CHAPTER III

PREPARATION AND PHYSICO-CHEMICAL CHARACTERISTICS OF N-MALEOYL CHITOSAN FILMS

3.1 Abstract

N-maleoyl chitosan (N-MC), a water-soluble derivative of chitosan, was synthesized by N-carboxyacylation of chitosan and maleic anhydride. N-MC films were prepared by solvent casting and kept in a desiccator for different time intervals in order to obtain N-MC films with different degrees of cross-linking. The swelling characteristic of N-MC films was investigated. When the aging time increased from 5 to 30 d, the swelling of the N-MC films decreased from ~475 to ~160% and the weight loss decreased from ~25 to ~8%, corresponding to the increase in the cross-linking density from $0.86 \times 10\text{-}6$ to $8.97 \times 10\text{-}6$ mol·cm-3. Additionally, the swelling behavior of the N-MC films was influenced by changes in either the pH or the ionic strength of the media.

(**Key-words**: Chitosan, N-maleoyl chitosan sodium salt, Solvent-cast film, Swelling behavior, Weight loss)

3.2 Introduction

Chitosan, an aminopolysaccharide, is a N-deacetylated product of chitin, which is an abundant polymer readily obtained from exoskeletons of shells of crustaceans, such as shrimps and crabs. Chitosan is soluble in an acidic aqueous solution of an organic acid, such as formic, acetic, propionic, lactic, or citric acid. The applicability of chitosan is therefore limited by its insolubility in neutral and basic aqueous solutions as well as common organic solvents. As a result, several chemical modifications of chitosan have been reported in the literature (Sashiwa and Aiba, 2004) to produce the derivatives that are soluble in a variety of solvent systems and exhibit wide applicability in biomedical fields. Several derivatives of the water soluble N-carboxyacyl chitosan have been synthesized by varying the types of the dicarboxylic anhydride, such as maleic, succinic, phthalic, and citraconic anhydride

(Sashiwa and Shigemasa, 1999; Hirano and Moriyasu, 2004).The sodium salt of these chitosan derivatives can be dissolved in neutral and basic aqueous solutions. Among the various derivatives of N-carboxyacyl chitosan, N-maleoyl chitosan (N-MC) is of great interest, due to its decreased solubility in an aqueous medium upon aging in air. This is expected to be a result of the slow reformation of H-bond as well as the formation of cross-linking and grafting reactions by their vinyl groups after preparation (Sashiwa and Shigemasa, 1999).This makes the sodium salt of N-MC (hereafter, Na N-MC) a self-cross-linkable water-based hydrogel polymer, which finds its usefulness in both pharmaceutical and biomedical fields. In the present contribution, Na N-MC was fabricated into films by solvent-casting technique. The physico-chemical properties of the obtained films were characterized in terms of their swelling and weight loss behavior at different aging time after film formation. Changes in the film properties, such as the swelling behavior and the mechanical properties, due to the variation in the pH, and the ionic strength of the medium were also investigated.

3.3 Experimental Section

3.3.1 Materials

Chitosan [degree of deacetylation = 95%; $Mn \approx 100,000$ Da; Seafresh Chitosan (Lab), Thailand] was treated with 50% w/w NaOH solution (KPT Cooperation, Thailand) at 110°C for 1 h in an autoclave prior to further use. Maleic anhydride (analytical grade) was purchased from Fluka. All other chemicals were of chemical reagent grade and used without further purification.

3.3.2 Synthesis of N-Maleoyl Chitosan Sodium Salt

Na N-MC was prepared by first dissolving 1.6 g of chitosan in 50 ml of 2% w/w acetic acid. The chitosan solution was then diluted with 300 ml of methanol. Exactly 3 g of maletic anhydride (i.e., 3 mol/glucosamine unit, GlcN) was dissolved in 100 ml of methanol prior to being added in the chitosan solution. After 30 min of stirring at room temperature, the mixture was allowed to settle at room temperature overnight to obtain gel. The gel was collected and repeatedly washed with ethanol and dried *in vacuo* overnight. The obtained product was the acid form of

N-MC. Na *N*-MC was obtained by treating the acid-form *N*-MC product with 250 ml of 0.1 M NaOH at room temperature overnight. The Na *N*-MC product was precipitated by adding 1500 ml of ethanol at room temperature. The precipitate was collected by filtration, washed with ethanol, and dried *in vacuo* overnight. The product was dissolved in distilled water, dialyzed (molecular weight cut-off at 4000 Da) in distilled water for 3-5 d and centrifuged at 10000 rpm for 10 min. The supernatant was finally freeze-dried to obtain a white sponge.

3.3.3 Characterization

3.3.3.1 Structural Characterization

The chemical integrity of the neat chitosan and the obtained Na *N*-MC films was investigated on a Thermo Nicolet Nexus 670 Fourier-transformed infrared spectrometer (FT-IR) using the transmittance mode at 32 scans and the resolution of 4 cm^{-1} over the wavenumber range of 4000-400 cm^{-1}. The degree of substitution of the obtained Na *N*-MC was determined by elemental analysis on a Perkin-Elmer PE2400 Series II with CHN mode.

3.3.3.2 Swelling and Weight Loss Characteristics

The swelling and the weight loss of the Na *N*-MC films were measured after they had been incubated in 5 ml of one of the media (see later for the preparation of different media) at 37°C. After 24 h or incubation, the films were taken out, blotted to remove the excess amount of the medium, and weighed immediately. The degree of swelling of the films that had been prepared at different ageing periods was calculated according to the following equation:

$$\text{Degree of swelling (\%)} = (W_s - W_d)/W_d \times 100, \qquad (1)$$

Where W_s is the weight of the films in the swollen state and W_o is the initial, dry weight of the film. For the weight loss, the films were subsequently dried and their dry weights were recorded to finally obtain the weight loss according to the following equation:

$$\text{Weight loss (\%)} = (W_d - W_o)/W_o \times 100, \qquad (2)$$

Where W_d is the weight of the dried films after the swelling measurement.

3.3.3.3 Crosslink Density

The cross-linking density of the Na N-MC films that had been prepared at different ageing periods was also determined based on the swelling method. In this case, distilled water was used as the medium. The cross-linking density (v) was calculated from the Flory-Rehner equation (Flory, 1953):

$$v = -\left(\frac{\ln(1 - \upsilon_{2m}) + \upsilon_{2m} + \chi_{sp}\upsilon_{2m}^{2}}{2(\upsilon_{2m}^{1/3} - 1/2\upsilon_{2m})\rho_1 V_0} \right) \qquad (3)$$

$$\upsilon_{2m} = \frac{W_d}{\upsilon_{equil} \times \rho_2} \qquad (4)$$

$$V_{equil} = \frac{W_d}{\rho_2} + \frac{W_s - W_d}{\rho_1} \qquad (5)$$

V_0 is the molar volume of the solvent (i.e., 18 $cm^2 \cdot g^{-1}$ for water), ρ_1 is the true density of N-MC (i.e., ~1.58 $g \cdot cm^{-3}$), ρ_2 is the density of water (i.e., 1.00 $g \cdot cm^{-3}$), χ_{sp} is the polymer-solvent interaction parameter, which can be estimated from the following equation:

$$\chi_{sp} = 0.34 + \frac{V_1(\delta_1 - \delta_2)^2}{RT} \qquad (6)$$

Where δ_1 and δ_2 are the solubility parameters or the cohesive energy densities of the solvent and the polymer, respectively (Krevelen,1976) (i.e., $\delta_1 = 47.9$ $MPa^{1/2}$ for water and $\delta_2 = 43.7$ $MPa^{1/2}$ for N-MC), V_1 is the partial molar volume of the solvent (i.e., 18 $cm^3 \cdot mol^{-1}$ for water), R is the universal gas constant (i.e., 8.314 $cm^3 \cdot MPa \cdot K^{-1} \cdot mol^{-1}$) and T is the absolute temperature (i.e., 298 K).

3.3.3.4 Effects of pH and ionic strength on swelling behavior

To study the effect of pH, the Na N-MC films that had been aged for 10 d were incubated in each of the media of different pH values, i.e., pH 1.0 (0.1 M HCl), pH 2.0 (0.01 M HCl), pH 4.0 (0.2 M CH_3COOH/0.5 M CH_3COONa), pH 5.5 (0.2 M CH_3COOH/0.5 M CH_3COONa), pH 7.4 (1.3 mM $NaHPO_4$/8.8 mM Na_2HPO_4), and pH 9.0(0.05 M Na_2HPO_4), for 24 h. In these cases, the ionic strength of the media was maintained at 1.2 M by an appropriate amount of NaCl. To study the effect of the ionic strength, the ionic strength of the media at three different values of pH (i.e., 2.0, 7.4, and 9.0) was varied between 0.15 and 2.0 M.

3.3.3.5 Mechanica Integrity

Mechanical properties in terms of the tensile strength and the elongation at break of the Na *N*-MC films that had been aged for different time intervals were investigated on a Llyod LRX universal testing machine. The specimens were cut from the films (rectangular shape, 1 cm × 10 cm). The gauge length and the crosshead speed were 50 mm and 50 mm min^{-1}, respectively.

3.4 Results and Discussion

3.4.1 Characterization of as-Synthesized Na *N*-MC

FT-IR spectra of the chitosan and the Na *N*-MC films (lower and upper curves, respectively) are shown in Figure 3.1. The characteristic absorption peaks of chitosan can be observed at 3456 cm^{-1} (O-H and N-H stretching), 2866 cm^{-1} (C-H stretching), 1586 cm^{-1} N-H bending), 1154 cm^{-1} (bridge -O- stretching), and 1093 and 1034 cm^{-1} (skeletal vibration involving the C-O stretching). In addition to the characteristic peaks of chitosan, the FT-IR spectrum of Na *N*-MC films shows new characteristic absorption bands at 1661 and 1565 cm^{-1}, which are specific to C=O stretching and N-H bending of the acyl groups, respectively.

The elemental analysis of the as-synthesized Na *N*-MC revealed the percentage of C, H, and N elements as ~38.2, ~6.1, and ~5.8%, respectively. Based on these values, the degree of substitution of the as-synthesized *N*-MC was estimated to be ~43.2%.

3.4.2 Physico-Chemical Properties of Na *N*-MC Films

4.4.2.1 Effect of Ageing Time

As previously mentioned, Na *N*-MC can undergo self-cross-linking reactions by their vinyl groups. Here, the effect of ageing time of the as-prepared Na *N*-MC films on their swelling and weight loss behavior after submersion in distilled water for 24 h was investigated. Figures 3.2 and 3.3 show the degree of swelling and the percentage of weight loss of Na *N*-MC films as a function of the ageing time. Evidently, both the degree of swelling and the percentage of weight loss decreased rather monotonically from ~475% and ~25%, respectively, at the ageing time of 5 d to ~160% and ~8%, respectively, at the ageing time of 25 and 30 d. The

observed decrease in the property values with increasing the ageing time (i.e., up to 25 d) corresponded to the increase in the cross-linking density of the Na N-MC hydrogel films from 0.86×10^{-6} to 8.97×10^{-6} mol·cm^{-3} (see Figure 3.4).

3.4.2.2 Effect of pH

Due to the presence of both the amino and the carboxylate groups (i.e., COO$^-$) in the as-synthesized Na N-MC, the sensitivity of the corresponding films towards the pH of the medium is of great interest. The swelling behavior of 10 d-aged Na N-MC films that were submerged in the medium of varying pH for 24 h is illustrated in Figure 3.5. Evidently, the degree of swelling of the films initially decreased from ~336% at pH 1.0 to reach a minimum value of ~154% at pH 5.5 and, finally increased to ~249% at pH 9.0 At pH < 5.5, electrostatic repulsion between the protonated primary amino groups (i.e., NH$_3$$^+$ was responsible for the high swelling ability of the films, while, at pH > 5.5, the electrostatic repulsion between the COO$^-$ groups was. On the other hand, electrostatic interaction between the NH$_3$$^+$and the COO$^-$ groups as well as the interactions between the free amino (i.e., NH$_2$) and the free carboxyl (i.e., COOH) groups should be responsible for the inability of the films to swell in the media at pH's 4 and 5.5.

3.4.2.3 Effect of Ionic Strength

The swelling behavior of 10 d-aged Na N-MC films that were submerged in the medium of varying ionic strength (i.e., between 0.15 and 2.0 M) at three different values of pH (i.e., 2, 7.4, and 9) for 24 h is also investigated and the results are graphically shown in Figure 3.6. At any pH, the Na N-MC films showed a monotonous decrease in their swelling ability with increasing the ionic strength. According to the Flory-Rehner theory (Flory, 1953), the swelling ratio is related to the ionic strength according to the following equation:

$$Q^{5/3} = [(\frac{i}{2V_\mu S^{1/2}})^2 + (\frac{1/2 - \chi}{V_1})]/(\upsilon_e / V_0) \qquad (7)$$

Where $Q^{5/3}$ is the swelling ratio, $i/2V_\mu$ is the concentration of the fixed charges in the original un-swollen state of the polymer, S is the ionic strength of the medium, υ_e is the effective number of chains in the network, and V_r is the volume of the relaxed network. According to Equation (7), the swelling ratio is

related to the reciprocal value of the ionic strength, suggesting that the swelling ability of hydrogel is a decreasing function of the ionic strength of the medium. Here, with increasing the ionic strength of the medium, the NH_3^+ groups (for the films that were incubated at pH 2.0) or the COO^- groups (for the films that were incubated at pH 9.0) or both (for the films that were incubated at pH 7.4) are screened by Na^+ or Cl^- or both, causing the films that had been incubated in the medium at a higher pH value to be denser than the ones that had been incubated in the medium at a lower one (Zhang, *et al.*, 2005).

3.4.3 Mechanical Properties of Na *N*-MC Films

Mechanical properties in terms of the tensile strength and the elongation at break of the Na *N*-MC films that had been aged for different time intervals, ranging from 5 to 30 d, were investigated and the results are graphically shown in Figures 3.7 and 3.8. The tensile strength of the films increased with the initial increase in the ageing period of 5 d, reached a maximum value at the aging period of ~15 d, and, finally, decreased with further increase in the ageing period. Clearly, as opposite trend was observed for the elongation at break, in which it decreased with the initial increase in the ageing period of 5 d, reached a minimum value at the aging period of ~15 d, and, finally, increased with further increase in the ageing period. The initial increase in the tensile strength or the initial decrease in the elongation at break of the Na *N*-MC films that had been aged for 5 to 15 d should be due to the increase in the cross-linking density. At longer ageing periods however, interaction of absorbed water molecules with the hydrophilic functional groups of Na *N*MC (Fringant, *et al.*, 1996) should be responsible for the observed decrease in the tensile strength or the observed increase in the elongation at break of the films, due to the plasticizing effect of the water molecules(Chang, Karim and Seow, 2006).

3.5 Conclusion

N-maleoyl chitosan sodium salt (Na *N*-MC), a self-cross-linkable, water-soluble derivative of chitosan, was successfully synthesized and fabricated into films by solvent-casting technique. The self-cross-linking ability of the Na *N*-MC films was assessed by observing their swelling and the loss in their weight after

submersion in distilled water at 37°C for 24 h. Both the swelling and the loss in the weight of the films decreased monotonically with the ageing time (i.e., from 5 to 25 and 30 d). These corresponded to the increase in the cross-linking density with the ageing time, as calculated from the swelling ability of the films. Effects of both the pH and the ionic strength of the media on the swelling behavior of the films that had been aged for 10 d were also investigated. The results showed that the Na N-MC films showed a minimum in their swelling ability when being submerged in the medium with the pH of 5.5, while they showed a monotonous decrease in their swelling ability with increasing the ionic strength of the media. Based on these properties, Na N-MC is a useful material for both pharmaceutical and biomedical applications, such as self-cross-linkable pH-sensitive drug carriers.

3.6 Acknowledgements

## 3.7 References

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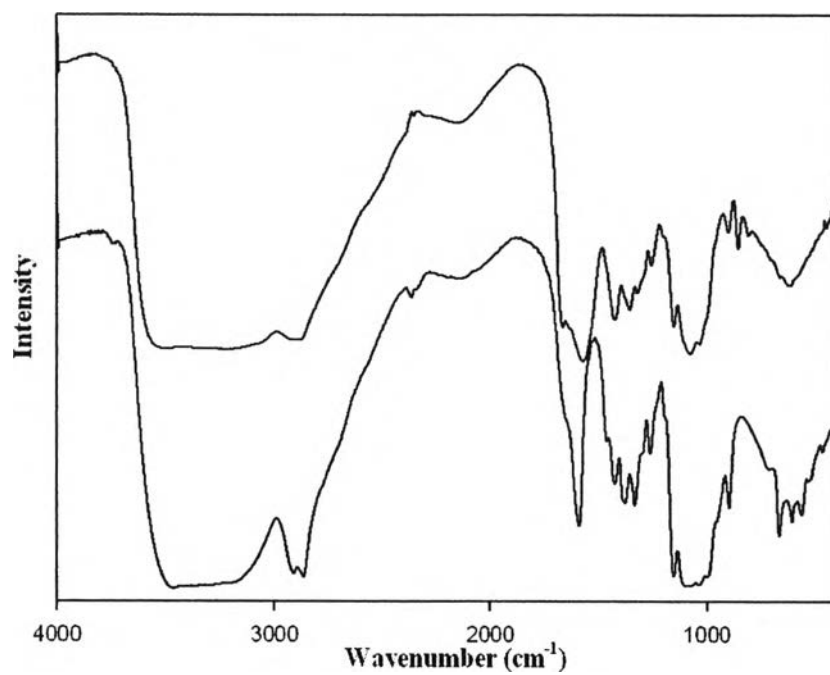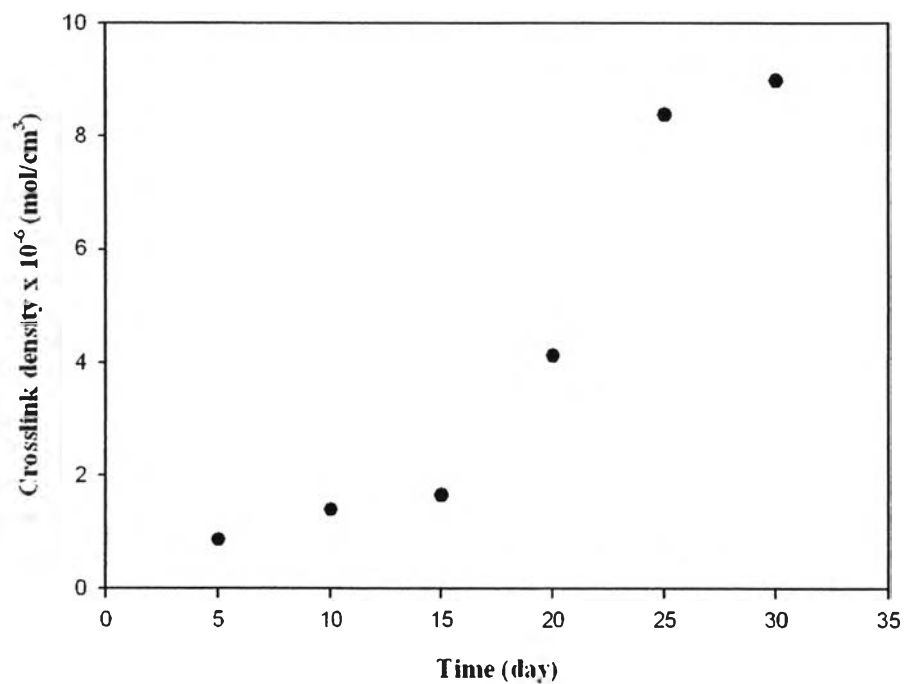
Figure 3.1 The FT-IR spectra of chitosan film (lower line) and Na *N*-maleoyl chitosan film (upper line).
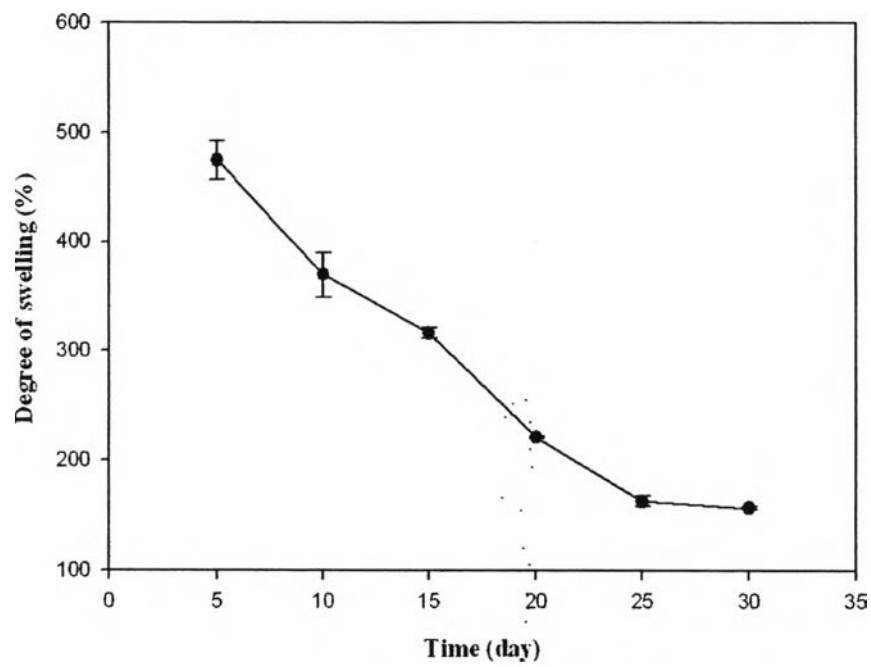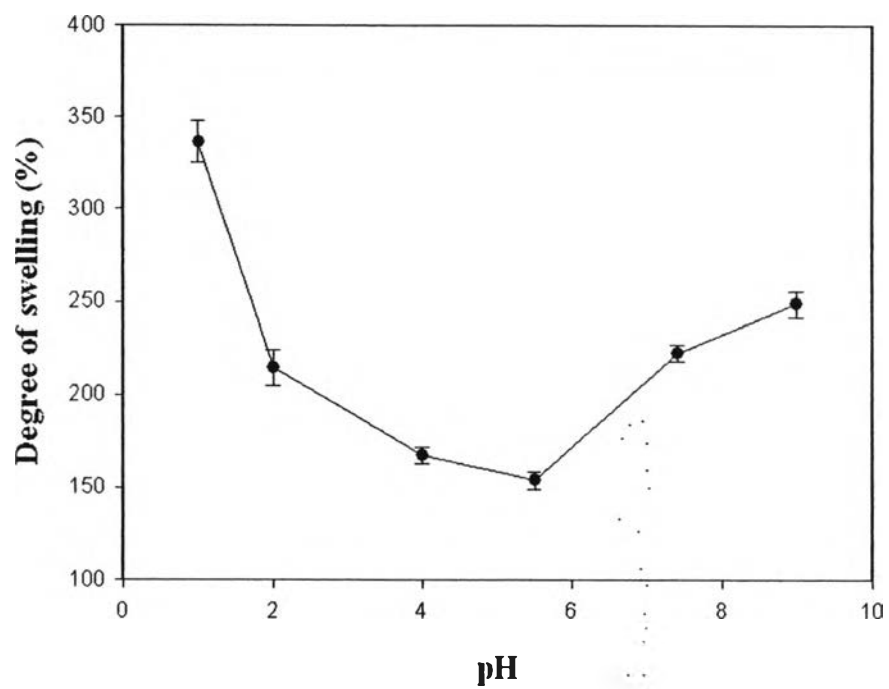
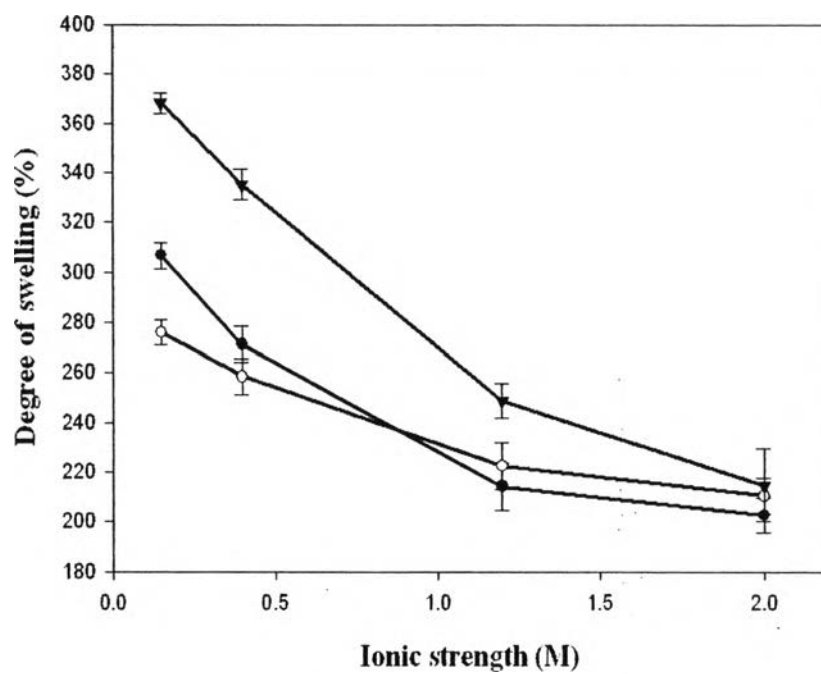**Figure 3.2** The degree of swelling at different storage time of Na *N*-maleoyl chitosan films.
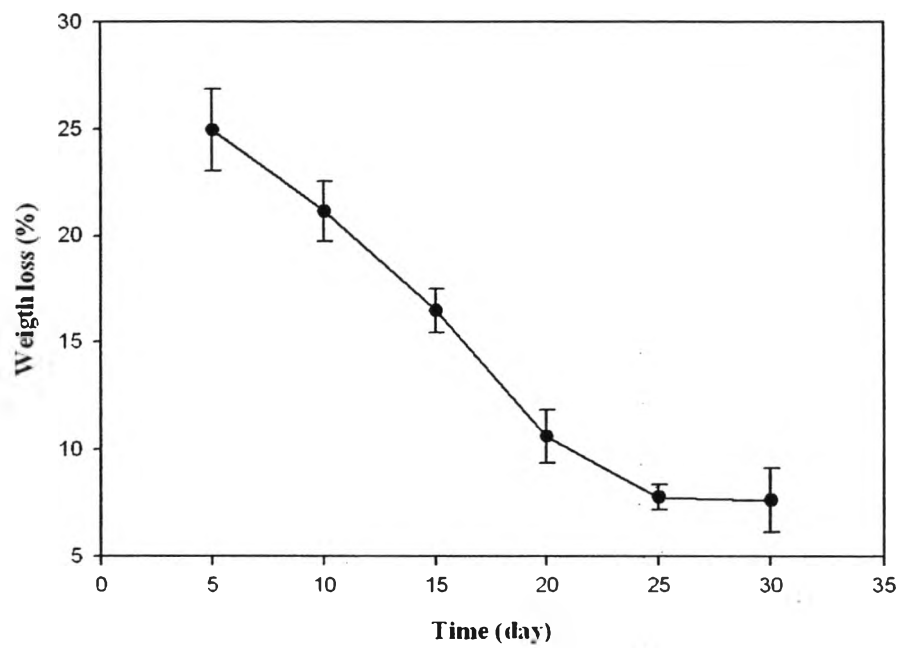
**Figure 3.3** The percentage of weight loss at different storage time of Na *N*-maleoyl chitosan films.

Figure 3.4 The crosslink density of Na N-maleoyl chitosan films at different time of storage.

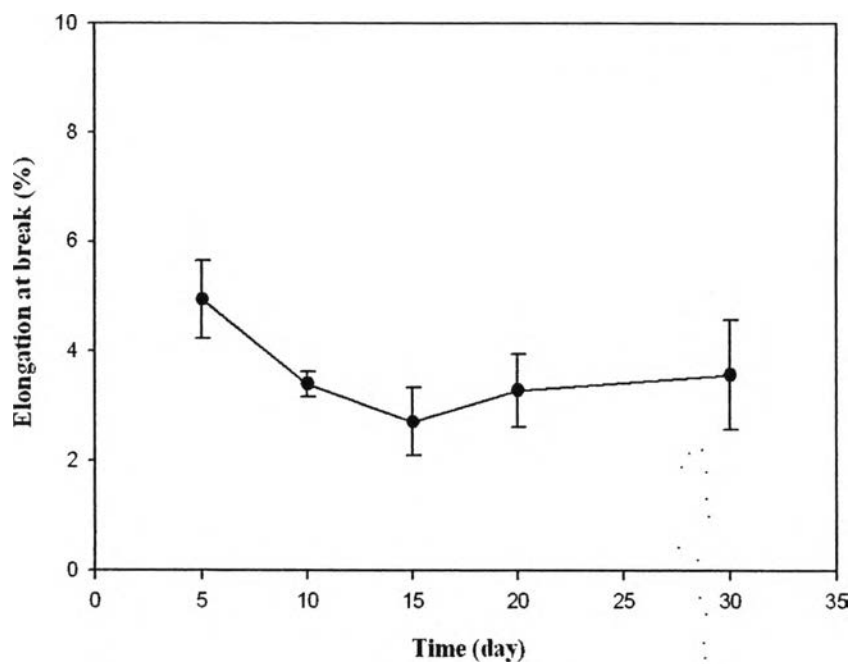**Figure 3.5** The degree of swelling at different pH of 10 days Na *N*-maleoyl chitosan films

Figure 3.6 The degree of swelling at different ionic strength of 10 days Na *N*-maleoyl chitosan films. (▼at pH= 9.0, o at pH=7.4,● at pH= 2.0)
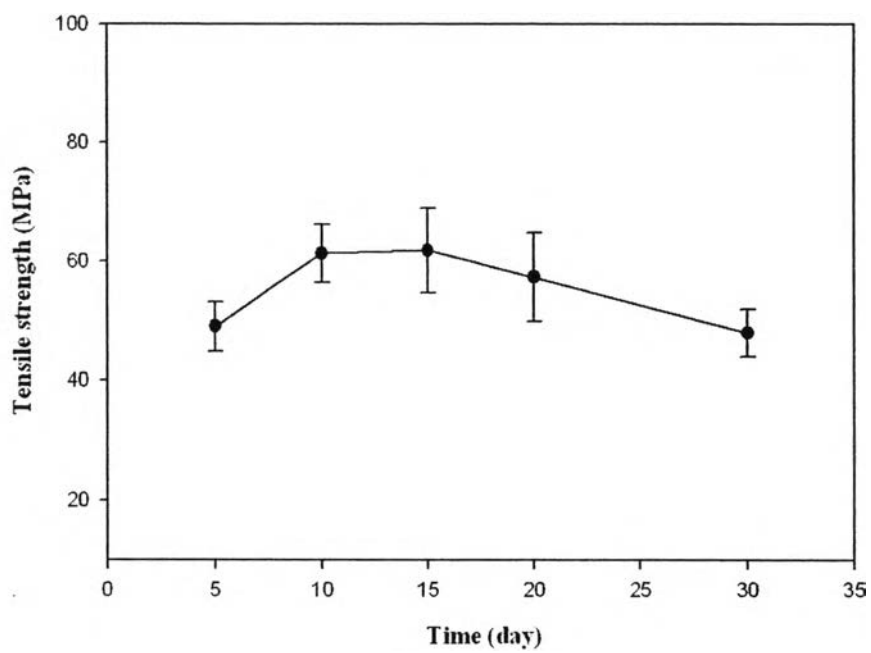
Figure 3.7 The tensile strength at different time of Na *N*-maleoyl chitosan films.

Figure 3.8 The elongation at break at different time of Na *N*-maleoyl chitosan films.