CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preparation of Chitin

Shrimp shells compose of three 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 merguiensis shrimp by demineralization with hydrochloric acid solution and deproteinezation 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

Materials	Yield* (%)
Shrimp shell	100
Chitin	34.17

*dry weight basis

Chitin has some extent of amino groups other than acetamide groups at C2 position of N-acetyl glucosamine repeating units. The degree of deacetylation of chitin depend 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 deproteinzation process under alkaline condition and heating. According to the method of Sannan et al., 1978, The degree of deacetylation of chitin calculated from FTIR (Figure 4.1) was 25.06%

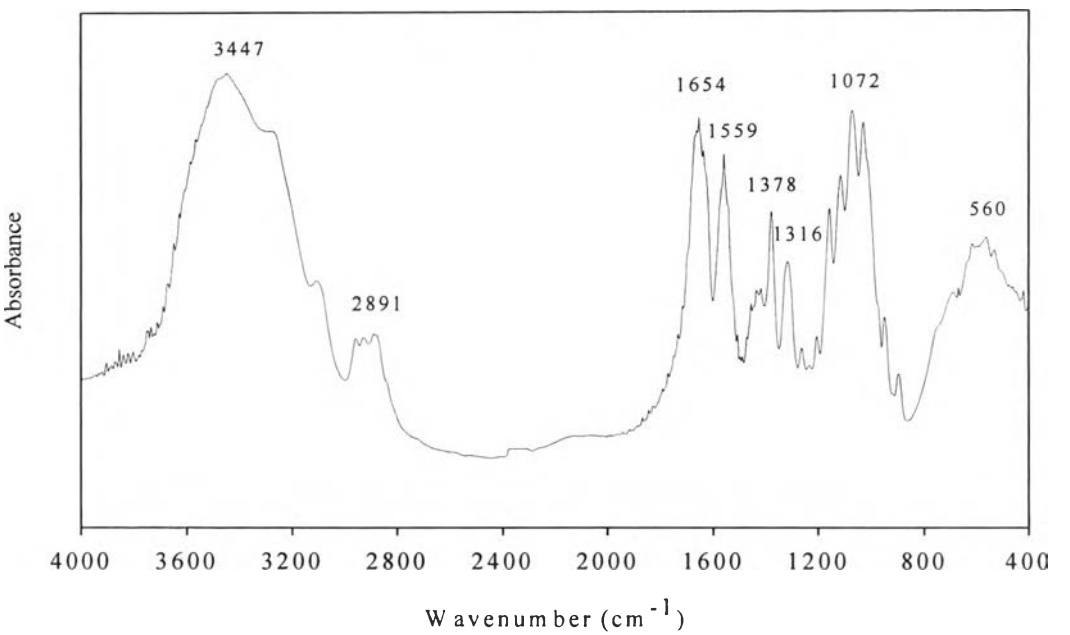

Figure 4.1 FTIR spectrum of chitin powder.

Figure 4.1 shows an IR spectrum of chitin. The characteristic absorption bands at 1654, 1559, and 1316 CM^{-1} due to the amide I, II, and III bands which assigned to C=O, N-H, and C-N stretching of acetamide groups, respectively. The sharp band at 1378 CM^{-1} has been assigned to the CH_3 symetrical deformation mode. The absorption at 2891 and 3447 CM^{-1} assigned to C-H and O-H stretching bands, respectively. The characteristic absorption bands of this study are similar to that of chitin which was reported by Sannan *et al.*, (1978). The molecular weight of chitin was determined by viscometric method. The intrinsic viscosity was obtained at 15.68 (100ml/g). The viscosity-average molecular weight of chitin was calculated to be 9.52×10^5 g/mol.

4.2 Preparation of CM-chitin

Although chitin is insoluble in common solvents, the dissolubility of chitin can be improved by chemical modification. CM-chitin, one of the water-soluble derivatives of chitin, was modified by carboxymethylation with monochloroacetic acid. The FTIR spectrum of CM-chitin is shown in Figure 4.2

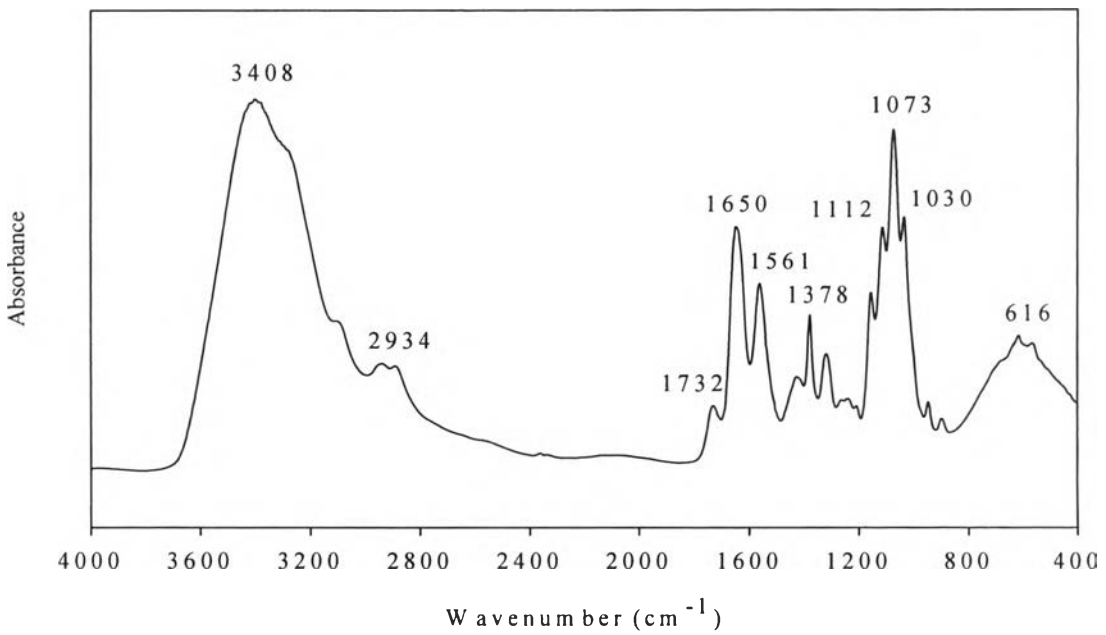

Figure 4.2 FTIR spectrum of CM-chitin film.

Figure 4.2 shows an IR spectrum of CM-chitin. The presence of carboxyl groups are indicated by absorption peak at 1732 cm^{-1} which are observed obviously in IR spectra of all CM-chitin after treating the Na-type CM-chitin with 2 N HCl. The characteristic absorption bands appear at 1650, 1560, 1320 and 1378 indicating a successful substitution of carboxymethyl groups which are amide I, amide II, amide III, and $-CH_3$ vibration bends, respectively. The C-O stretching vibrations appear at 1030 and 1070 cm^{-1} corresponding to the primary hydroxyl group. The secondary amide vibration band at 1561 cm^{-1} conform the NH deformation. The strong, broad band of spectrum at 3408 and 3290 cm^{-1} are assigned to the hydrogen-bonded –OH and –NH bands.

The molecular weight of CM-chitin was determined by viscometric method. According to the method of Kanebo (1982), the molecular weight of CM-chitin was derived from its intrinsic viscosity. The intrinsic viscosity was obtained at 6.28 (100ml/g). The viscosity-average molecular weight of chitin was calculated to be 7.93×10^4 g/mol. The degree of substitution of CM-chitin was estimated to be 0.37.

4.3 Effect of Drug Concentration on Drug Release

To investigate the effect of drug concentration on drug release, CM-chitin films containing 0.1%, 0.2%, and 0.5% by weight of the model drugs and chitosan films containing 0.2%, 0.5% and 1.0% by weight of the model drugs were used. Salicylic acid and theophylline were used as model drugs in this study.

The drug release profiles of CM-chitin and chitosan films with different drug concentrations are shown in figures 4.3-4.6. For CM-chitin films containing salicylic acid and theophyline, the amounts of released drug were increased with the increasing of time and reached the equilibrium after 20 hours. The total amounts of released drug at equilibrium for 0.1%, 0.2%, and 0.5% loading of salicylic acid were 0.37, 0.73, and 1.63 mg respectively. In addition, the total amounts of released drug at equilibrium for 0.1%, 0.2%, and 0.5% loading of theophylline were 0.36, 0.70, and 1.58 mg respectively.

For chitosan films containing salicylic acid and theophyline, the drug release profiles of drug loaded-chitosan films were similar to those of drug loaded CM-chitin films. The total amounts of released drug at equilibrium for 0.2%, 0.5%, and 1.0% loading of salicylic acid were 0.59, 1.98, and 3.56 mg respectively. In addition, the total amount of released drug at equilibrium of 0.2%, 0.5%, and 1.0% theophylline were 0.37, 1.57, and 3.42 mg, respectively.

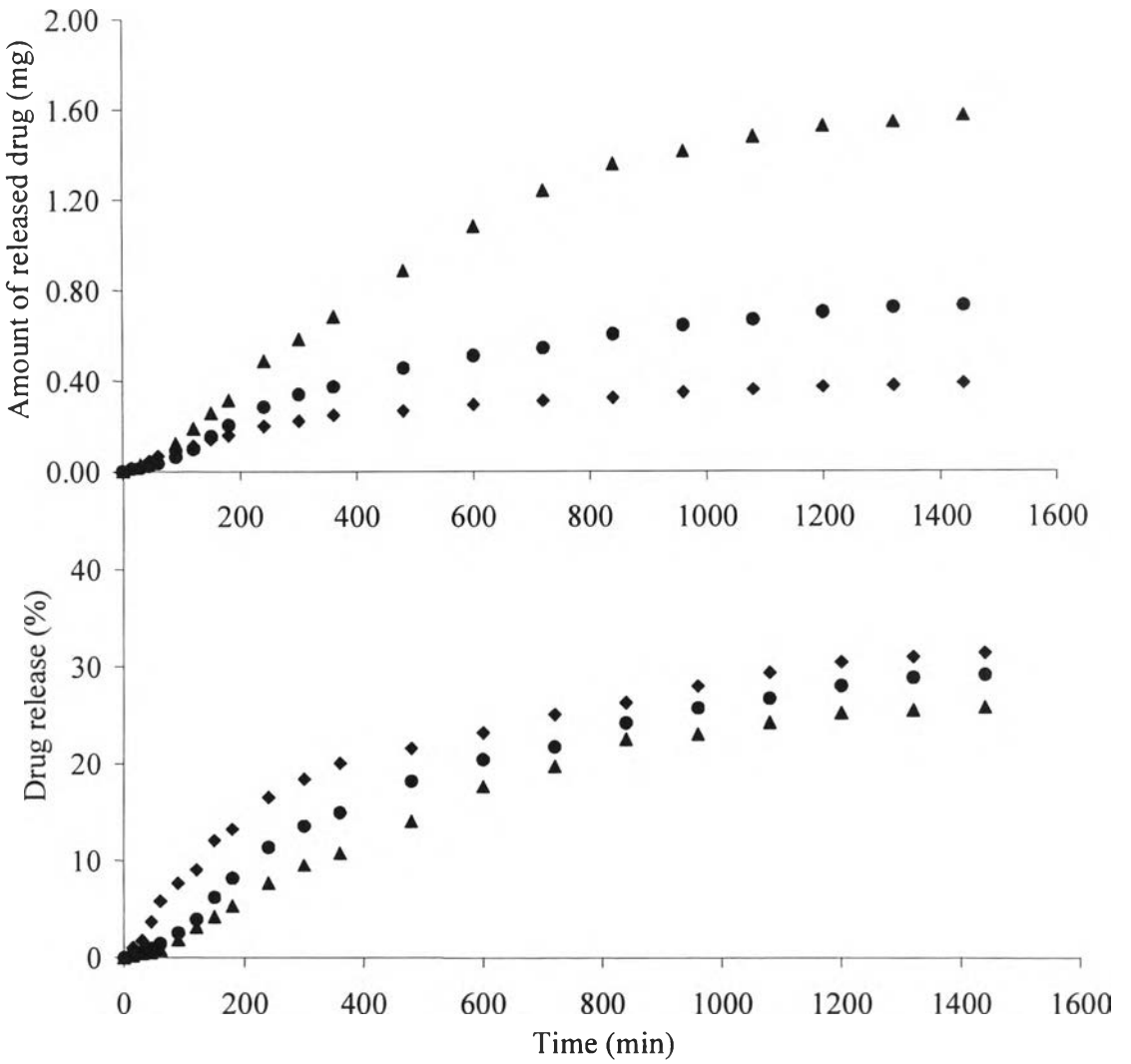

Figure 4.3. Salicylic release profiles of CM-chitin films containing different amount of drug concentration at 37°C and pH 5.5. (◆) 0.1% salicylic acid, (●) 0.2% salicylic acid, (▲) 0.5% salicylic acid.

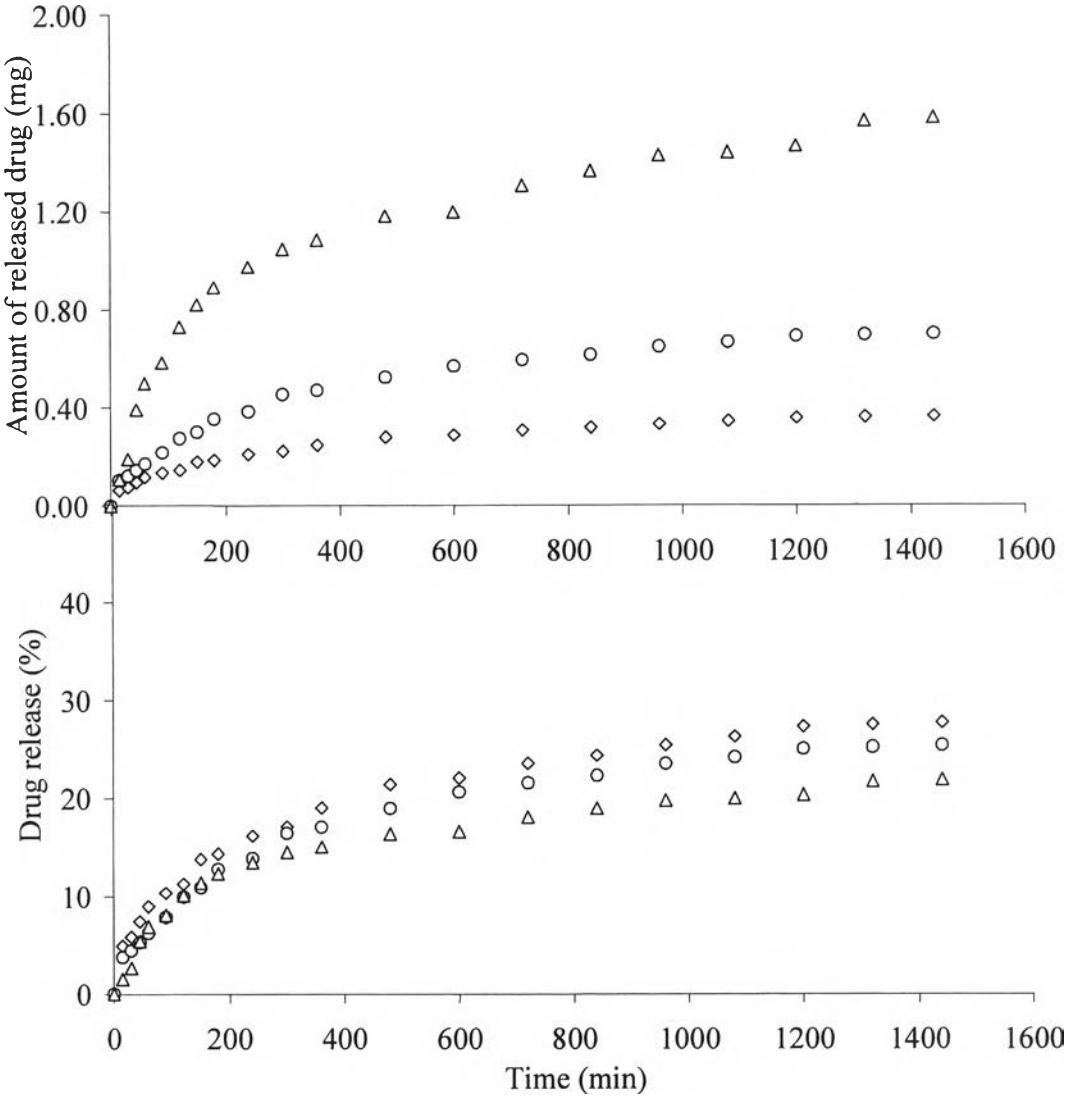

Figure 4.4. Theophylline release profiles of CM-chitin films containing different amount of drug concentration at 37°C and pH 5.5. (◇) 0.1% theophylline theophylline, (O) 0.2% theophylline, (□) 0.5% theophylline.

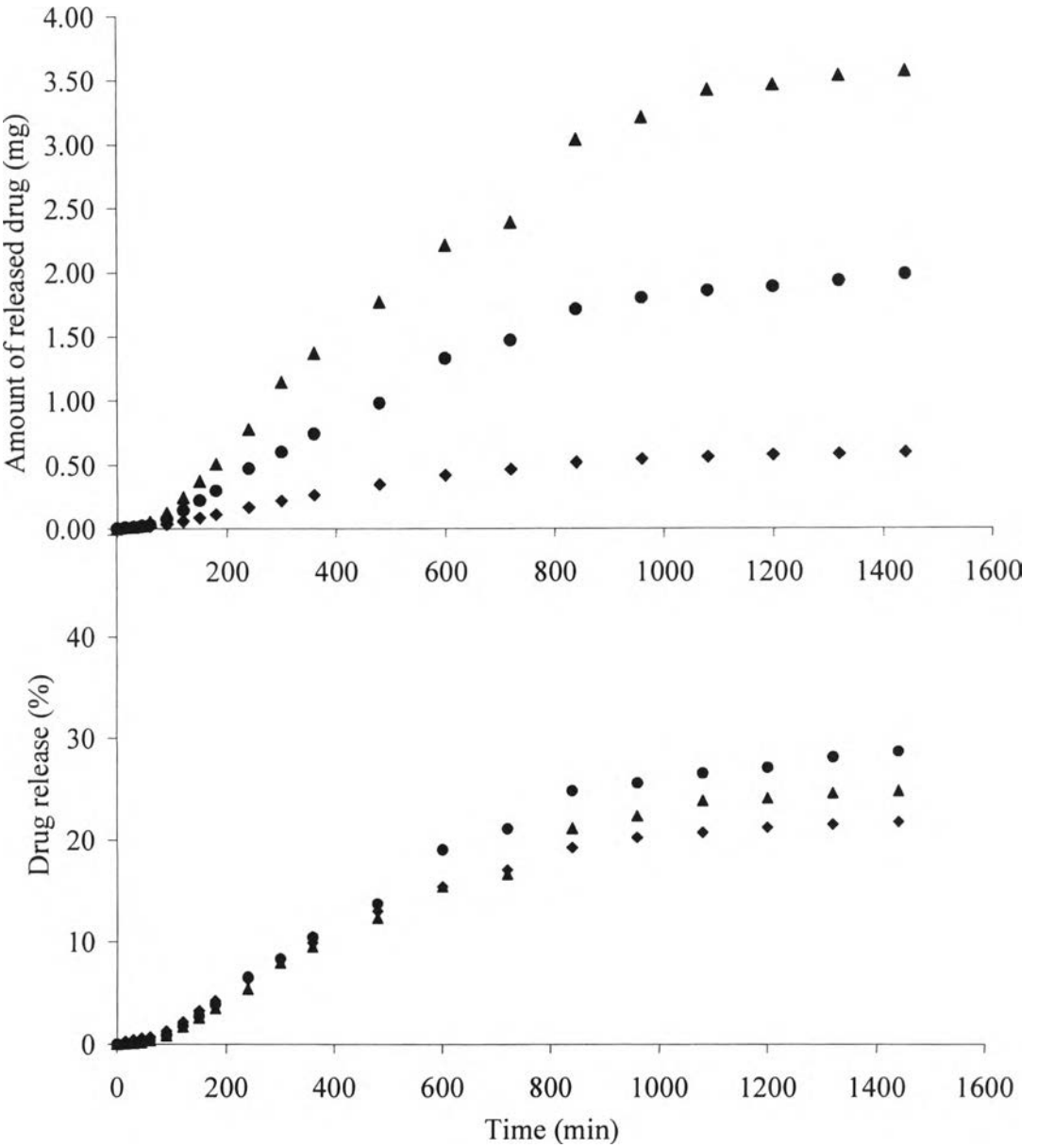

Figure 4.5. Salicylic release profiles of chitosan films containing different amount of drug concentration at 37°C and pH 5.5. (◆) 0.2% salicylic acid, (●) 0.5% salicylic acid, (▲) 1.0% salicylic acid.

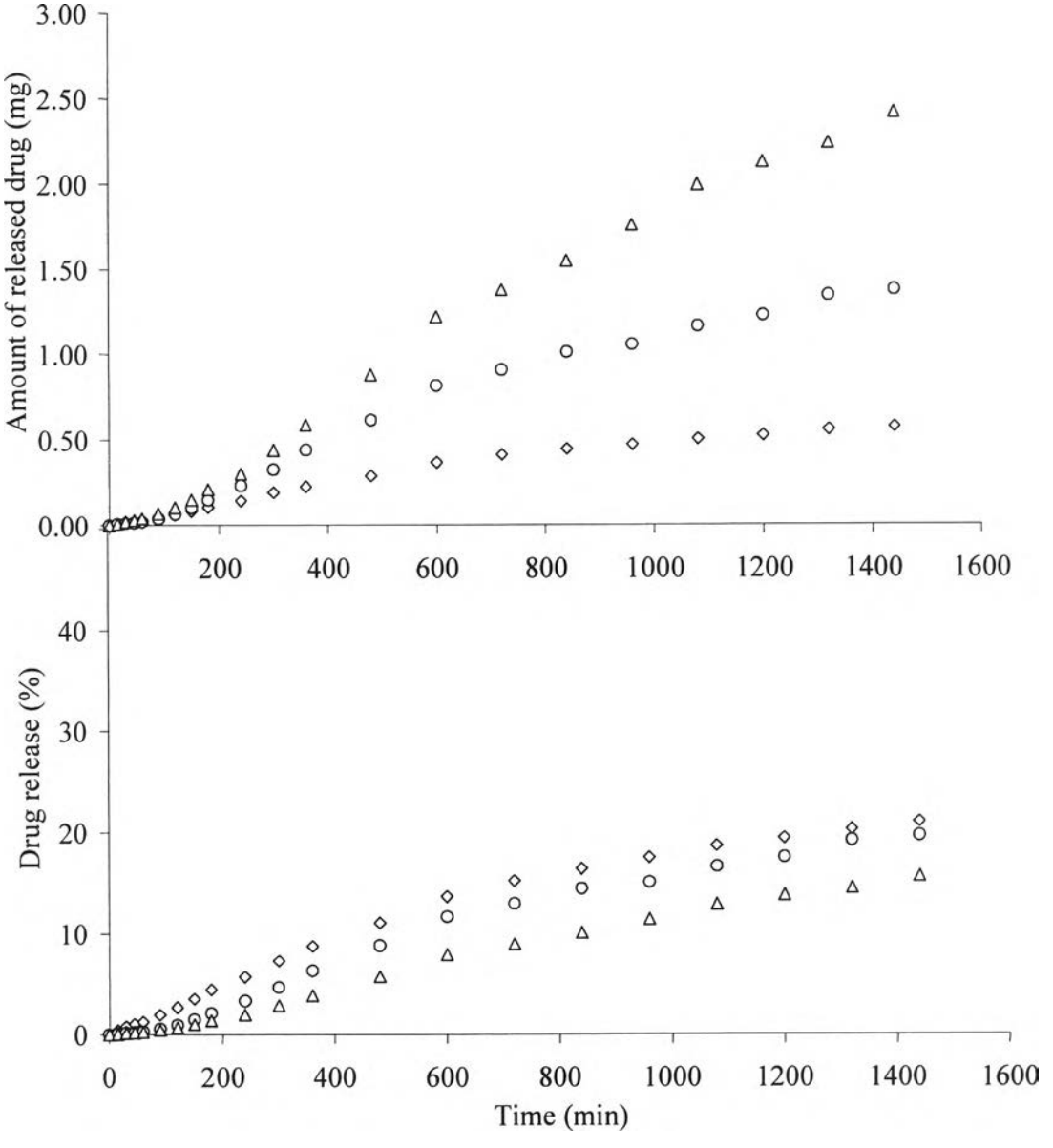

Figure 4.6. Theophylline release profiles of chitosan films containing different amount of drug concentration at 37°C and pH 5.5. (◇) 0.2% theophylline theophylline, (O) 0.5% theophylline, (□) 1.0% theophylline. .

Table 4.2. Occurrence of solid particles of drug precipitation in CM-chitin film

Drug	Film appearance				
	Amount of drug in the film (%)				
	0.1	0.2	0.3	0.4	0.5
Salicylic acid	-	-	+/-	+/-	+
Theophylline	+/-	+	++	+++	++++

- : Transparent film without any appearance of solid particles of drugs
+/- : Transparent film with small amount of solid particles of drugs
+ : Turbid film

Table 4.3. Occurrence of solid particles of drug precipitation in chitosan film

Drug	Film appearance									
	Amount of drug in the film (%)									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Salicylic acid	-	-	-	-	-	-	+/-	+/-	+/-	+
Theophylline	-	+	++	++	++	++	+++	+++	+++	++++

- : Transparent film without any appearance of solid particles of drugs
+/- : Transparent film with small amount of solid particles of drugs
+ : Turbid film

It was found that the amounts of released drug were increased with increasing the drug concentrations. However, the increase of drug concentration did not increase the percentages of drug released from the films. In fact, the percentages of drug released from the films were decreased with increasing the drug concentrations. At higher drug concentrations, it appears that phase separation of drug within the films possibly occurs during evaporation of the solvent from the

films. This was manifested by the visible presence of solids particles in the films after drying (Table 1-2). It was found that the higher the drug concentrations in the polymer solution were, the more solid particles of drug appeared in the films, and the lower the percent release of drug obtained. It is known that a drug can be released from a polymer matrix by the penetration of the dissolution medium from outside into the polymer matrix and then the diffusion of the dissolved drug out of the polymer matrix. These two factors could affect the release of drugs from the films. (Oungbho *et al.*, 1997). Therefore the percentages of released drug were decreased as the drug content in the films increased because the precipitation of drug in the film reduced the amount of dissolved drug that could diffuse out of the films during the film swelling process.

These results are similar to those of Gupta and Kumar (2000) who reported that the total amount of released diclofenac sodium was higher from the highly drug-loaded chitosan beads in comparison to that from beads loaded with low concentrations of drug, but the percentages of drug released from chitosan beads decreased with higher loading of diclofenac sodium.

4.4 Effect of Drug Type on Drug Release

The percentages of model drugs released from CM-chitin and chitosan films are summarized in Table 4.4.

For both polymer matrices, the amount of salicylic acid released from the films is higher than for theophylline. Several factors can affect the drug release characteristic from polymer matrices. The molecular weight of drug is one factor. The molecular weights of salicylic acid and theophylline are 138.12 and 180.16 respectively. In general, the lower the molecular weight of the drug, the easier is drug penetration through the polymer matrix, and the higher is the amount of drug released. Salicylic acid has a lower molecular weight than theophylline. Accordingly, it follows that salicylic acid can be released from the films at a higher amount than theophylline.

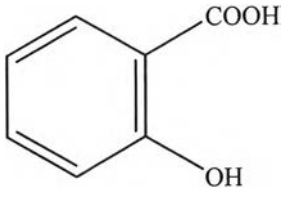

Figure 4.7. Structure of salicylic acid.

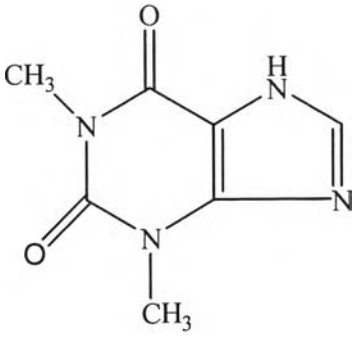

Figure 4.8. Structure of theophylline.

Table 4.4. The percentages of drug released from pure CM-chitin and chitosan

Polymer Matrix	Drug	Drug Concentration (%)	Drug Release[a] (%)
CM-chitin	Salicylic acid	0.1	31.38
		0.2	29.67
		0.5	28.51
	Theophylline	0.1	27.67
		0.2	25.71
		0.5	21.89
Chitosan	Salicylic acid	0.2	21.78
		0.5	28.69
		1.0	24.85
	Theophylline	0.2	21.06
		0.5	19.59
		1.0	15.66

a the average value from three experiments

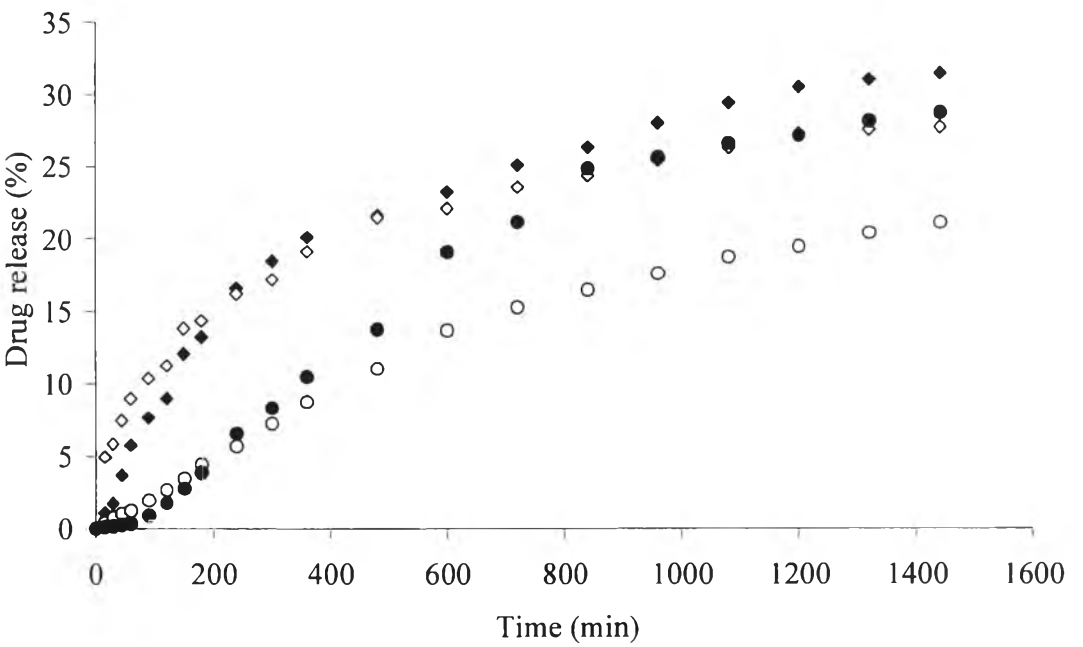

Figure 4.9 Comparison of drug release profiles of CM-chitin and chitosan films at 37°C and pH 5.5: (◆) 0.1% salicylic loaded-CM-chitin film, (◇) 0.1% theophylline loaded-CM-chitin film, (●) 0.5% salicylic loaded-chitosan film, (O) 0.1% theophylline loaded-chitosan film.

Another factor that can affect the penetration of drug from the polymer films is drug-polymer interaction. The chemical structures of the model drugs are shown in figures 4.7 and 4.8. The drug release profiles of salicylic acid and theophylline from CM-chitin and chitosan films are shown in figure 4.9. It can be seen in figure 4.9 that the total amount of salicylic acid released from the films is greater than for theophylline but the release of salicylic acid during the initial period is slower than that of theophylline. It is possible that salicylic acid may interact with the amino group on CM-chitin and chitosan to form a salicylate salt. Because of the neutrality of theophylline, such drug-polymer interaction may not occur for theophylline. This result is in good agreement with the study of Puttipipatkhachorn *et al.* (2001) who studied the interaction between salicylic acid and chitosan by using Fourier transform spectroscopy and ^{13}C NMR spectroscopy. An interaction was observed between the carboxylic groups of salicylic acid and the amino group of chitosan. Furthermore, these authors also studied the drug-polymer interaction

between theophylline and observed no interaction between theophylline and chitosan. Therefore, it appears only salicylic acid has an interaction with the polymer matrices. It therefore seems possible that the initial lag time for release of salicylic acid is required for dissociation of the drug from the polymer matrix before release of the drug can occur.

4.5 Effect of Crosslinking Agent Concentration on Drug Release

Salicylic acid–loaded CM-chitin and chitosan films containing different concentrations of glutaraldehyde, i.e., 0.005%, 0.01%, and 0.05% glutaraldehyde, were used to investigate the effect of crosslink density on drug release. The results are shown in figure 4.10 and 4.11. The percentages of drug released from 0.1% salicylic acid loaded-CM-chitin films containing with 0.005%, 0.01%, and 0.05% glutaraldehyde at equilibrium were 34.15, 31.38, and 26.47%, respectively. Moreover, the percentage of drug released from 0.5% salicylic acid loaded-chitosan films containing 0.005%, 0.01%, and 0.05% glutaraldehyde at equilibrium were 31.67, 28.69 and 25.36%, respectively. Thus, the amount of released salicylic acid decreased with increase of glutaraldehyde concentrations for both polymer matrices.

Since crosslinking restricts the swelling ability of the films, the swelling behavior of the films containing different concentrations of glutaraldehyde was investigated and the results are shown in Table 4. The degrees of swelling of salicylic acid-loaded CM-chitin and chitosan films were decreases with increase of glutaraldehyde concentrations in the films. With increase of crosslinking agent, the crosslink reaction of amino groups in CM-chitin and chitosan with aldehyde groups in glutaraldehyde to form imine bonds is amplified. Yao *et al.* (1994) investigated the swelling kinetics and release characteristics of hydrogels formed from glutaraldehyde-crosslinked chitosan/polyether interpenetrating polymer networks (semi-IPN) hydrogels and reported that the drug release was highly dependent on the degree of swelling of the gel, which increased for semi-IPN with lower levels of crosslinker.Moreover, Ko *et al.* (2002) studied the effect of the amounts of crosslinking agent on the drug release behavior and reported that the release of drug from chitosan microparticles and observed it to decrease with increased crosslinking.

Therefore, it can be concluded that the swelling behavior and, consequently, the drug release level of CM-chitin and chitosan films depends inversely on the concentration of crosslinking agent.

In addition, the drug release level is controlled not only by the swelling behavior of the polymer matrix, but may also be affected by a physical erosion of the film. This process is associated with macroscopic changes in the appearance of the device, changes in the physicomechanical properties of the polymer material, deformation or structural disintegration, or weight loss, which may lead to the eventual loss of function. To test this possibility, the weight loss of the films after drug release were determined, and the results are shown in Table 5. Since significant weight loss of was observed for each film, we conclude that drug release from the films may also be facilitated by an erosion process.

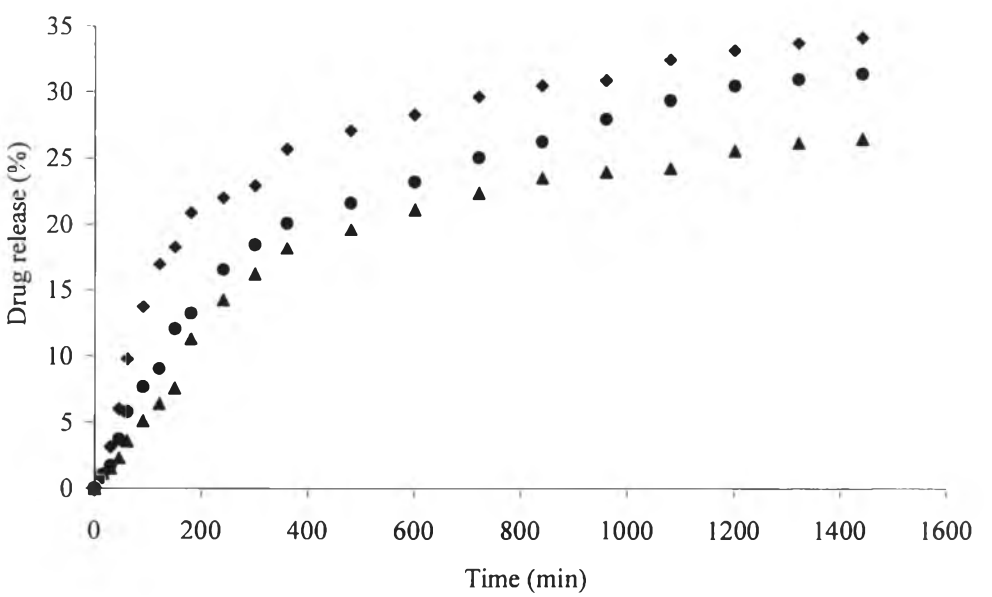

Figure 4.10. Salicylic acid release profiles of CM-chitin films crosslinked with different concentration of glutaraldehyde at 37°C and pH 5.5. The salicylic concentration in the films was 0.1%. (◆) 0.005% glutaraldehyde, (●) 0.01% glutaraldehyde, (▲) 0.05% glutaraldehyde.

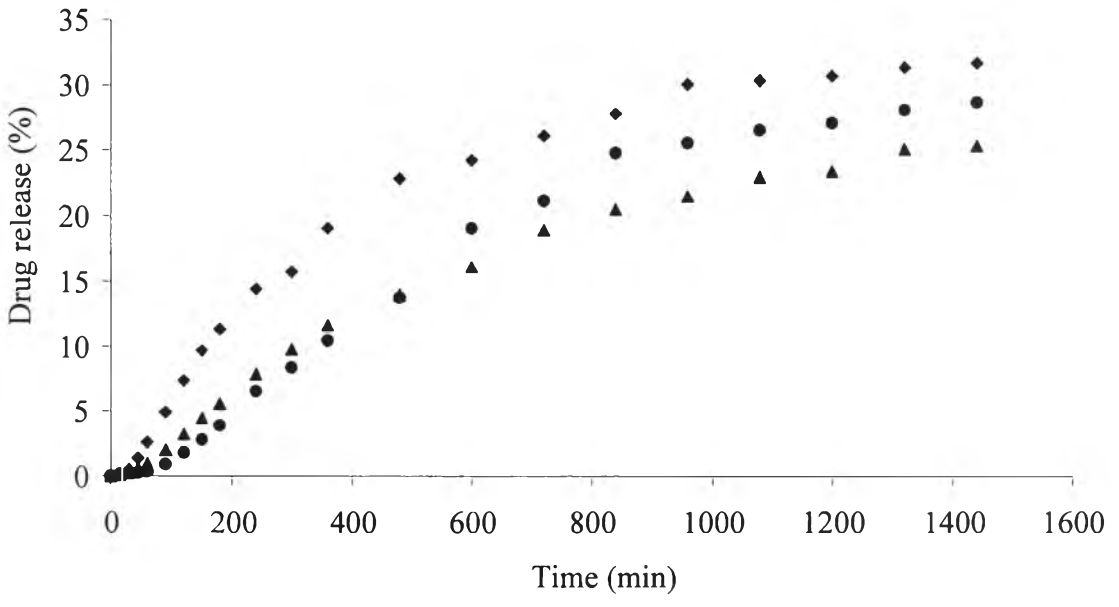

Figure 4.11. Salicylic acid release profiles of chitosan films crosslinked with different concentration of glutaraldehyde at 37°C and pH 5.5. The salicylic concentration in the films was 0.5%. (◆) 0.005% glutaraldehyde, (●) 0.01% glutaraldehyde, (▲) 0.05% glutaraldehyde.

Table 4.5. Degree of swelling and percent weight loss of salicylic acid–loaded CM-chitin and chitosan films containing various glutaraldehyde concentrations

Polymer Solution	Degree of swelling[a] (%)			Weight Loss [a](%)		
	Glutaraldehyde concentration			Glutaraldehyde concentration		
	0.005%	0.01%	0.05%	0.005%	0.01%	0.05%
CM-chitin	461	432	410	16.01	13.78	8.54
Chitosan	445	421	397	15.39	10.74	5.47

a the average value from three experiments

Table 4.6. Percent weight loss of salicylic acid–loaded blend films

Polymer	Blend Ratio	Weight Loss[a] (%)
CM-chitin/PVA	100:0	13.78
	75:25	16.27
	50:50	20.82
Chitosan/PVA	100:0	10.74
	75:25	12.70
	50:50	18.41
CM-chitin/PVP	100:0	13.78
	75:25	27.01
	50:50	36.33
Chitosan/PVP	100:0	10.74
	75:25	17.20
	50:50	18.85

a the average value from three experiments

4.6 Effect of Releasing Time on Drug Release

The release profiles of salicylic acid from CM-chitin, chitosan and their blend films with PVA and PVP are illustrated in figure 4.12-4.15. The release of salicylic acid from the films is fast initially and and then slowly approaches the equilibrium state. For CM-chitin and chitosan films, the release of salicylic acid reaches the equilibrium after 22 hours. In case of the blends of CM-chitin and chitosan with PVA and PVP, the salicylic acid release reaches the equilibrium after 18 hours. It was observed that the release rate of pure CM-chitin and chitosan films is slower, and reaches equilibrium more slowly than for the blend films. It is known that the release of drug from hydrogels occurs by a swelling-controlled mechanism. According to this, the amount of drug released should depend on the degree of swelling of the films. The swelling behavior of CM-chitin, chitosan, and their blend

films with PVA and PVP as a function of release time are shown in figure 4.16-4.18. The degree of swelling of each films increases quickly during the initial period and rapidly reaches the equilibrium. This can be explained as due to the fact that, when the films come in contact with the pigskin, saturated with buffer solution, a large amount of water is rapidly absorbed in the film leading to a highly swollen hydrogel state. After this the dissolved drug can diffuse through polymer matrix and escape from the film until the equilibrium state is reached.

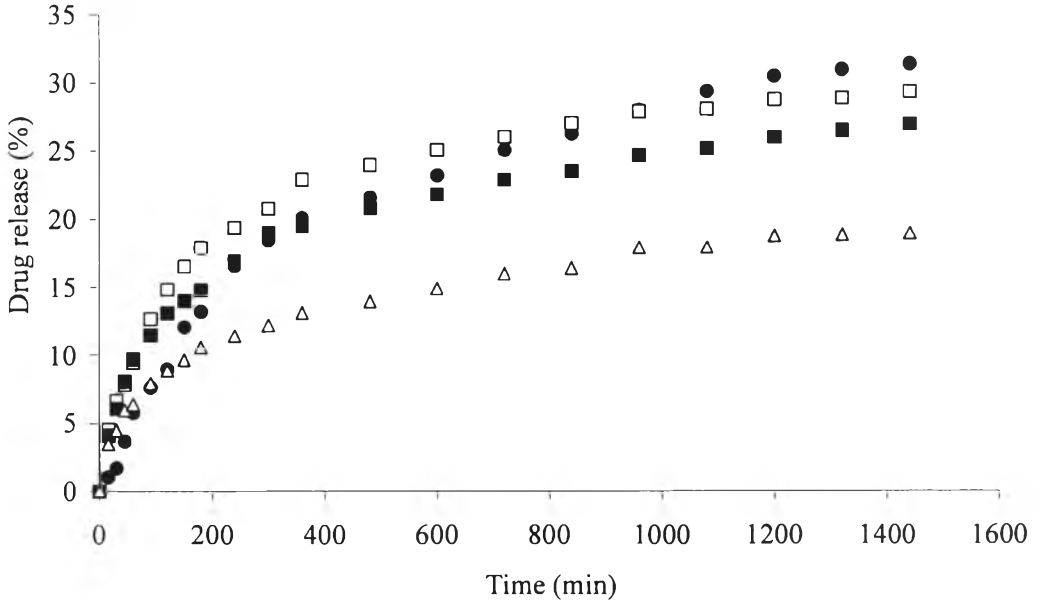

Figure 4.12. Drug release profiles for CM-chitin and CM-chitin/PVA blend films with 0.1% salicylic acid at 37°C and pH 5.5. CM-chitin:PVA (●) 100:0 (CM-chitin), (□) 75:25, (■) 50:50, (△) 0:100 (PVA).

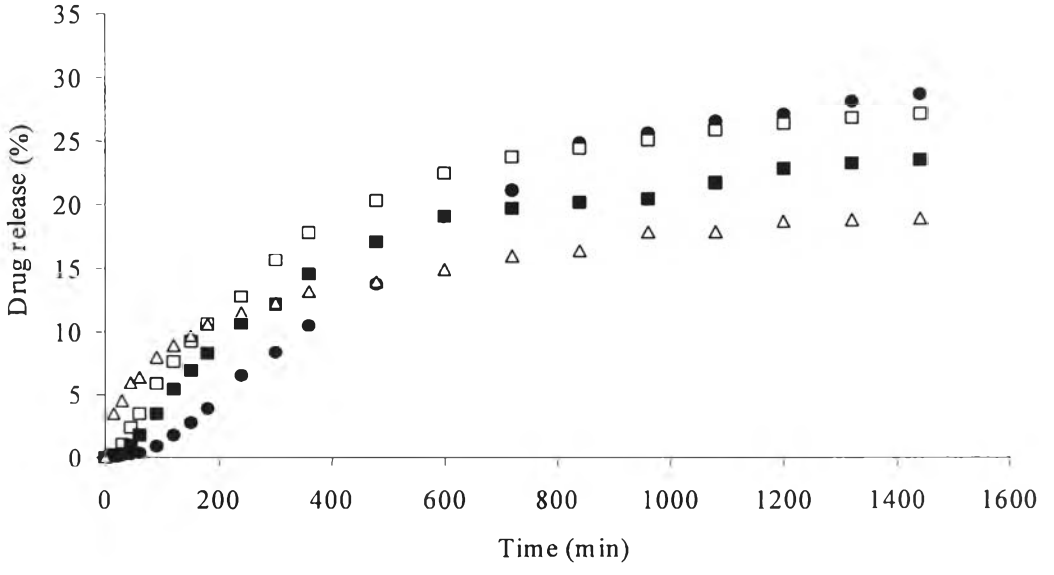

Figure 4.13. Drug release profiles for chitosan, PVA, and chitosan/PVA blend films with 0.5% salicylic acid at 37°C and pH 5.5. chitosan:PVA (●) 100:0 (chitosan), (□) 75:25, (■) 50:50, (△) 0:100 (PVA).

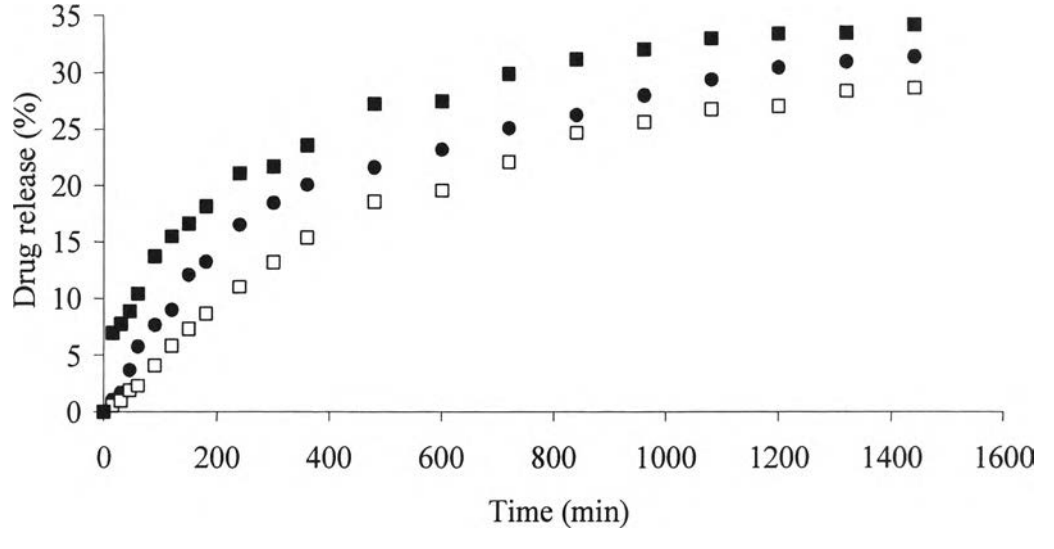

Figure 4.14. Drug release profiles for CM-chitin and CM-chitin/PVP blend films with 0.1% salicylic acid at 37°C and pH 5.5. CM-chitin:PVP (●) 100:0 (CM-chitin), (□) 75:25, (■) 50:50.

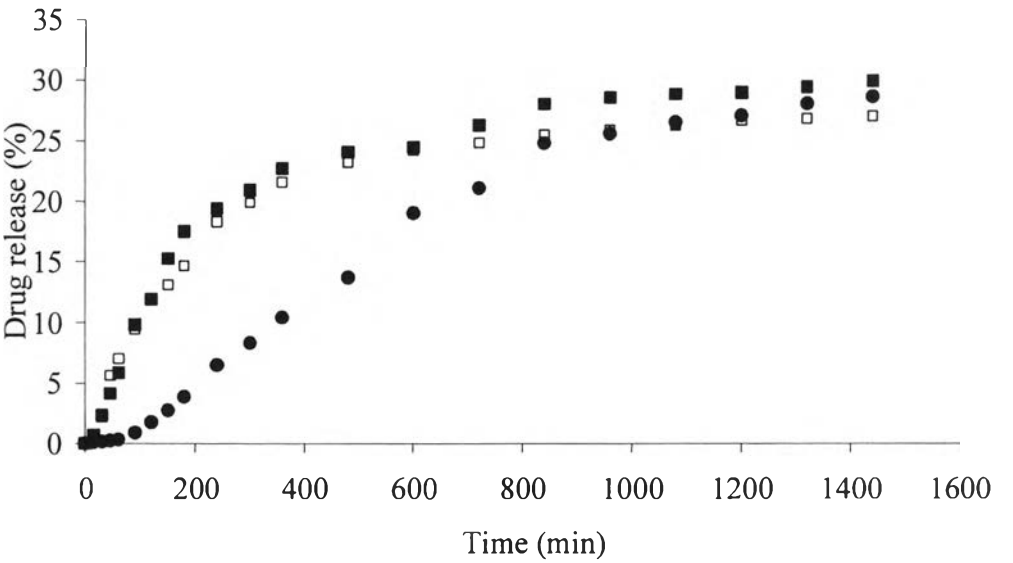

Figure 4.15. Drug release profiles for chitosan and chitosan/PVP blend films with 0.5% salicylic acid at 37°C and pH 5.5. chitosan: PVP (●) 100:0 (chitosan), (□) 75:25, (■) 50:50.

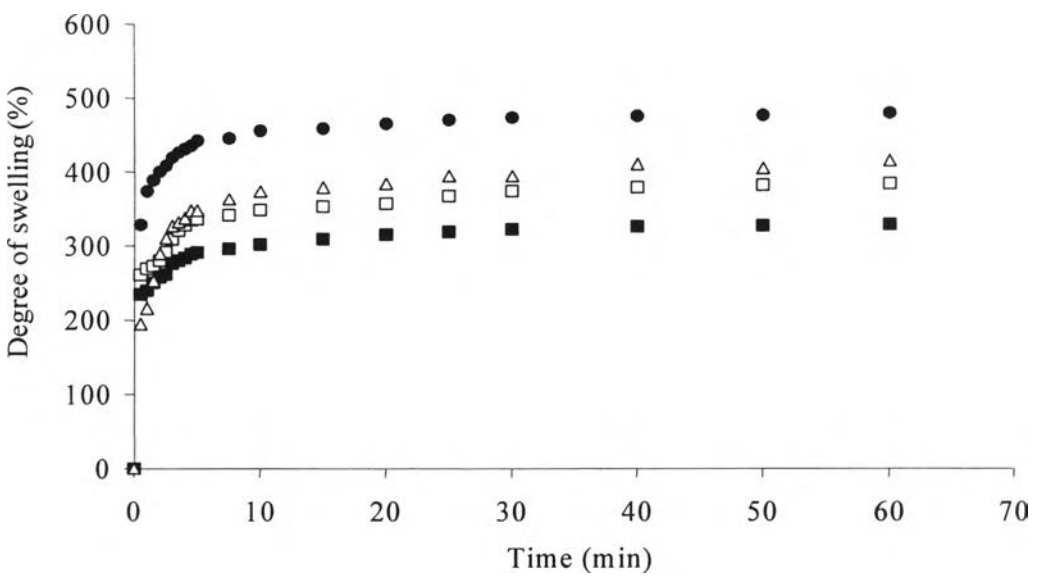

Figure 4.16. Degree of swelling of CM-chitin/PVA blend films with different blend composition. CM-chitin:PVA (●) 100:0 (CM-chitin), (□) 75:25, (■) 50:50, (△) 0:100 (PVA).

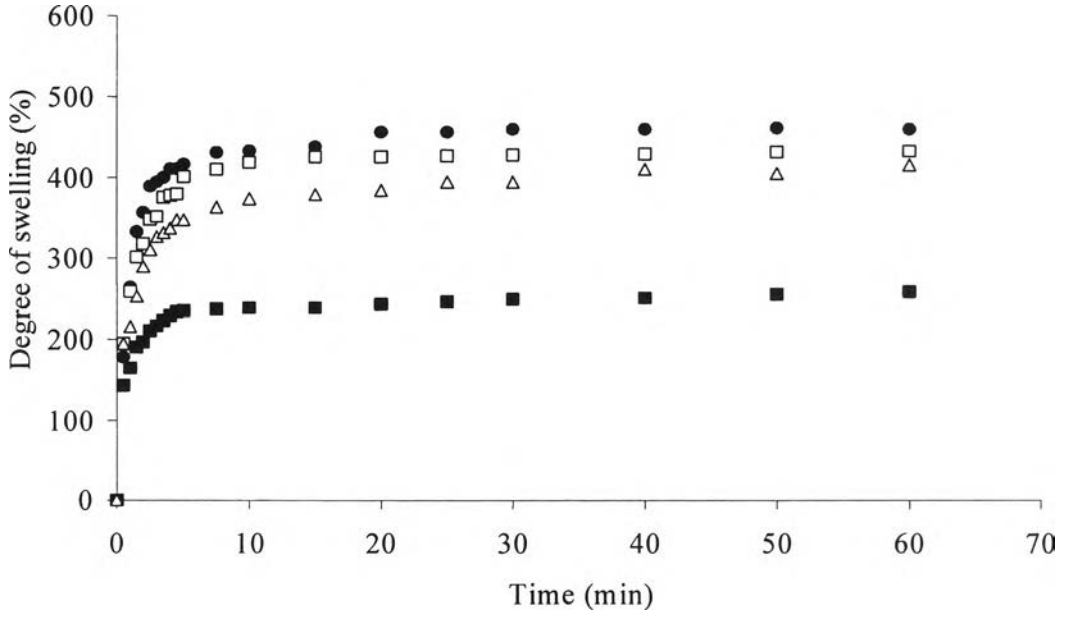

Figure 4.17. Degree of swelling of chitosan/PVA blend films with different blend composition. Chitosan:PVA (●) 100:0 (chitosan), (□) 75:25, (■) 50:50, (△) 0:100 (PVA).

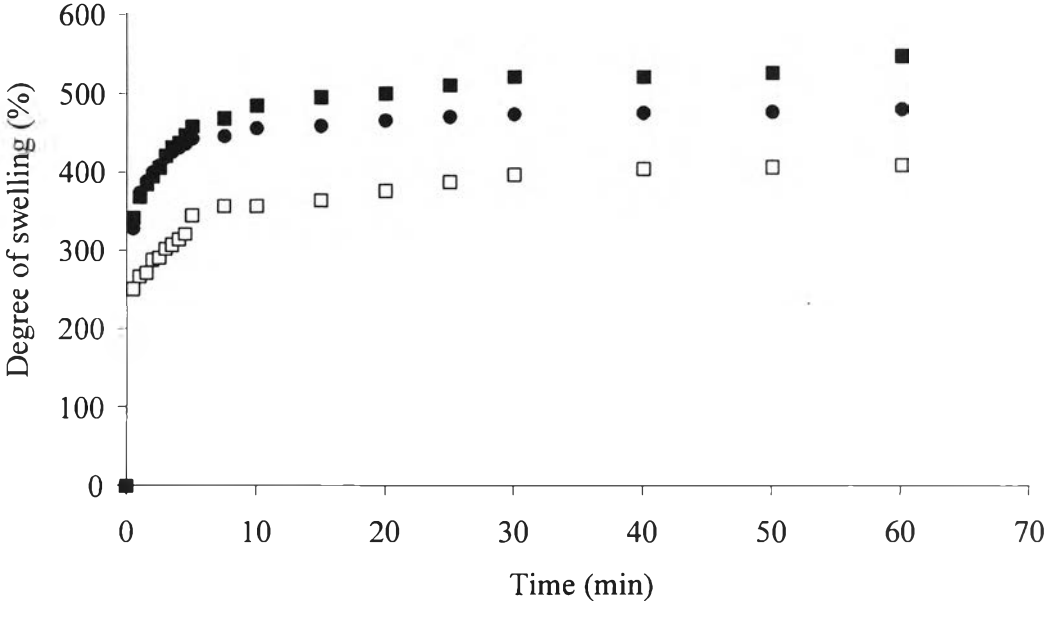

Figure 4.18. Degree of swelling of CM-chitin/PVP blend films with different blend composition. CM-chitin:PVP (●) 100:0 (CM-chitin), (□) 75:25, (■) (50:50).

4.7 Effect of Blend Composition on Drug Release

PVA is a synthetic polymer that can form a hydrogel and is widely used in biomedical applications. The drug release profiles of CM-chitin/PVA and chitosan/PVA blend films containing salicylic acid are shown in figures 4.12 and 4.13. These blend films were prepared at four different blend ratios of CM-chitin to PVA and chitosan to PVA, i.e., 100:0, 75:25, 50:50, and 0:100.

As seen in figure 4.12, for CM-chitin/PVA blend films, the releasing rate of pure PVA film was faster than that of pure CM-chitin, but the percentages of salicylic acid released from pure PVA film was less than that from pure CM-chitin. For both blends; with 25% and 50% PVA, the release rate was slightly faster than for pure PVA and CM-chitin films. However, the percentages of salicylic acid released from the blend films decreased with increasing PVA contents.

As evident in figure 4.13, a similar pattern of behavior was observed for chitosan/PVA blend films. Although the release rate of salicylic acid from pure PVA film was faster than from pure chitosan, the percentage of salicylic acid released from pure PVA film was less than that released from pure chitosan film. Accordingly, blending PVA with chitosan or CM-chitin resulted in an increase of release rate and decrease of the released amount of salicylic acid as compared to pure chitosan or CM-chitin. This can be explained in terms of degree of swelling of the films. The degree of swelling of CM-chitin, chitosan, and the blend films is shown in figures 4.16 and 4.17. The degree of swelling of the films increases very quickly during initial period and reaches equilibrium within 10 min. The degree of swelling of pure CM-chitin and chitosan films is higher than for both blend films, 75% and 50%, and decreases with the increasing PVA content.

The drug release profiles of CM-chitin/PVP and chitosan/PVP blend films containing salicylic acid are shown in figures 4.14 and4.15. These blend films were prepared at three different blend ratios of CM-chitin to PVP and chitosan to PVP, i.e. 100:0, 75:25, and 50:50. The drug release of pure PVP films was not investigated because the PVP rapidly absorbs moisture in the air and becomes very sticky. In addition, the PVP film does not retain its shape when the water content in the film increases.

As seen in figure 4.14, for CM-chitin/PVP blends, the release rate of films with 50% CM-chitin content were faster than for pure CM-chitin and for the film with 25% PVP. Furthermore, the percentages of salicylic acid released from the pure CM-chitin film and the blends with 25% and 50% CM-chitin were 31.38, 34.17, and 28.63%, respectively. Thus, the films with 50% PVP content gave the highest amount of released salicylic acid and the blend films with 25% PVP gave the lowest amount of released salicylic acid.

As seen in figure 4.15, for chitosan/PVP blends, the release rates of the films with both 25% and 50% PVP were higher than for pure chitosan, although there was little difference in the equilibrium released amounts of salicylic acid from pure chitosan and the blends. These observations can again be explained in terms of degree of swelling of the films. The degree of swelling of these films is shown in figure 4.18. The chitosan/PVP blend films with PVP content of 50% and 25% did not retain their shapes for longer than two minutes when they were immersed in buffer pH 5.5, therefore, the data is not shown. The degree of swelling of the CM-chitin/PVP blends was very fast during the initial period and reached the equilibrium with in 10 min. The degree of swelling of the blend films with 50% PVP was higher than that of pure CM-chitin and the blend film with 25% PVP content.

Several researchers have reported the effect of swelling behavior on drug release characteristics. Gupta and Kumar, (2000) investigated the drug release of diclofenac sodium (DFS) from chitosan beads and microgranules. They reported that the release of the drug depends greatly on the swelling behavior. Furthermore, Ridbud *et al.* (2000) concluded that the release of amoxicillin from the air-dried and freeze-dried chitosan/PVP hydrogels was related to the degree of swelling of the hydrogels.

In the present study, the amounts of drug released from the blend films increased with increasing degree of swelling.