CHAPTER IV

DYE ADSORPTION AND DESORPTION CHARACTERISTICS OF CM-CHITIN/CHITIN WHISKER BIONANOCOMPOSITE FILMS

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4.1 Abstract

Carboxymethyl chitin (CM-chitin)/chitin whisker (CTW) bionanocomposite films were fabricated by solution casting technique. Glyoxal was used as a crosslinking agent for retaining shape of the films in wet state. The effects of crosslinking concentrations on physical properties of the films were evaluated in terms of swelling behaviour and weight loss. Methylene blue, a cationic dye and methylorange, an anionic dye, were selected as model compounds in order to examine the adsorption and desorption characteristics of the films. The effects of solvent of dye, blend composition and types of dye on the dye adsorption of the films were investigated as a function of adsorption time. The CM-chitin film crosslinking with glyoxal could form hydrogel in wet state and had high degree of swelling with low percentage of weight loss. The presence of CTW in the crosslinked CM-chitin films resulted in the improvement in shape stability. Furthermore, CM-chitin films had higher adsorption capacities for the methylene blue than methyl orange because of the interaction between carboxymethyl group and cationic group of the dye. The existence of chitin whisker in composite films exhibited the lower adsorption and desorption due to the low degree of swelling.

Keywords: CM-chitin/Chitin whisker/Wound dressing/Dye/Adsorption/Desorption

4.2 Introduction

Nowadays, biodegradable materials, especially those from natural polymers, have received more attention in biomedical applications. It is known that wound dressing is one of the most important tools in wound management. In recent years, wound dressings carrying active ingredients have been developed for the better wound therapy by promoting the wound healing process. The active ingredients in wound dressings will be delivered to wound sites in a consistent and sustained over long periods of time without the need for frequent dressing change (Boateng *et. al,* 2007). These devices usually are made from hydrophilic polymers which can serve as vehicles for the release of active ingredients. The release of active ingredients from polymeric formulations is controlled by one or more physical processes such as hydration of the polymer by fluids, swelling to form a gel, diffusion of active ingredients through the swollen gel. Therefore, hydrogel dressing seems feasible to play a part in controlled release of active ingredients from the dressings when they come into contact with wound exudate. Hydrogel is a three dimensional crosslinked hydrophilic polymeric network containing mainly of water which is excellent for helping to create moist environment that able to clean and remove necrotic tissue (Yoshii *et. al,* 1999). In addition, hydrogel dressing is not to stick to wounds, then, it can be easily taken out without damaging the wound and provide less pain for patients. As a result, hydrogel material is very suitable for development modern wound dressing which facilitate in wound healing.

Chitin, poly (β-(1-4)-N-acetyl-$\square$-glucosamine), is known to be able to biodegrade by the nature and in the body. Chitin possesses interesting biological properties which made it attractive to use as biomaterial. However, highly crystalline structure of chitin causes a problem in solubility which can be solved by chemical modification. The introduction of carboxymethyl (CM) groups into chitin leads to the formation of anionic derivatives which would be useful in biomedical fields. Moreover, CM-chitin is able to form hydrogel by cross-linking that makes it can provide a moist environment to a wound, resulting in an effective wound therapy. There are many researches that has been supported the use of CM-chitin as wound dressing in various forms such as water-soluble chitin hydrogel (Cho *et al.,* 1999),

carboxymethyl modified surface of chitin beads (Yusof *et al.*, 2000), CM-chitin films (Wongpanit *et al.*, 2005), etc. Thus, CM-chitin is suitable raw material to fabricate as biomedical devices.

However, the high degree of swelling of hydrogels results in the cracking of materials and lead to burst release of active ingredients. So, the additional substance is required to hold the structure and slow down the release of active ingredients. Chitin whisker, the crystalline nanofibrils chitin with high aspect ratio providing stiffness and strength, is remarkable to improve the properties of hydrogel. In previous report, chitin whisker was used in biomedical application as the wound medicament composite (Muzzarelli *et al.*, 2007). In addition, chitin whisker was also used to improve dimensional stability in the composites (Wongpanit *et al.*, 2007).

The aim of this research is to fabricate bionanocomposite films consisting of CM-chitin and chitin whiskers for wound care system via solution casting technique by using glyoxal as a crosslink agent. Moreover, the effects of crosslinking concentrations on physical properties of the films were evaluated in terms of swelling behavior and weight loss. Methylene blue, a cationic dye and methyl orange, an anionic dye, were selected as model compounds in order to examine the adsorption and desorption characteristics of the films. The effects of solvent of dye, blend composition and types of dye on the dye adsorption of the films were investigated as a function of adsorption time. Moreover, the effects of lysozyme and the ionic strength in released media on the dye desorption of the films were observed as a function of desorption time.

4.3 Experiment

4.3.1 Materials and Chemicals

Shells of *Metapenaeus dobsoni* shrimp were supported from Surapon Food Co. Ltd. Analytical grade hydrochloric acid 37 %w/w and analytical grade anhydrous sodium hydroxide pellets were purchased from Labscan. Analytical grade glacial acetic acid (J.T.Baker), commercial grade acetone, analytical grade monochloroacetic acid (Fluka), analytical grade ethanol (Labscan), glyoxal 40% in H_2O (Sigma-Aldrich), molecular biology grade Tris-HCl (Scharlau Chemie S.A.),

Lysozyme from chicken egg white (Fluka), Methylene blue C.I. 52015 (UNILAB) and Methyl orange C.I. 13025 (LABCHEM) were used.

4.3.2 Preparation of CM-Chitin

Twenty gram of chitin powder was suspended in 400 g of 42%w/w NaOH solution. The suspension was placed in vacuum oven for 30 min to reduce pressure in the sample. Then, 640 gram of crushed ice was added to the suspension under vigorous stirring at 0-5 °C for 30 min. A pre-cooled acid solution, containing 108 g of momochloroacetic acid in 280 g of 14%w/w NaOH solution was slowly dropped into the mixture under stirring. The reaction temperature was kept at 0-5 °C for 30 min. The solution was settled at room temperature overnight and was then neutralized by glacial acetic acid. Afterwards, the sample was transferred to dialysis bags and placed in running water for 2 days, followed by distilled water for 1 day. The dialysate was centrifuged at 10,000 rpm for 10 min to remove insoluble material. The CM-chitin solid was obtained from the supernatant by adding it drop-wise into cool acetone and then centrifugation to separate the precipitate. Then, the product was washed with ethanol and dried at 50 °C.

4.3.3 Preparation of Chitin Whisker Suspension

Chitin whisker suspension was prepared by acid hydrolysis method (Nair and Dufrense, 2003; Wattanapanit, 2008). Chitin flakes were hydrolyzed with 3N HCl, which the ratio of chitin: HCl was 1g: 30mL, under continuously stirring at 104°C for 6 hours. The suspension was later diluted with distilled water, followed by centrifugation at 10,000 rpm for 10 minutes and repeated for two times. Afterward, the suspension was dialyzed in distilled water until neutral. The suspension was stored in refrigerator before used.

4.3.4 Preparation of CM-Chitin/Chitin Whiskers Bionanocomposite Films

CM-chitin solution was prepared at concentration of 2 %wt. Glyoxal solution, the crosslinking agent, was diluted from 40 %w/w to 1 g/L and then mixed with the CM-chitin solution at various pH to find the suitable condition for crosslinking. The mixture was transferred to the polystyrene petri dish (diameter: 25 mm) and dried at 40 °C to obtain CM-chitin films. Moreover, the glyoxal concentration in the solution was varied from 0.25-2.0 mM based on total volume.

Hence, CM-chitin/CTW composite films were prepared by mixing CM-chitin solution with chitin whisker (CTW) suspension by varying wight ratio of CM-chitin:CTW as following: 90:10, 80:20, 70:30, 60:40, 50:50 and 40:60 under stirring until homogeneous solution was obtained. Afterwards, the composite solution was crosslinked with glyoxal by the same method as CM-chitin film.

4.3.5 Preparation of Dyes Stocks

The dyes used in this study were Methylene blue, a cationic dye and Methyl Orange, an anionic dye. The dye stock solutions were prepared by dissolving dyes in the solvents to the concentration of 500 ppm. The experimental solvents were the mixture of distilled water and ethanol having volume ratio as following: 100:0, 80:20, 50:50 and 20:80. The known concentrations of the dyes were dilute from the dyes stocks to make the calibration curve. The absorbance of each solution was measured at the maximum wavelength of each solvent. Consequently, the dye stock was diluted to 25 ppm as an initial concentration.

4.3.6 Characterization

Chemical structures of chitin, CM-chitin and crosslinked CM-chitin were determined by FTIR. The viscosity-average molecular weight of CM-chitin was evaluated by viscosity measurement with Ubbelohde type viscometer at 25 °C. The morphology of CTW was observed from TEM. The microstructure of the surface and cross-section of the composite films were investigated by SEM. The dimension stability of samples in wet state was also evaluated in term of degree of swelling and weight loss by the following equations:

$$\text{Degree of swelling (\%)} = \frac{W_s - W_d}{W_d} \times 100 \tag{1}$$

$$\text{Weight loss (\%)} = \frac{W_d - W_i}{W_d} \times 100 \tag{2}$$

where W_d is the weight of dried sample, W_s is the weight of the sample in the swollen state and W_i is the weight of dried sample after immersion in water for 24 hours.

4.3.7 Dye Adsorption Characteristics

In order to investigate the adsorption and desorption characteristics of the composite films, methylene blue and methyl orange were used as the cationic and anionic model compounds. The composite films were placed in the glass bottles containing 40 ml of dye solutions in various solvents at room temperature. At various time, 100 µL of dye solution was taken out and transferred to the well plate. The absorbance of the sample was measured by the UV-VIS Spectrophotometer (Tecan, Infinite M200). The λ_{max} for methylene blue in the mixture of distilled water and ethanol having volume ratio as following: 100:0, 80:20, 50:50 and 20:80 were 668, 664, 662 and 660 nm, respectively. Moreover, the λ_{max} for methylene blue in the mixture of distilled water and ethanol having volume ratio as following: 100:0, 80:20, 50:50 and 20:80 were 466, 468, 448 and 430 nm, respectively. The accumulative adsorption of the films was calculated from following equation (Jeon *et al.*, 2008):

$$\text{Accumulative adsorption} = \frac{C_0 - C_t}{C_0} \times 100 \qquad (3)$$

where C_0 (ppm) and C_t (ppm) are the initial concentration of dye and the concentration of dye at time t, respectively. The amounts of dyes sorbed by the films were calculated from following equation (Gong *et al.*, 2005):

$$q_s = \frac{(C_0 - C_t)V}{W} \qquad (4)$$

where q_s (mg/g) is the amount of dye sorbed by the film, V (l) is the initial volume of dye solution and W(g) is the weight of the film. Note that ppm is equal to mg/l.

In addition, the effect of blend compositions to the adsorption characteristic was also evaluated from the CM-chitin/CTW composite films at the weight ratio of 90:10, 80:20 and 70:30.

4.3.8 Dye Desorption Characteristics

The films containing the dye were placed in 40 ml of 0.01M 2-Amino-2-hydroxymethyl-1,3-propanediol hydrochloride or Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) solution at pH 7.4 with shaking rate 100 rpm at 37°C, and compared condition with and without 0.01 %wt. lysozyme. The buffer solution was taken out at the various time and transferred to the well plate and the absorbance of the sample was later measured. In case of the presence of lysozyme, the mixture was centrifuged at 10000 rpm for 5 min before transferring to the well plate. The concentration of dye release was determined in compared with the standard curve. Furthermore, the effect of ionic strength to the desorption characteristics was estimated by varying the concentration of NaCl in buffer solution as 0.01, 0.1 and 0.5 M. The amounts of dyes desorbed from the films were calculated from the equation modified from Gong et al. (2005) as following:

$$q_d = \frac{(C_{tb})V_b}{W} \tag{5}$$

where q_d(mg/g) is the amount of dye desorbed from the film, C_{td}(ppm) are the concentration of dye in buffer solution time t, V_b(l) is the volume of buffer solution and W(g) is the weight of the film. Note that ppm is equal to mg/l.

Then, the percentage of dye desorption was determined from following equation:

$$\text{Dye desorption (\%)} = \frac{\text{total dye desorption capacity}}{\text{total dye adsorption capacity}} \times 100 \tag{6}$$

$$\text{Dye desorption (\%)} = \frac{q_d}{q_s} \times 100 \tag{7}$$

4.4 Results and discussion

4.4.1 Characterization of Raw Materials

4.4.1.1 Yields Production

In general, chitin from animals occurs associated with other constituents, such as lipids, calcium carbonate, proteins and pigments, so, the shrimp shells were treated with chemicals to extract chitin. The shrimp shells were first cleaned and treated with HCl and NaOH to remove calcium and other minerals, and proteins, respectively. Demineralization occurs according to the following reaction (Belgacem et al., 2008):

$$CaCO_3 + HCl \rightarrow CO_2 + CaCl_2 + H_2O$$

Afterwards, chitin was ground to be powder for use to synthesized CM-chitin and also crushed to be the small flakes for use in chitin whisker preparation. The yields of chitin, CM-chitin and chitin whisker production was represented in Table 4.1.

Table 4.1 Yields of chitin, CM-chitin and chitin whisker production

Substances	Dry Weight (g)
Shrimp shells	100
Demineralized shrimp shells	37.85
Chitin	31.25
➢ CM-chitin	27.38
➢ Chitin whisker	23.74

4.4.1.2 Characterization of Chitin and CM-Chitin

FT-IR spectra of chitin and CM-chitin (H-form) are represented in Figure 4.1. It can be seen that the spectra of CM-chitin showed the sharp peak at 1732 cm^{-1} corresponded to carboxylic group (-COOH) of CM-chitin.

Moreover, intensity of primary hydroxyl groups at 1070 cm^{-1} slightly decreased on carboxymethylation (Tokura, et al., 1983). Therefore, it can be concluded that the carboxymethylation was mainly occurred at hydroxyl groups of C-6 position.
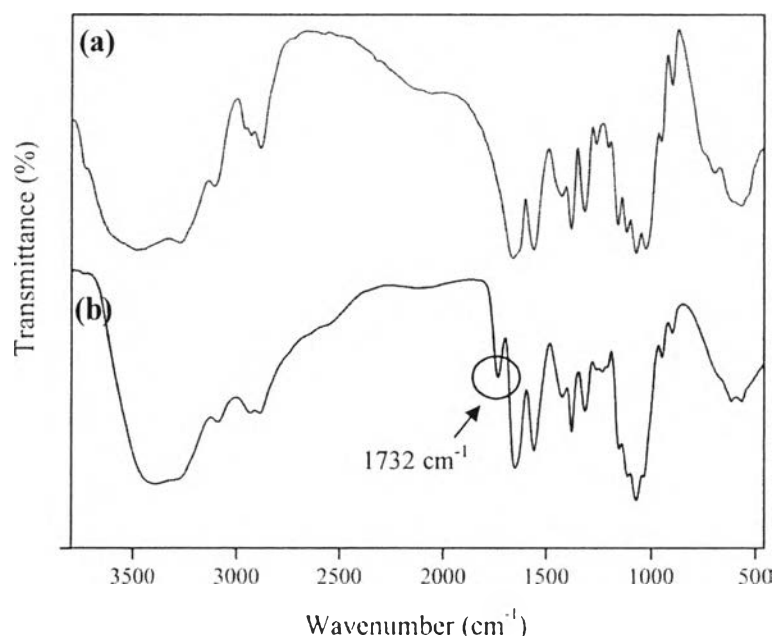

Figure 4.1 FT-IR spectra of (a) chitin and (b) CM-chitin.

Degree of deacetylation (DD) of chitin was determined following the method of Sannan et al. (1977) which calculated from the ratio of absorbance of amide II band at 1550 cm^{-1} and C-H band at 2878 cm^{-1}. In this synthesis, %DD of chitin was found to be 30%.

Degree of substitution (DS) of chitin was evaluated from the relationship between weight ratio of carbon to nitrogen and DD (Huang et al., 2010). The DS value of CM-chitin was determined to be 0.45.

In addition, the number average molecular weight of CM-chitin was estimated by applying the viscosity equation proposed by Kaneko et al. (1982) which was found to be 66000.

4.4.1.3 Characterization of Chitin Whisker

The protonation of the amino groups (-NH$_3^+$) in chitin after acid hydrolysis using hydrochloric acid resulted in positive charges appearing at the

surfaces of chitin whisker. Hence, the repulsion forces of these cationic charges led to the stable colloidal suspension of chitin whisker in water (Marchessault *et al.*, 1959). The chloride ions (Cl⁻) from HCl did not play important role on charges because chloride ions were easily eliminated by washing with distilled water for several times (Akira *et al.*, 1999). The individual whisker, which has a rod-like structure, exhibited a broad distribution in both width and length as shown in Figure 4.2. The length of the chitin fragments ranged from 130 to 990 nm, while the width ranged from 12 to 57 nm. The average length (L) and width (d) of these whiskers were 447 and 34 nm, respectively, and the average aspect ratio (L/d) was 13. However, the previous work reported the dimensions of chitin whisker from shrimp shells (Wattanapanit *et al.*, 2008) gave the average width, length, and aspect ratio about 46, 343, and 7.5 nm, respectively.
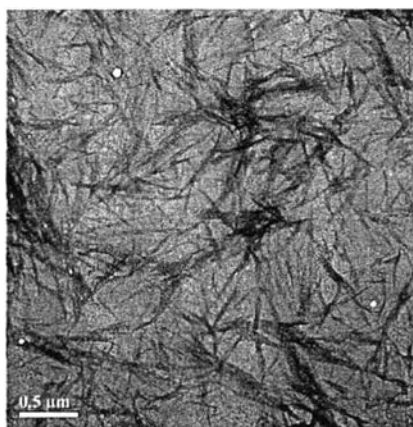
Figure 4.2 TEM micrograph of chitin whisker suspension.

4.4.2 Characterization of Glyoxal Crosslinked CM-Chitin Films

4.4.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 4.3 exhibits FT-IR spectra of non-crosslinked and glyoxal crosslinked CM-chitin films with various concentrations of glyoxal. Certainly, the spectrum of the native CM-chitin films is mainly similar to that all of the crosslinked CM-chitin sponge. Generally, glyoxal or dialdehyde reacts with hydroxyl groups of polymer chains (Yang *et al.*, 2004), so that the absorption peak of -OH at 1110 cm⁻¹ was slightly decreased as increasing of glyoxal concentrations.

Furthermore, the boarder peak of amide II at 1550 cm^{-1} was observed when the glyoxal concentration increased. Then, these FT-IR results suggested that effective linkage of glyoxal in CM-chitin structure involved with the acetamide and hydroxyl group in CM-chitin.
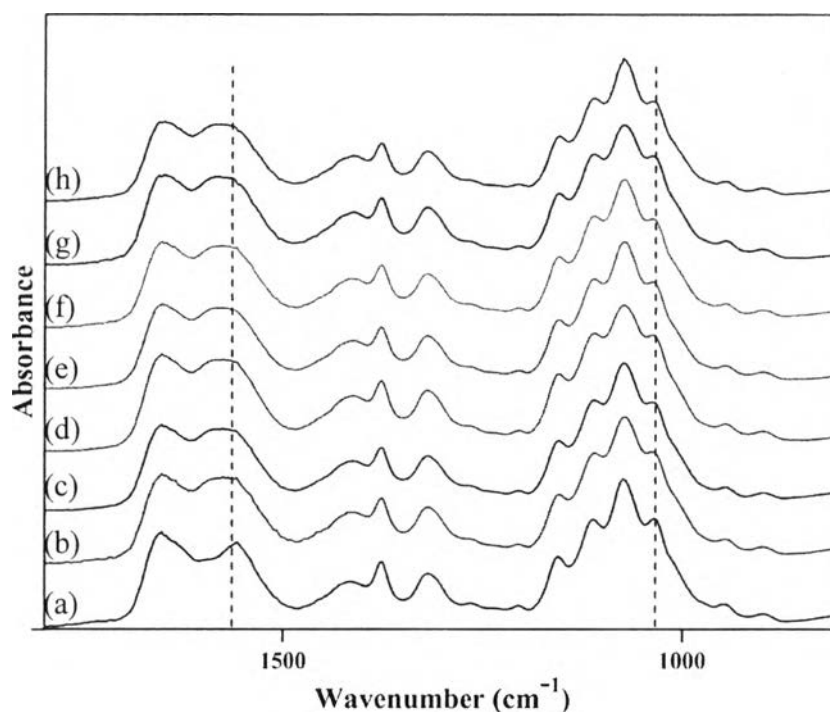

Figure 4.3 FTIR spectra of glyoxal crosslinked CM-chitin films for the various concentrations of glyoxal of (a) 0 (b) 0.5 (c) 0.75, (d) 1.0, (e) 1.25, (f) 1.5, (g) 1.75 and (h) 2.0 mM.

4.4.2.2 Water Absorption Properties

Due to there is no report the using of glyoxal as a crosslinking agent for CM-chitin, the suitable pH for crosslinking reaction was necessary to study. Figure 4.4 and 4.5 exhibits the effect of pH of the CM-chitin solution on the degree of swelling and weight loss of CM-chitin films. The degree of swelling of crosslinked CM-chitin is increased with the increasing of pH until to 6 and then suddenly reduced. The weight loss of crosslinked CM-chitin tends to slightly decrease as the increasing of pH until to 6 and then increased. The result

indicated that at pH 6, CM-chitin films represented the highest absorbency and showed the lowest weight loss. Hence, crosslinking of CM-chitin with glyoxal was performed at pH 6. According to the FTIR result, crosslinking reaction involved with the acetamide and hydroxyl group in CM-chitin which have the nucleophilic position of unshared pair of electrons on nitrogen and oxygen. Consequently, as the acidity of CM-chitin solution increased, the acetamide and hydroxyl group could be protonated to present non-nucleophilic property (Yang *et al.*, 2004).
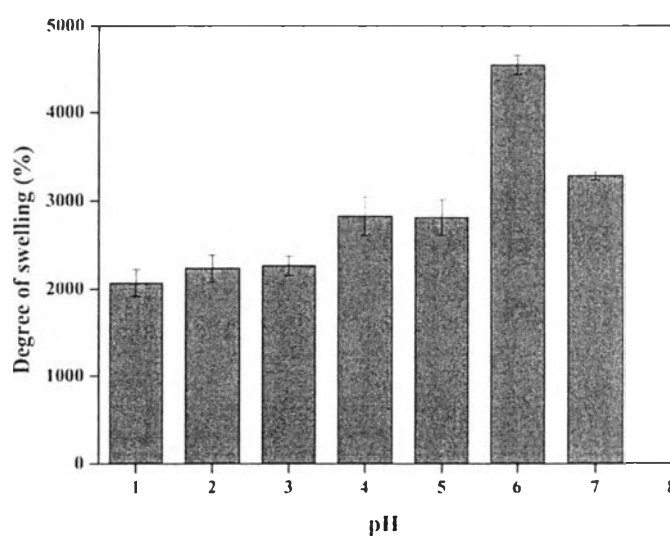
Figure 4.4 Degree of swelling of CM-chitin films cross-linked with 0.5 mM glyoxal as a function of pH of CM-chitin solution after immersion the samples in distilled water for 24 hr at room temperature.
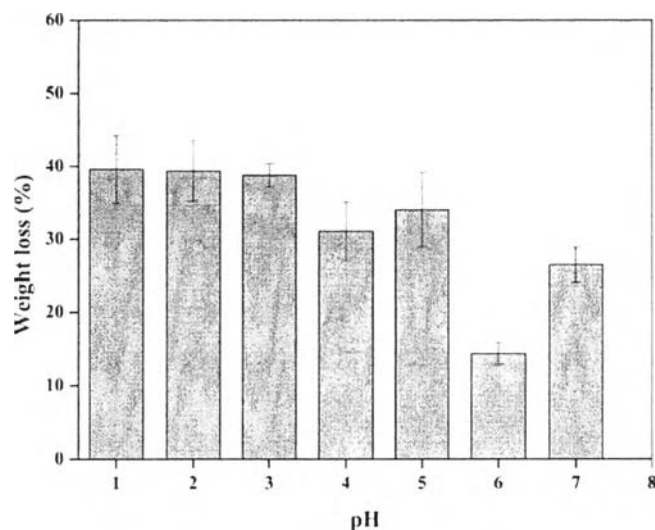
Figure 4.5 Percentage of weight loss of CM-chitin films cross-linked with 0.5 mM glyoxal as a function of pH of CM-chitin solution after immersion the samples in distilled water for 24 hr at room temperature.

It is well known that dimensional stability of a hydrophilic material in wet state can be improved by crosslinking. From the swelling test, swelling behavior of CM-chitin film without crosslinking was not detectable because CM-chitin dissolved in the testing media within a short period of time. According to Figure 4.6 and 4.7, the degree of swelling and the percentage of weight loss tended to decrease with the increasing of glyoxal concentration. However, in case of the very low glyoxal concentration, the crosslinked CM-chitin film exhibits the lower degree of swelling because there are some parts of the film dissolve into water and lead to the high percentage of weight loss. Since CM-chitin consists of the rich hydrophilic groups, so CM-chitin could absorb high amount of water which results in the degree of swelling more than 1000 times the mass of polymer.
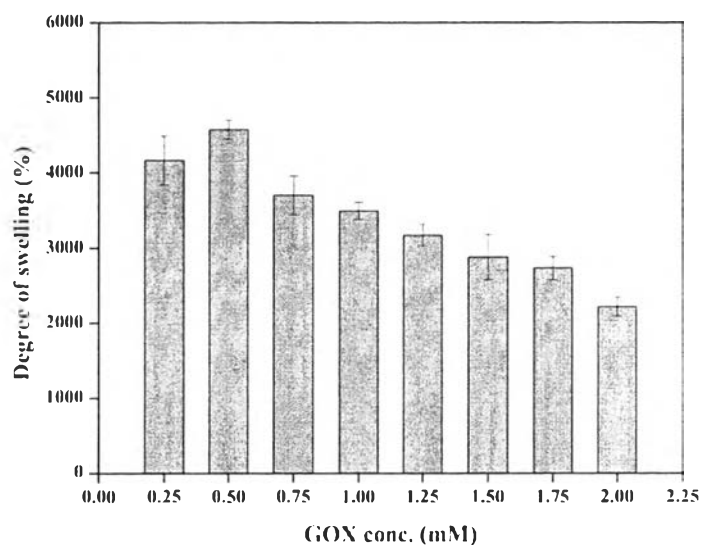
Figure 4.6 Degree of swelling of CM-chitin films cross-linked with different glyoxal concentrations after immersion in distilled water for 24 hr at room temperature.
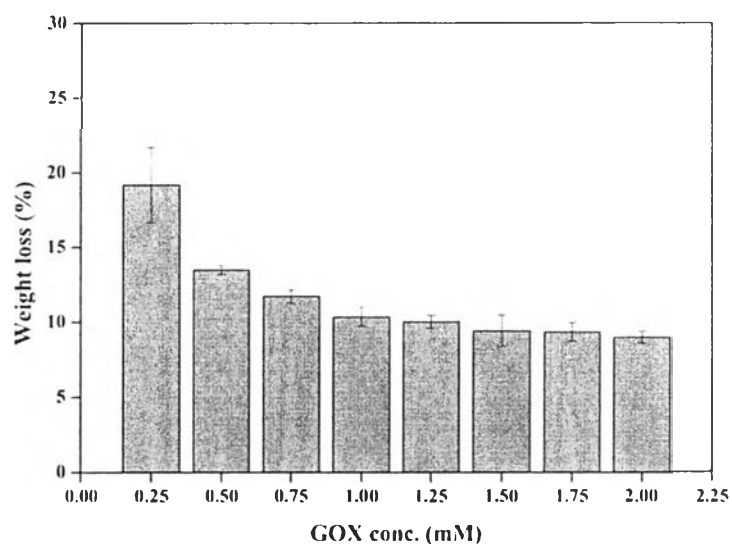

Figure 4.7 Percentage of weight loss of CM-chitin films cross-linked with different glyoxal concentrations after immersion in distilled water for 24 hr at room temperature.

In addition, the higher amount of crosslinking agent exhibited the higher crosslinking density, resulting in the decreasing of weight loss. The

percentage of weight loss was found to be steady at the glyoxal concentration more than 1 mM. This can be indicated that the high concentration of glyoxal crosslinked CM-chitin still exhibited some hydrophilic groups which was not crosslinked and can interact with water. Hence, the percentage of weight loss of CM-chitin could not reach to zero. As a result, the concentrations of crosslinking agent at 0.50 and 0.75 mM were selected to prepare CM-chitin/CTW composite films due to they provided the high degree of swelling and the acceptable of percentage of weight loss.

4.4.3 Characterization of Glyoxal Crosslinked CM-Chitin/CTW Composite Films

4.4.3.1 Water Absorption Properties

The degree of swelling and percentage weight loss of CM-chitin/CTW films cross-linked with 0.5 and 0.75 mM glyoxal are illustrated in Figure 4.8 and 4.9, respectively. As the content of CTW in the composite films increased, the decreasing in degree of swelling of the composite films was observed. The existence of CTW in the composite films affected to the reduction of degree of swelling and the percentage of weight loss much more than the raising of crosslinking concentration. In soy protein isolate films reinforced with α-chitin whiskers from crab shells (Lu *et al.*, 2004), the equilibrium percentage degree of swelling also decreased with increasing whisker content. The reduction in the degree of swelling could be a result of the strong hydrogen bonding between CM-chitin chains and the high crystalline nanofibrils of chitin. These results can be concluded that the presence of CTW in CM-chitin films could remarkably improve the shape stability of the composite films.
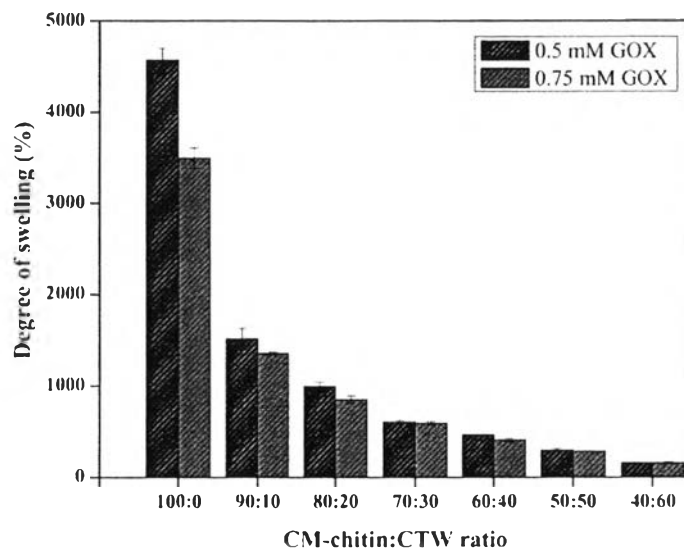
Figure 4.8 Degree of swelling of CM-chitin/CTW films cross-linked with 0.5 and 0.75 mM glyoxal as a function of blend ratios of CM-chitin to CTW after immersion in distilled water for 24 hr at room temperature.
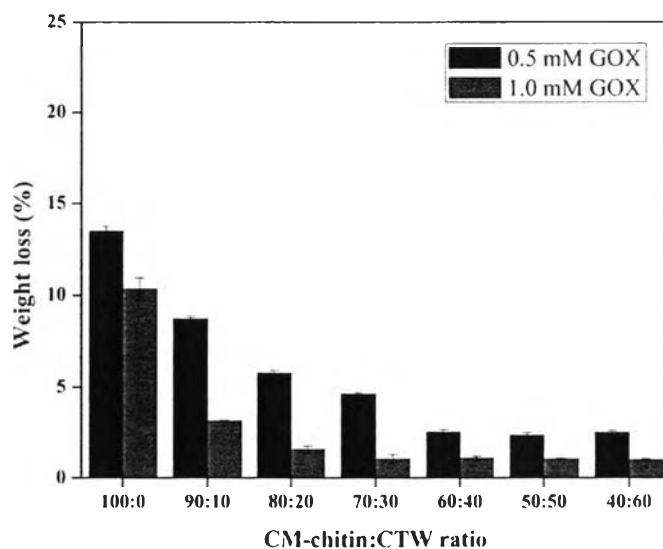

Figure 4.9 Percentage of weight loss of CM-chitin/CTW films cross-linked with 0.5 and 0.75 mM glyoxal as a function of blend ratios of CM-chitin to CTW after immersion in distilled water for 24 hr at room temperature.

4.4.3.2 Structural Morphology

From the SEM micrograph of the composite films surface (Figure 4.10), the CM-chitin film without chitin whisker exhibited the smooth surface. As the chitin whisker content was higher, the higher roughness on the film surface was obtained.

Moreover, the cross-sectional SEM micrographs of surface of CM-chitin/CTW composite films are shown in Figure 4.11. The SEM images exhibited that CM-chitin film represented dense with stripe. Furthermore, the incorporation of chitin whisker presented the coarser feature.
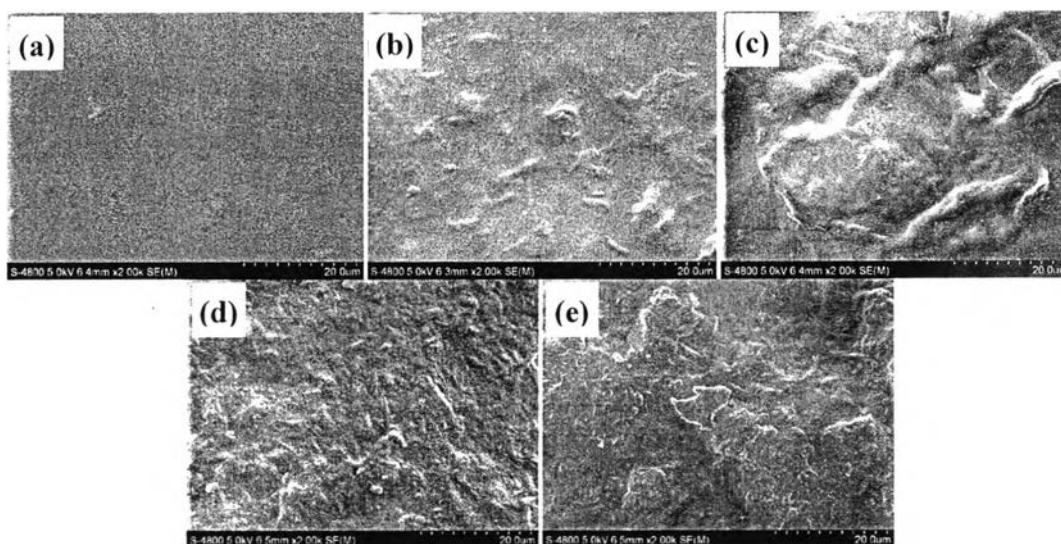

Figure 4.10 SEM images of surface of CM-chitin/CTW composite films with the weight ratio of CM-chitin:CTW as (a) 100:0, (b) 90:10, (c) 80:20, (d) 70:30 and (e) 50:50.
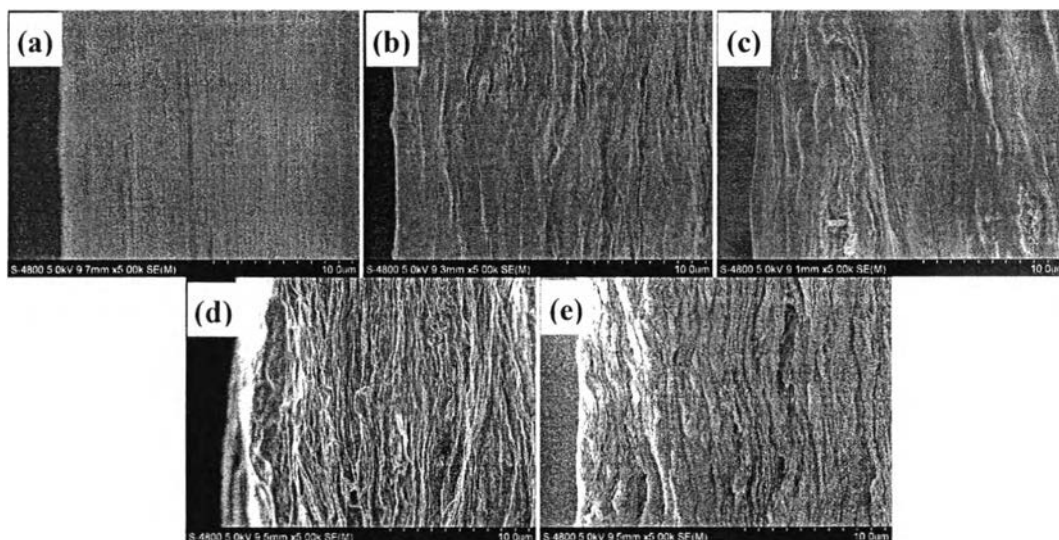
Figure 4.11 Cross-sectional SEM micrographs of surface of CM-chitin/CTW composite films with the weight ratio of CM-chitin:CTW as (a) 100:0, (b) 90:10, (c) 80:20, (d) 70:30 and (e) 50:50.

4.4.4 Dye Adsorption Behavior of CM-Chitin/CTW Composite Films

4.4.4.1 Effect of Dye Solvents

The CM-chitin/CTW films were fabricated as the hydrogel dressings containing the active ingredients and methylene blue, a cationic dye and methyl orange, an anionic dye, were selected as model compounds. The substances were incorporated into hydrogel matrices by post-loading. The model component was absorbed into the matrix after the hydrogel matrix was formed. As water and ethanol (EtOH) are the common solvents for the drug and substances used in pharmaceutical (Domanska, 2009), the effect of dye solvents to the dye adsorption characteristics were studied. The mixture of distilled water and EtOH having different volume ratios were selected as the dye solvents. The cumulative adsorption of methylene blue and methyl orange on CM-chitin films in various solvents are exhibit in Figure 4.12 and 4.13, respectively. From the results, the dye adsorption percentage decreased when the volume fraction of EtOH increased. Due to EtOH was non-solvent of CM-chitin, CM-chitin film exhibited the low degree of swelling resulting in the low amount of adsorption. Therefore, the suitable solvent of CM-chitin for dye adsorption is distilled water. Moreover, the dye solvents also affected

to the initial adsorption rate of CM-chitin film. The initial adsorption rate and of CM-chitin film tended to decreased as the increasing of the volume fraction of EtOH as represented in Table 4.2. In addition, the amount of dye adsorption of CM-chitin films in compare with the weight of the sample also shows in Table 4.2. The higher amount of dye could be adsorbed on the films in the mixture with the high content of distilled water.

Furthermore, the charge of model compound also affected to the adsorption characteristic of CM-chitin film. CM-chitin was in an anionic form which has ionic interaction with positive charge of methylene blue and has repulsion force with negative charge of methyl orange. In agreement with Gong *et al.* (2005), they reported the important functional group of the biomass in the adsorption of dyes that the carboxyl group inhibited the adsorption of anionic dyes because of its negative charge and the carboxyl group was major functional group in the adsorption of cationic dyes. Moreover, the hydroxyl group was important functional group in the adsorption both of cationic and anionic dyes. Hence, the CM-chitin films could adsorb the cationic compound better than the anionic compound. The other types of interaction between gel and dyes which could affect to the dye adsorption may be hydrophobic and hydrogen bonds (Karadag *et al.*, 1997). Hydrophobic effects are specifically aqueous solution interactions, which in the present case will involve the aromatic rings and the methyl groups on the dye molecules and the methine groups on the gel.
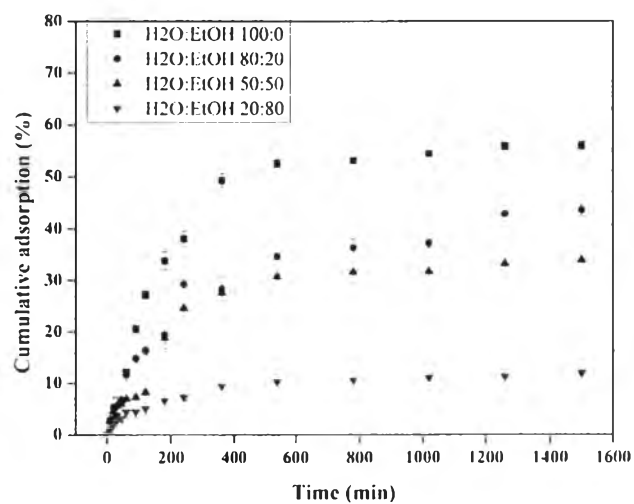
Figure 4.12 The cumulative adsorption of methylene blue on CM-chitin films in various solvents.
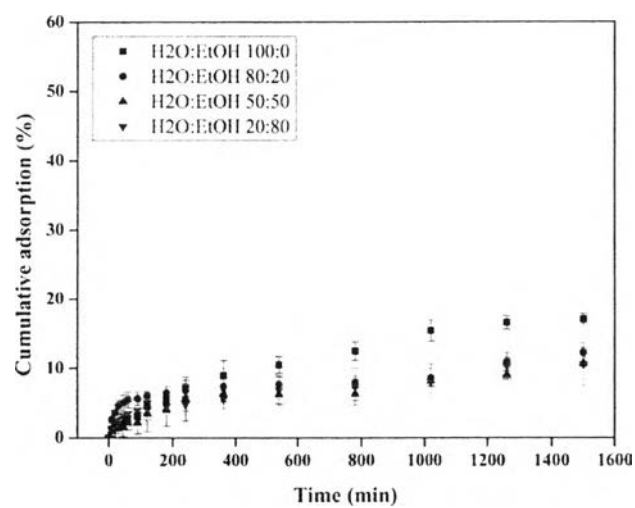

Figure 4.13 The cumulative adsorption of methyl orange on CM-chitin films in various solvents.

Table 4.2 The initial dye adsorption rate and amount of dye adsorption of CM-chitin films in various solvents

Solvent (H$_2$O:EtOH)	Dye types			
	Methylene Blue		Methyl Orange	
	Initial Adsorption Rate (ppm/min)	Amount of Dye Adsorption (mg/g)	Initial Adsorption Rate (ppm/min)	Amount of Dye Adsorption (mg/g)
100:0	0.0464	15.3576	0.0094	6.2191
80:20	0.0240	9.6674	0.0095	3.0918
50:50	0.0199	8.3892	0.0077	2.8466
20:80	0.0072	3.1411	0.0057	2.7410

4.4.4.2 Effect of Blend Compositions

The cumulative adsorption of methylene blue and methyl orange on CM-chitin/CTW composite films at various weight ratios are represented in Figure 4.14 and 4.15, respectively. Due to the presence of CTW in the composite films affected to the degree of swelling of the films, the dye adsorption of the composite films was decreased as the increasing of CTW content. Furthermore, the higher CTW content also resulted in the reduction of the initial dye adsorption rate and amount of dye adsorption as shown in Table 4.3.

In case of methyl orange which represented the negative charge, it could interact with the positive charge on the CTW surface but the interaction was not so strong to affect to the amount of dye adsorption. This is due to the result of the lower degree of swelling which remarkably consequence to the adsorption capacity.
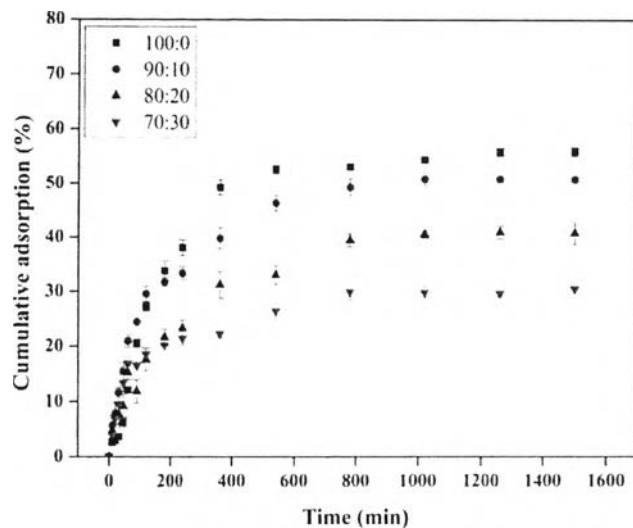
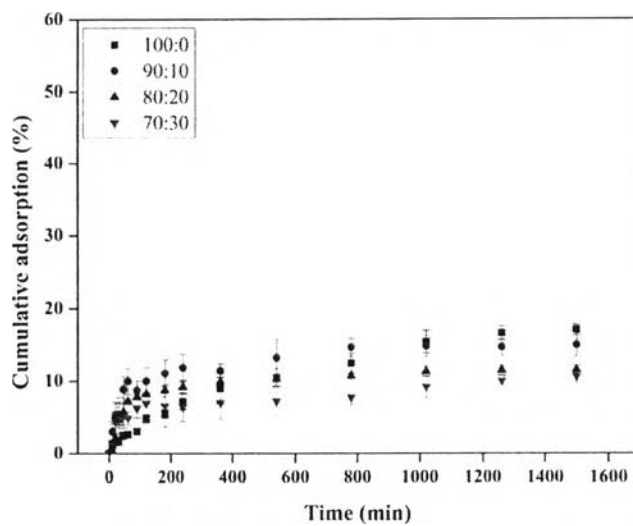**Figure 4.14** The cumulative adsorption of methylene blue on CM-chitin/CTW composite films at various weight ratios.

Figure 4.15 The cumulative adsorption of methyl orange on CM-chitin/CTW composite films at various weight ratios.

Table 4.3 The initial dye adsorption rate and amount of dye adsorption of CM-chitin/CTW films at various weight ratios

CM-Chitin:CTW Ratio	Dye types			
	Methylene Blue		Methyl Orange	
	Initial Adsorption Rate (ppm/min)	Amount of Dye Adsorption (mg/g)	Initial Adsorption Rate (ppm/min)	Amount of Dye Adsorption (mg/g)
100:0	0.0464	15.3576	0.0088	6.2191
90:10	0.0254	12.8632	0.0073	4.4347
80:20	0.0171	9.2232	0.0084	3.2446
70:30	0.0123	7.3303	0.0074	2.9568

4.4.5 Dye Desorption Behavior of CM-Chitin/CTW Composite Films

4.4.5.1 Effect of Blend Composition and The Presence of Lysozyme

The cumulative desorption of methylene blue and methyl orange from CM-chitin/CTW composite films at various weight ratios in tris-HCl buffer solution without and with 0.001 %w/v of lysozyme are represented in Figure 4.16 and 4.17, respectively. The experiment was set in the tris-HCl buffer solution which is the same pH at the human skin. When the polymer was hydrated by fluid, they could form the swollen gel which allowed the diffusion of the substance through the gel. Therefore, the dye compounds were continuous released. From the results, the composite films showed the high released rate in the first hour which called burst release (Huang and Brazel, 2003). The burst release is the important characteristic of the hydrogel materials. The incoporation of CTW did not show the significantly different on the adsorption rate at the initial time. This might be due to the CM-chitin can form hydrogel fast in the buffer solution, then, the CTW could not improve the releasing rate. Nevertheless, in wound dressing application, it has been shown that many drugs need to be administered at varying rates and for some drugs, such as those used at the beginning of wound treatment, an initial burst provides immediate relief followed by prolonged release to promote gradual healing (Huang and Brazel,

2001). Consequently, the burst release of these composite films could be the benefit in the wound dressing containing the active ingredients. In addition, the existence of CTW in the composite films exhibited the lower cumulative desorption because of the lower degree of swelling. Hence, it can be indicated that the desorption capacity of the composite films was the dependent with the degree of swelling of the hydogel.
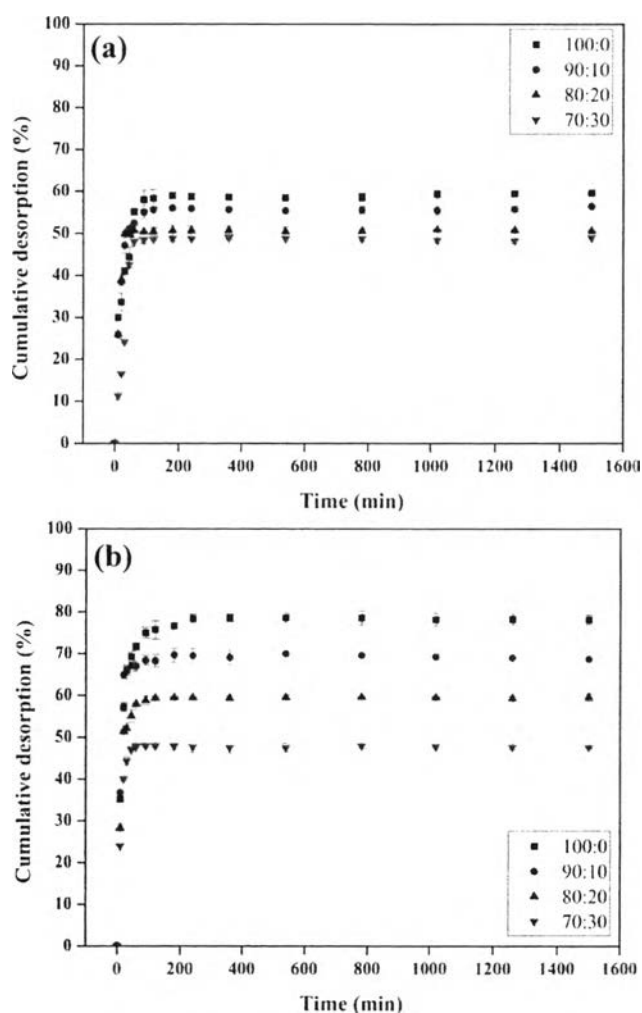

Figure 4.16 The cumulative desorption of methylene blue from CM-chitin/CTW composite films at various weight ratios in tris-HCl buffer solution (a) without lysozyme and (b) with 0.001 %w/v of lysozyme.

It is well known that lysozyme is the enzyme in human body fluids (Hankiewicz and Swierczek, 1974) which can be found in the wound. Then, the effect of the presence of lysozyme in the buffer solution was studied at the

concentration of 0.001 %w/v which was the normally amount found in human serum (Hankiewicz and Swierczek, 1974). Since CM-chitin and CTW can be digested by lysozyme (Watanabe *et al.*, 1990; Muzzareli *et al.*, 2007), so the presence of lysozyme could affected the the release of substance from the composite films. From the Figure 4.16, the composite films exhibited the higher desorption capacity in the buffer solution with lysozyme. The presence of lysozyme supported the erosion mechanism and then resulted in breakage of the film structure. The composite films were digested into small piece within 12 hours and the composite with the high amount of CTW tended to be digested slower than the neat CM-chitin. In agreement with Hjerde *et al.* (1997), they studied the rate of degradation of CM-chitin and they reported that the long-range electrostatic interactions between the negatively charged of CM-chitin chain and the positively charged lysozyme could affected the digestion CM-chitin. Moreover, lysozyme could hydrolyse (1→4) glycosidic linkage in chitin but the hydrolysis proceeds very slowly in highly crystalline chitin (Hjerde *et al.*, 1997). Therefore, the presence of CTW could slow down the degradation rate.

However, the cumulative desorption was not reach to 100% due to the ionic interaction between the polymer and the dye compound. In case of methyl orange, the desorption rate was not so high in the initial time. This mighe be due to the low dye adsorption capacity, so the amount of dye in the films was low as well. Then, the amount of dye release was slowly increase.
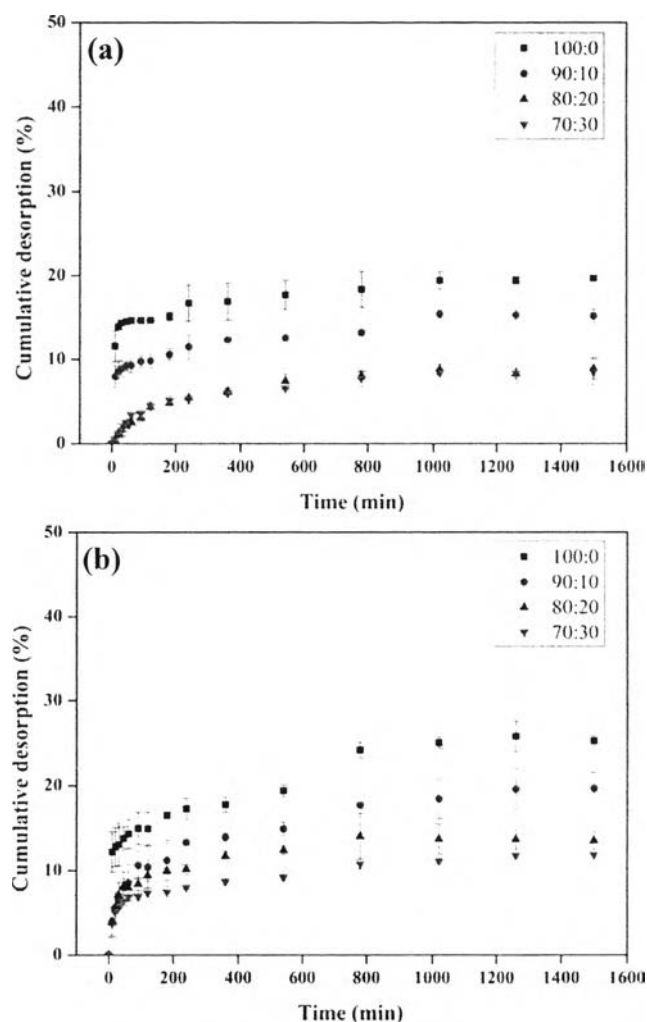
Figure 4.17 The cumulative desorption of methyl orange from CM-chitin/CTW composite films at various weight ratios in tris-HCl buffer solution (a) without lysozyme and (b) with 0.001 %w/v of lysozyme.

4.4.5.2 Effect of Ionic Strength

Generally, the human body fluids consist of many ions which are beneficial to the body system such as sodium, calcium, potassium etc. Then, the effect of ionic strength in the media to the releasing characteristic should be studied. The composite films with the ratio of 100:0 and 90:10 were selected to study this effect because of their high desorption capacity. The cumulative desorption of methylene blue and methyl orange from CM-chitin/CTW composite films in various concentrations of NaCl in tris-HCl buffer solution is exhibited in Figure 4.17 and

4.18, repectively. As the amount of ions in media increased, the amount of dye released was higher. The ions could obstruct the ionic interaction between the polymer and the dye compound and resulted in the increasing of dye desorption. However, the dye desorption in various ionic strength was not significantly different especially in case of methyl orange. This can be concluded that the presence of lysozyme affected to the dye desorption more than the increasing of ionic strength of the media.
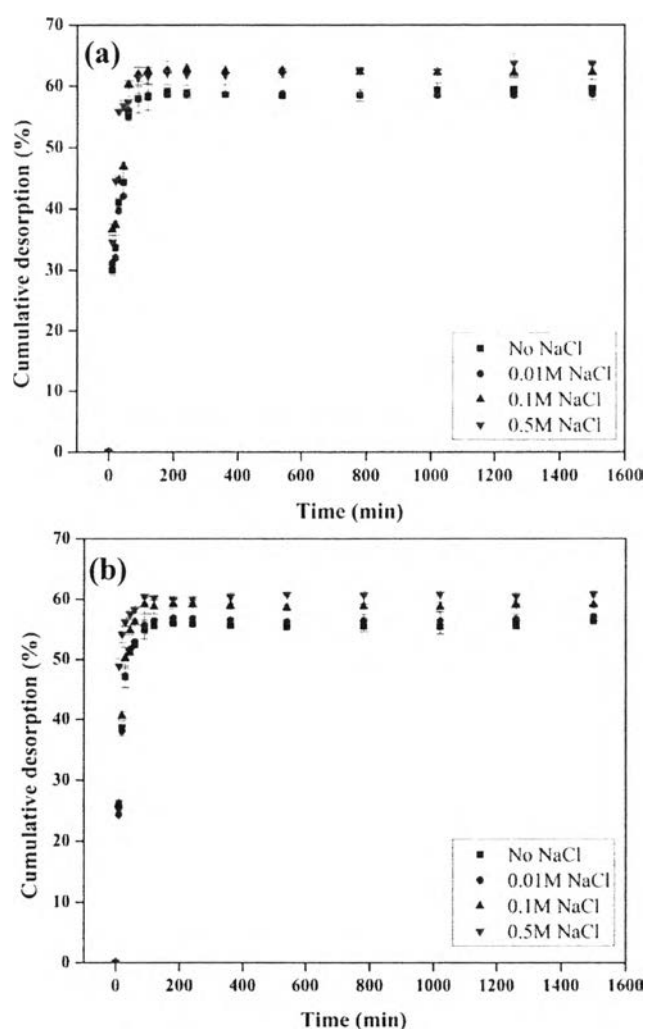

Figure 4.18 The cumulative desorption of methylene blue from CM-chitin/CTW composite films at the weight ratio of (a) 100:0 and (b) 90:10 in various concentrations of NaCl in tris-HCl buffer solution.

Figure 4.19 The cumulative desorption of methyl orange from CM-chitin/CTW composite films at the weight ratio of (a) 100:0 and (b) 90:10 in various concentrations of NaCl in tris-HCl buffer solution.
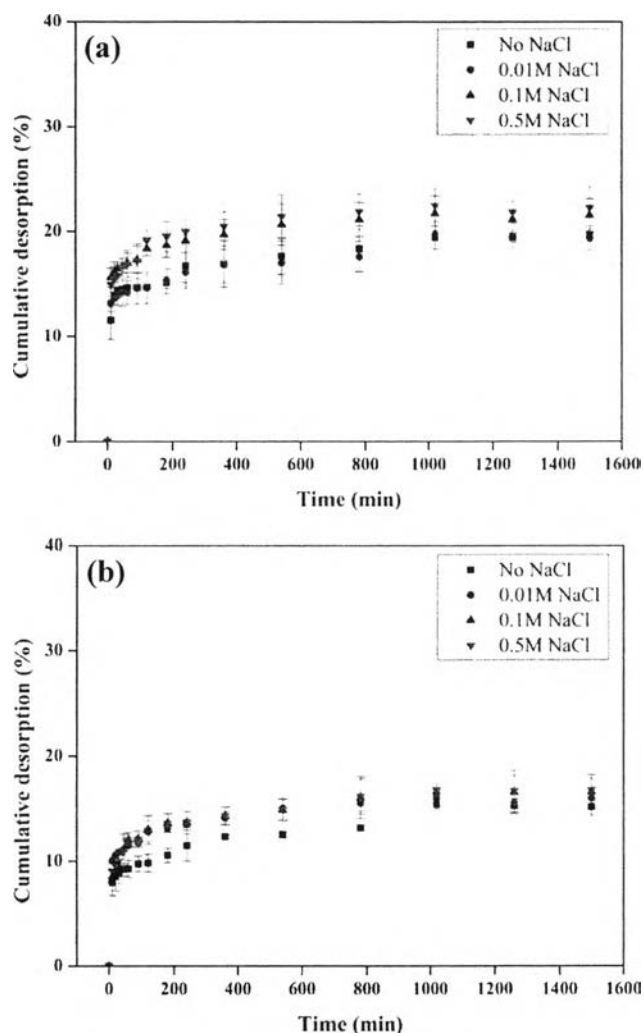
4.5 Conclusions

CM- chitin was successfully synthesized by carboxymethylation reaction at O-6 position of chitin. CM- chitin films can be prepared by solution casting technique and the clearly thin films were obtained. CM- chitin films were effectively crosslink by using glyoxal and the optimum pH in crosslinking reaction was 6. The presence of chitin whisker in CM-chitin/CTW composite films resulted in low degree of swelling and weight loss which led to the improvement of shape stability. For the

use as wound dressing carrying active ingredients, CM- chitin could adsorbed cationic compound higher than anionic compound because of the ionic interaction between carboxymethyl group of CM-chitin and cationic group of the compound. Moreover, the existence of chitin whisker in composite films exhibited the lower adsorption and desorption due to the low degree of swelling. CM-chitin can be degraded by lysozyme within 12 hrs and then led to the higher desorption capacity. In addition, the increasing of ionic strength in the media could enhance the dye desorption capacity as well. In conclusion, CM-chitin/CTW composite films showed a potential to be used as a hydrogel based wound dressing material and the optimum blend ratio is 90:10.

4.6 Acknowledgements

4.7 References

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