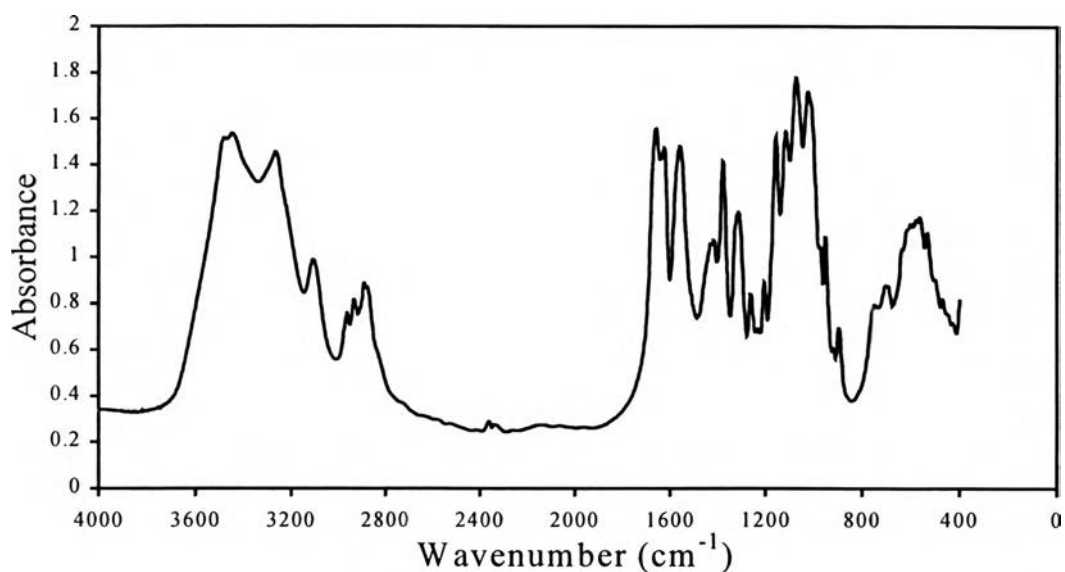When A_{1550} and A_{2878} are absorbances at 1550 cm^{-1} and 2878 cm^{-1}, respectively.

Figure 4.1 FTIR spectrum of chitin powder.

Chitin has some extent of amino groups rather 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 sujected to N-deacetylation deproteinization process under alkaline condition and heating. According to the method of Sannan *et al.* (1978), the degree of deacetylation of chitin was calculated from FTIR to be 21.34%.

4.1.3 Determination the Molecular Weight of Chitin

The method used to determine the molecular weight of chitin was based on viscosity measurement by the method of Rutherford *et al.* (1978). The viscosity-average molecular weight of chitin was determined based on Mark-Houwink equation (eqn. 3.2)

$$[\eta] = 8.93 \times 10^{-4} M^{0.71} \tag{3.2}$$

Where $\quad [\eta]$ = Intrinsic viscosity (l/g)

$\quad$ M = Viscosity-average molecular weight

The intrinsic viscosity was determined from the Y-intercept of the plot between $[\eta_{sp}]/c$ versus chitin concentration (g/l) and ln $[\eta_{rel}]/c$ versus chitin concentration (g/l). The intrinsic viscosity was obtained at 20 l/g. The viscosity-average molecular weight of chitin was 3.14×10^6 g/mole.
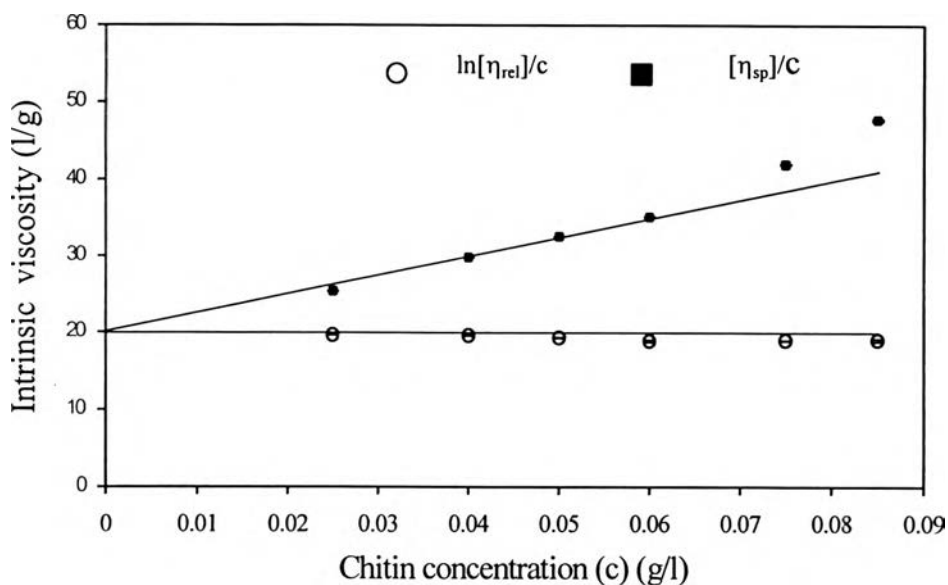
Figure 4.2 The relative viscosity [η_{rel}] and the reduce viscosity [η_{red}] as a function of chitin concentration.

4.2 Film preparation

The cosolvent for dissolving chitin and cellulose is LiCl/DMAc. Dissolution of chitin and cellulose requires swelling of the structure by mean of reagents capable of penetrating between adjacent chains and breaking hydrogen bonds thus leading to complete dispersion of the constituent polymer chains.

Chitin films were prepared by solvent evaporation of chitin solution. Care has to be exercised during solvent evaporation to prevent film shrinkage. The shrinkage also occurred with cellulose films due to the characteristic of polysaccharide polymers. Aiba *et al.* (1985) investigated the effect of coagulation solvents on chitin membranes prepared by casting from solution of 100:100:10:3 parts of N,N-dimethylacetamide(DMAc):N-methylpyridone(NMP):LiCl:chitin. It was found that coagulation of chitin

membranes in acetone resulted in opaque films. Films coagulated in methanol, ethanol, and a mixture of 2-propanol and water had moderate shrinkage and were not flat. The films coagulated in 2-isopropanol were flat and transparent. In this study, pure and the chitin blend films were coagulated in 2-isopropanol. The resulting blend films had moderate shrinkage and were transparent. The thickness of the films measured by digital thickness gauge was in the range of 10-50 μm.

4.3 FTIR analysis of the blend films

FTIR spectroscopy is one of the most powerful techniques for investigation of multi-component systems, because it provides information on the blend composition as well as on the polymer-polymer interaction (Lee *et al.*, 1994). Figure 1 shows the FTIR spectra of chitin, cellulose and the blend films measured at wavenumber ranging from 2000-400 cm^{-1}. The peaks at around 1660 cm^{-1}, 1625 cm^{-1} and 1554 cm^{-1} indicated the amide bond of chitin as shown in Figure 1(a). The peaks at 1660 cm^{-1} and 1625 cm^{-1} were assigned to the amide I band of chitin. The peak at 1660 cm^{-1} was assigned to $-C=O$ stretching, when $-C=O$ is linked by hydrogen bond to -NH group and the peak at 1625 cm^{-1} was due to $-C=O$ stretching, when $-C=O$ is bonded to hydroxyl group (Bianchi *et al.*, 1995). The peak at 1554 cm^{-1} indicated the amide II band of chitin (Kurita *et al.*, 1993). Cellulose exhibited the boarder peak at the wavenumber of 1613-1656 cm^{-1} as shown in Figure 1(f). It possibly attributed to $-C=O$ stretching, where $-C=O$ is bonded to hydroxyl group. The FTIR spectra of chitin/cellulose blend films [Fig. 1(b-e)] are characterized by the presence of absorption bands of the pure components, whose intensities are roughly related to the blending ratio.
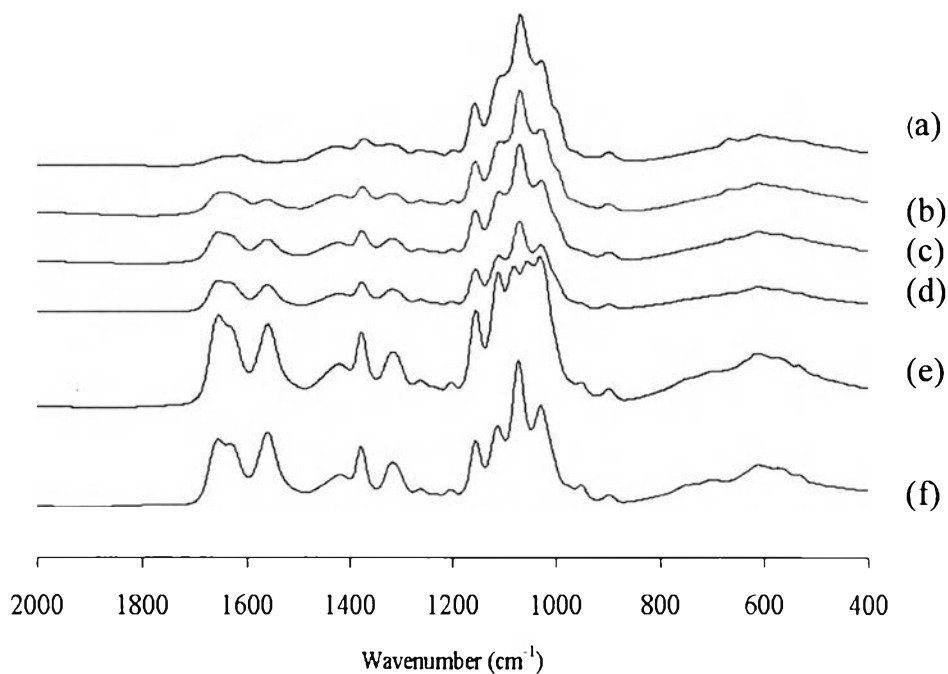
Figure 4.3 The FTIR spectra of the chitin/cellulose blend films: (a) Chitin (CT); (b) CT/CE 80/20; (c) CT/CE 60/40; (d) CT/CE 40/60; (e) CT/CE 20/80; and (f) Cellulose (CE).

4.4 X-ray analysis of blend films

Wide-angle x-ray diffraction (WAXD) patterns of the films were measured to compare the crystalline structure of pure chitin and cellulose films with the blend films. The x-ray diffraction pattern of the films is shown in Figure 2. The crystalline structure of chitin and cellulose films appeared to be nearly the same positions of 2θ degree. Figure 2(a) shows that chitin exhibited crystalline peaks at around $2\theta = 9.8°$ and $2\theta = 19.3°$. Cellulose showed the crystalline peak at $2\theta = 9.8°$ and $2\theta = 19.8°$. The x-ray diffraction pattern of cellulose was similar to that of chitin, but cellulose had boarder peak than chitin. In the x-ray diffraction pattern of the blend films, no peak

other than those of chitin and cellulose were observed. For the blends, their reflection patterns are similar to those of chitin and cellulose with small reducing in their intensity. The boarder pattern indicated that the lower crystallinity or less packing in the main chain as compared to pure chitin and cellulose films (Kim *et al.*, 1996)
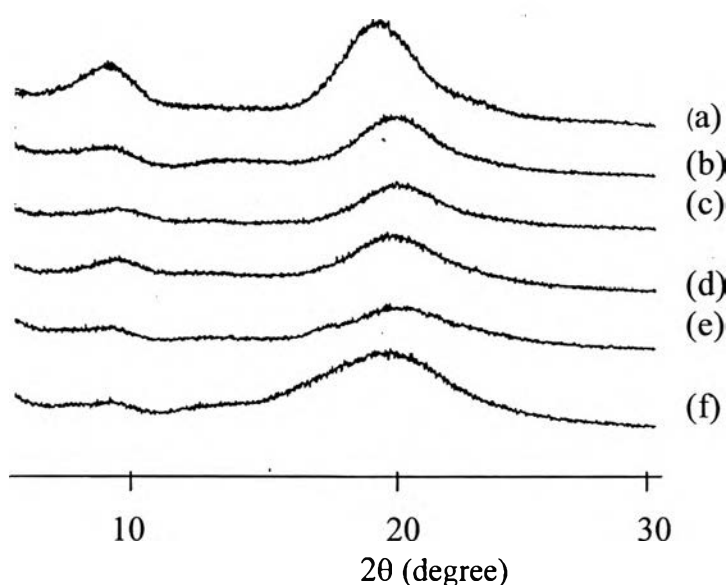

Figure 4.4 The x-ray diffraction pattern of chitin/cellulose blend films:
(a) Chitin (CT); (b) CT/CE 80/20; and (c) CT/CE 60/40; (d) CT/CE 40/60;
(e) CT/CE 20/80; and (f) Cellulose (CE).

4.5 Thermal Gravimetric Analysis

Figure 4.5 shows the degradation temperature (T_d) of chitin/cellulose blend films as a function of cellulose content. The T_d of a cellulose film was at 314.4 °C and that of chitin film was at 263.2 °C. As the cellulose content in the blend films increased, the T_d of the blend films increased. The T_d of the blend films was in the range of 263.2-314.4°C. It was found that the T_ds of pure cellulose and the blend film were higher that of pure chitin film. It may

films with 80% cellulose content versus temperature (°C) is shown in Figure 4.6. The T_d of the blend films occurred at one temperature over a temperature range between the T_d of chitin and cellulose films. It was suggested that there was good intermolecular interaction between chitin and cellulose. If there was no intermolecular interaction, the resulting T_d would be expected to occur at two temperatures. The first temperature occurred at T_d of chitin and the second temperature occurred at T_d of cellulose (Williamson *et al.*, 1998).
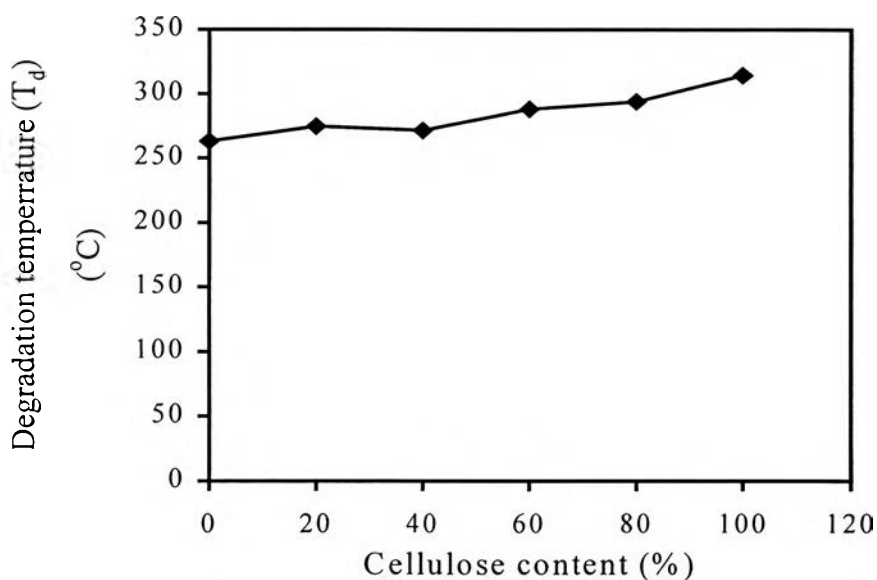

Figure 4.5 The degradation temperature of the chitin/cellulose blend films as a function of cellulose content
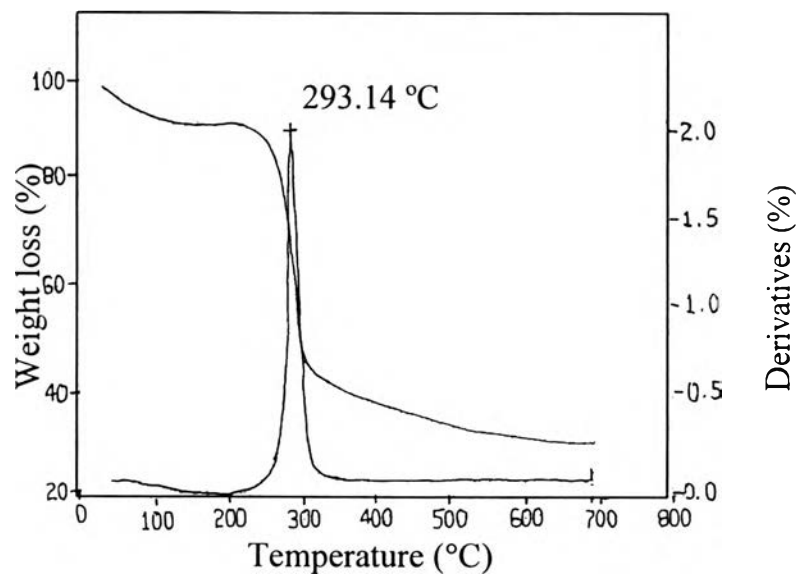
Figure 4.6 The degradation temperature of the blend films when the blend composition was 80 : 20 (Chitin : Cellulose).
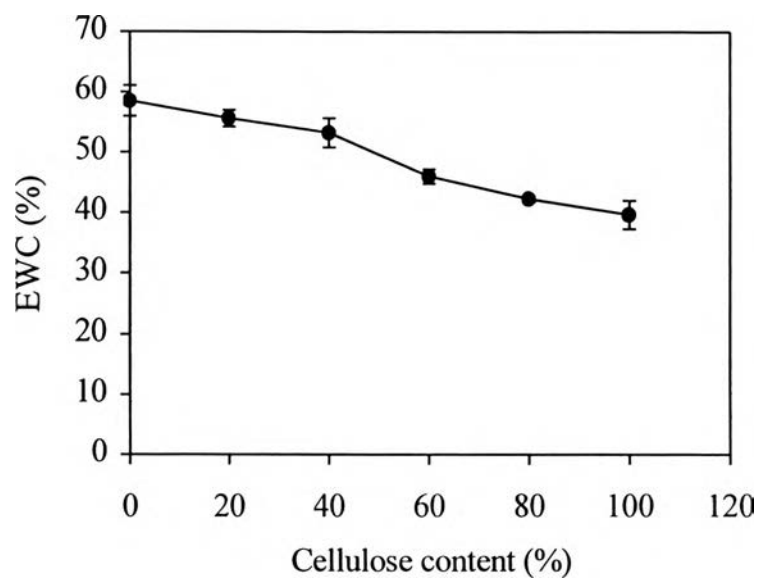
4.6 Equilibrium Water Content

Figure 4.7 The Equilibrium Water Content (EWC) of the chitin/cellulose blend films as a function of cellulose content.

Figure 4.7 illustrates the equilibrium water content (EWC) of the films as a function of cellulose content. The EWC of chitin films was approximately 60% while the EWC of cellulose films was around 40%. It was found that the EWC of the blend films decreased as cellulose content increased. The EWC of the blend films was in the rage of 40-60%. Khor *et al.* (1997) suggested that the ability to absorb water of chitin films depended on the preparation method to prepare the films. Chitin chains in the films were in the loose arrangement and weak association to adjacent chitin chains. The reducing of hydrogen bonding of chitin chains in the films resulted in increasing of water compatibility of the films. The increasing of cellulose content in the blend films resulted in decreasing of water absorption ability of the blend films. It was possibly due to the hydrogen bonding formation between the acetamide group of chitin with hydroxyl group of cellulose (Bianchi *et al.*, 1995).

4.7 Effect of salt type on the degree of swelling of chitin/cellulose blend films.

The effect of salt type on the degree of swelling of the blend films as a function of cellulose content is showed in Figure 4.8. The salt solutions used in this study were NaCl, LiCl, $CaCl_2$, $AlCl_3$ and $FeCl_3$ solution. The concentration of the salt solutions was 0.25 M. Among these salts, the blend films immersed in LiCl solution had the highest degree of swelling when the blend composition was 60% cellulose content. For the other salt solution, the blend films also had the maximum degree of swelling at 60% cellulose content. The blend film immersed in $AlCl_3$ solution had the degree of swelling next to LiCl solution. The effects of NaCl, $CaCl_2$ and $FeCl_3$ solutions on the degree of swelling of the blend films were nearly the same.

solutions on the degree of swelling of the blend films were nearly the same. For the blend films immersed in water, the degree of swelling continuously decreased as cellulose content increased.
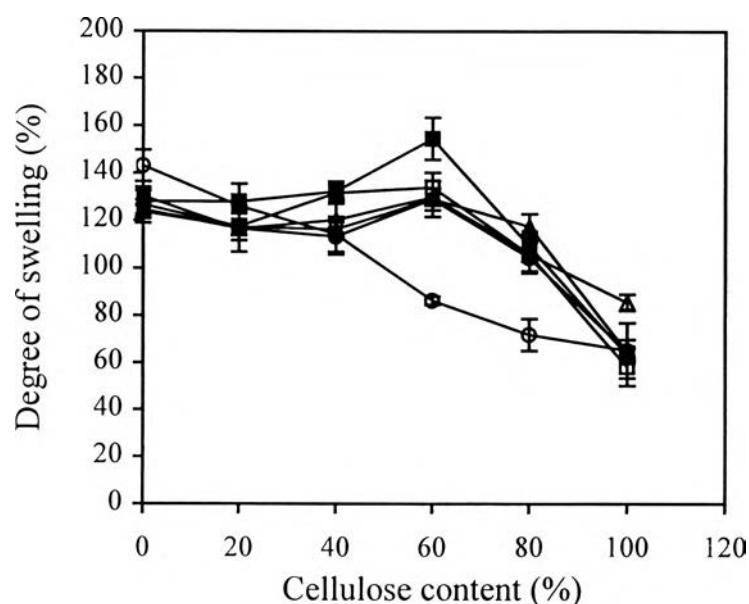

Figure 4.8 The effect of salt on the degree of swelling of the chitin/cellulose blend films as a function of cellulose content when the salt concentration was 0.25 M:

● 0.25 M NaCl; ■ 0.25 M LiCl; ▲ 0.25 M $CaCl_2$;

□ 0.25 M $AlCl_3$; △ 0.25 M $FeCl_3$; and ○ water.

Swelling behavior of chitin/cellulose blend films in various types of salt solution was 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. The degree of swelling of semi-IPN increased substantially with increased ionic valence. Semi-IPN exhibited the maximum degree of swelling in the solutions of trivalent salts (Al^{3+}), the lower degree

minimum degree of swelling was obtained in the solutions of monovalent salts (K^+, Na^+).

For cellulose films and the blend films with cellulose content less than 40%, the effect of different salt types on the change in degree of swelling of the blend films was small and the degree of swelling of the films immersed in salt solutions were closed to the degree of swelling of the films immersed in water. The effect of salt type on the change in the degree of swelling of the blend films immersed in salt solutions was obviously observed for the blend films with 60% and 80% cellulose content. It may be concluded that the chitin/cellulose blend films had good ion sensitivity when the blend compositions were 60% and 80% of cellulose content.

4.8 Effect of salt concentrations on chitin/ cellulose blend films.

Figure 4.9 shows the effect of salt concentrations on the degree of swelling of blend films when the blend composition was 60% cellulose content. The salt solutions used in this study were NaCl, LiCl, $CaCl_2$, $FeCl_3$, and $AlCl_3$ solutions. The salt concentrations were 0.05, 0.125, 0.25 and 0.5M. Among these salts, the blend films immersed in LiCl solution had the maximum degree of swelling when the salt concentration was 0.125M. For the other salt solutions, the maximum degree of swelling was also obtained when the salt concentration was 0.125 M. At salt concentrations was higher than 0.125 M, the degree of swelling of the blend films decreased and became rather constant when salt concentration was higher than 0.25 M. It could be explained by Donnan effect(1996). At 0.125M salt solutions, the difference of ion concentrations between inner and outer part of the films was high leading to different osmotic pressure, which resulted in the high degree of swelling.
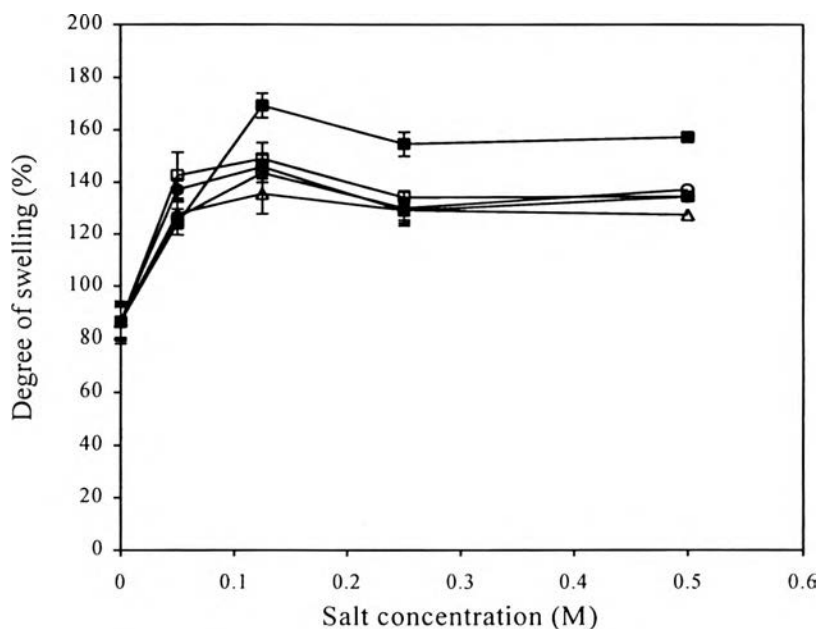
Figure 4.9 The degree of swelling of chitin/cellulose blend films when the blend composition was 60% cellulose content as a function of salt concentrations:

● NaCl;   ■ LiCl;   △ CaCl₂;   □ AlCl₃;   ○ FeCl₃.

4.9 Effect of pH on the degree of swelling of chitin/cellulose blend films

The effect of pH on the degree of swelling of chitin/cellulose blend films with various blend compositions is shown in Figure 4.10. The degrees of swelling of pure cellulose films were rather constant for the whole pH range from pH 3 to 10, while the degree of swelling of pure chitin films increased substantially in acidic pH (pH < 7) solutions and became constant at alkaline pH. The degree of swelling of the blend films decreased as the pH of the solution increased. The reason to explain the effect of pH on the degree of swelling of chitin films is that in acidic pH (pH < 7) solutions, the amine groups of chitin molecules are ionized leading to the dissociation of the

adjacent chains. By comparison to cellulose, the cellulose molecules have no functional groups that can serve as additional ionizable sites(Williamson *et al.*, 1999).
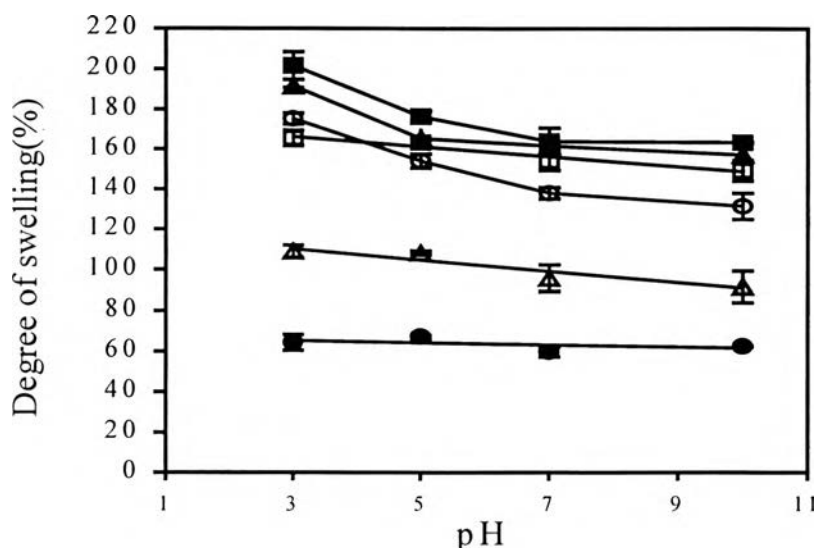

Figure 4.10 The degree of swelling of the chitin/cellulose blend films various blend compositions as a function of pH:

O Chitin (CT); ■ CT/CE 80/20; ▲ CT/CE 60/40;

△ CT/CE 40/60; □ CT/CE 20/80; ● cellulose (CE).

For chitin films and the blend films with 80% and 60% chitin content, the degree of swelling of the blend films increased as the pH of the solution decreased from pH 7 to pH 3. The reason for the increasing of the degree of swelling at acidic pH may be due to the Donnan equilibrium (Chen *et al.*,1997) between these blend films that carried fixed $-NH_3^+$ at acidic pH and the external solution phase. When the blend compositions were 20% and 40% chitin content, the blend films showed poor pH sensitivity for the whole pH range. It may be due to the poor ionic complex formation between $-NH_3^+$ and salt ions in these blend films. It could say that the chitin/cellulose blend

films showed the pH sensitivity property when the blend compositions were 80% and 60% of chitin content.

4.10 Mechanical properties of the blend films

Because polymeric materials, such as films, may be subjected to various kinds of stress during use, the determination of the mechanical properties will be useful for practical uses. Figure 4.11 shows the tensile strength of chitin /cellulose blend films as a function of cellulose content. The maximum tensile strength was obtained for the blend film with 80% cellulose content. When the cellulose content increased from 0 to 80%, the tensile strength increased from 60 to 105 MPa. The blend films had tensile strength higher than pure chitin and cellulose film.
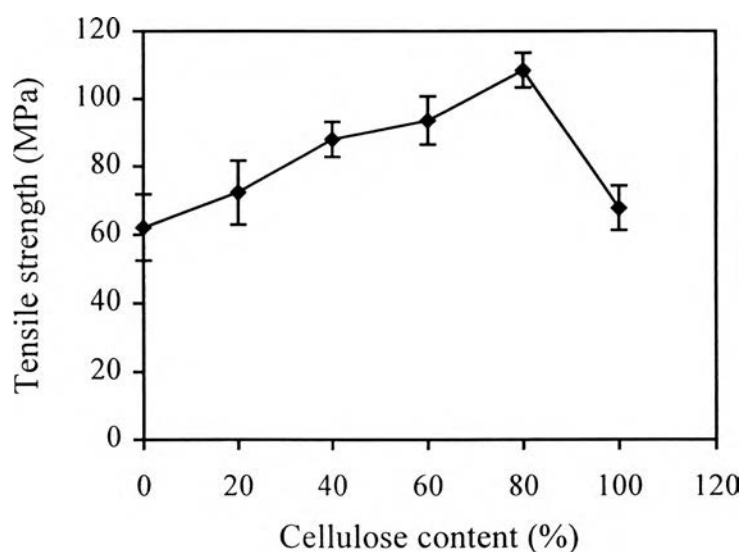

Figure 4.11 The tensile strength of the chitin/cellulose blend films as a function of cellulose content.

Figure 4.12 shows the elongation at break of the blend films as a function of cellulose content. The maximum elongation at break was obtained for the blend film with 80% cellulose content. When the cellulose

content increased from 0 to 80%, the elongation at break increased from 29 to 82%. The blend films had elongation at break higher than pure chitin and cellulose film.
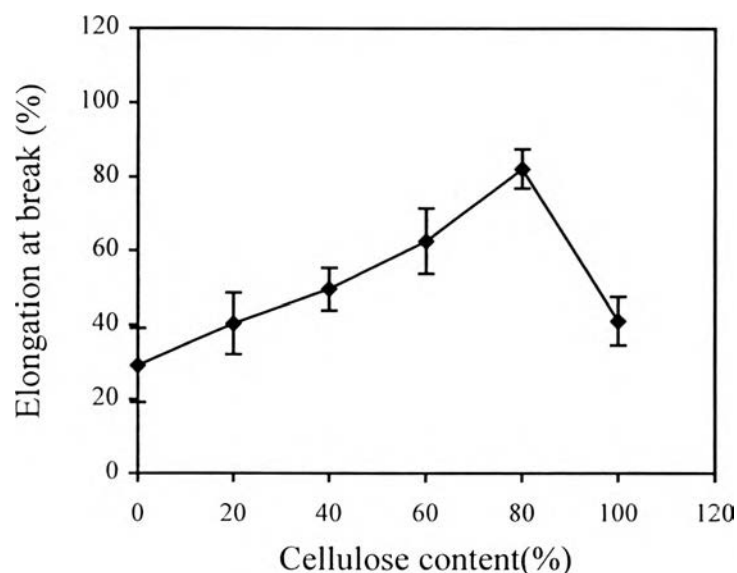

Figure 4.12 The elongation at break of the chitin/cellulose blend films as a function of cellulose content.

Hasegawa *et al.*(1992) prepared the blend films of chitosan and cellulose from the solution of trifluoroacetic acid (TFA) solvent system. They found that the blend films had the maximum tensile strength and elongation at break at 30% chitosan content. In addition, they also prepared chitosan/cellulose blend films from the solution of chloral /dimethylformamide (DMF) (Hasegawa *et al.*, 1994). Both tensile strength and elongation at break had maximum values at 20% chitosan content. They reported that the enhancement of mechanical properties suggested to be the occurrence of specific interaction between chitosan and cellulose molecules based on their structural similarity.

It was found that the improvement of mechanical properties of the chitin/cellulose blend films was similar to that of chitosan/cellulose blend films. The reason for the improvement in the mechanical properties of the chitin/cellulose blend films might be due to the occurrence of some specific interactions between chitin and cellulose molecules based on their structure similarity. These interactions were supposed to be hydrogen bonding formations between the hydroxyl groups of chitin and the acetamide groups of cellulose.

4.11 Oxygen permeability

Figure 4.13 shows the oxygen permeability rate of the chitin/cellulose blend films. The oxygen permeability rate of chitin film was 27.48 cm^3/m^2 d bar and that of cellulose film was 8.88 cm^3/m^2 d bar. The blend film had a remarkable reducing in oxygen permeability rate as compared to pure chitin films. The minimum oxygen permeability rate was obtained for the blend film with 20% cellulose content. The oxygen permeability rates of the blend films were slightly decreased as cellulose content increased from 20% to 100 %. The dramatically decreasing of oxygen permeability rate of the blend film as compared to pure chitin films is possibly due to the cellulose content in the blend films increased when the oxygen permeability rate of cellulose film was lower than chitin film.
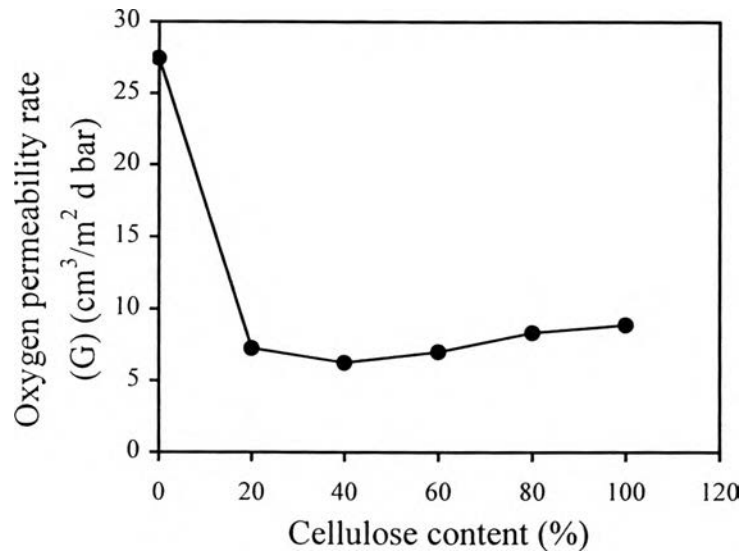
Figure 4.13 The oxygen permeability rate (G) of chitin/cellulose blend films as a function of cellulose content.

4.12 Surface morphology

The surface morphology of the chitin/cellulose blend films is shown in figure 4.14. The surface of chitin film [Figure 4.14(a)] was rough. In contrast, a smooth surface was obtained for cellulose film [Figure 4.14(f)]. The blend films had smoother surface than chitin film as shown in Figure 4.14 (b-f). The rough surface that was observed in the chitin film is attributed to the hydrophobic fibrous nature of chitin (Khor *et al.*, 1997).
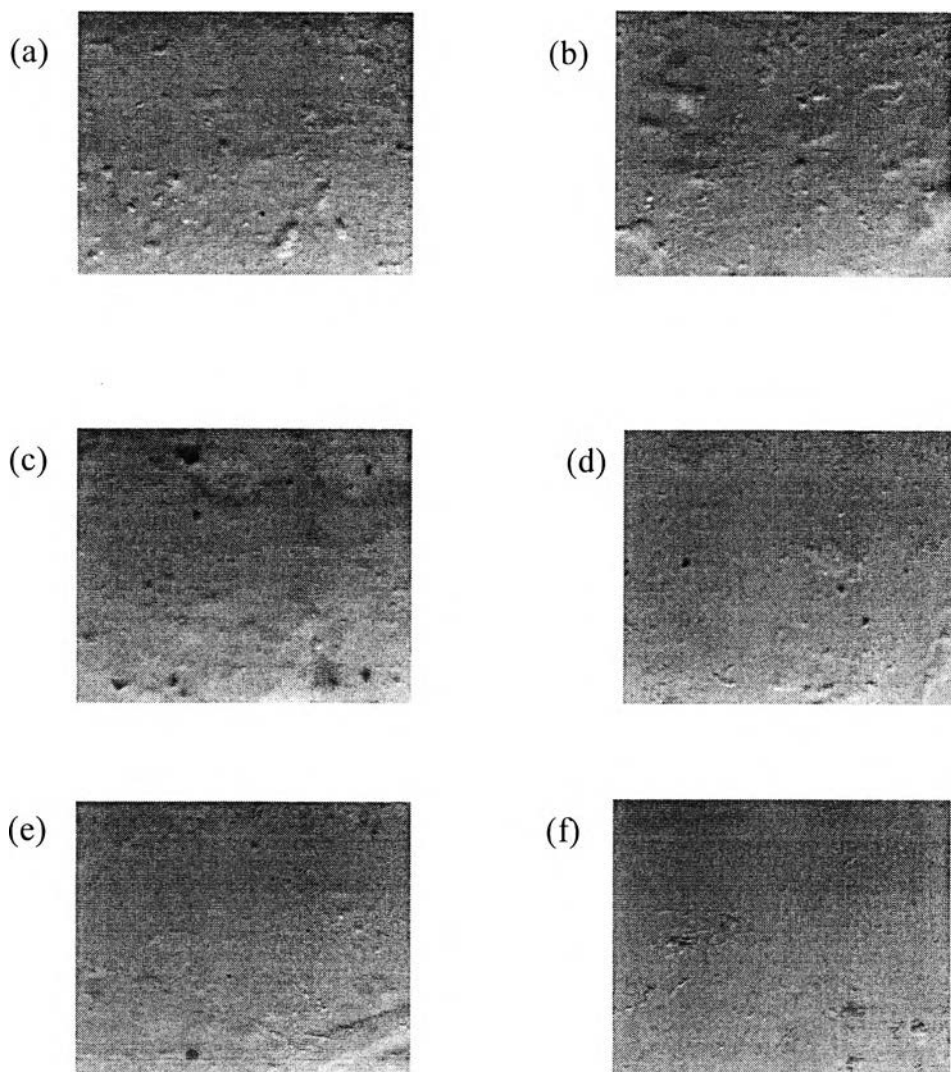
Figure 4.14 The optical microscope photograph of chitin/cellulose blend films:
(a) Chitin (CT); (b) CT/CE 80/20; (c) CT/CE 60/40; (d) CT/CE 40/60;
(e) CT/CE 20/80; and (f) Cellulose (CE).