amoxicillin trihydrate are 2.17 mg/ml, 8.3 mg/ml, 21 mg/ml and 1-10 mg/ml, respectively (Brittain, 1994 and Florey, 1975). From our results, although the solubility of diclofenac was higher than that of salicylic acid, the releasing amounts of diclofenac were less than those of salicylic acid. It might be explained that drug release property resulted from a combination of various factors, e.g. molecular weight of drug, interaction between drug and polymer matrix and solubility of drug. However, in this study, molecular weights of model drugs seem to be a dominant factor that influenced drug release property of chitosan/silk fibroin blend films.

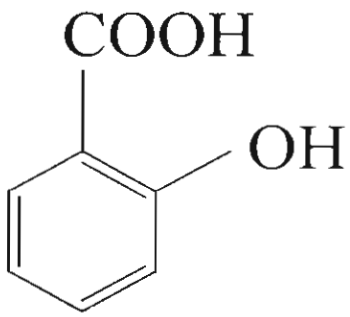

Figure 3.3 Structure of salicylic acid.

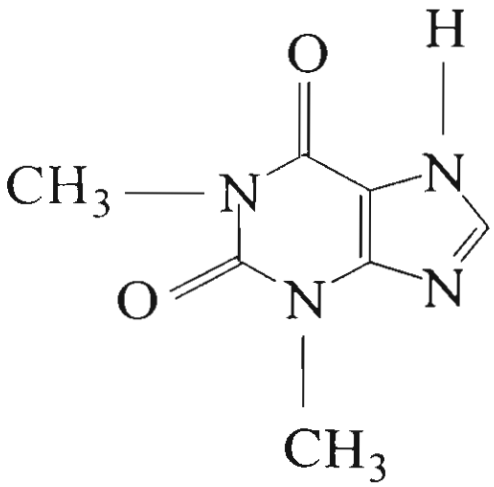

Figure 3.4 Structure of theophylline.

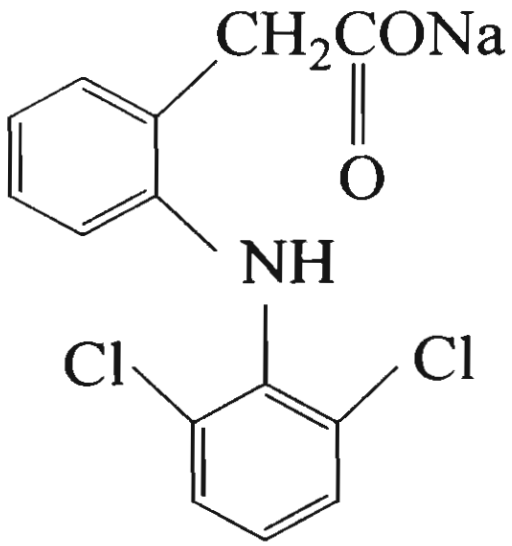

Figure 3.5 Structure of diclofenac sodium.

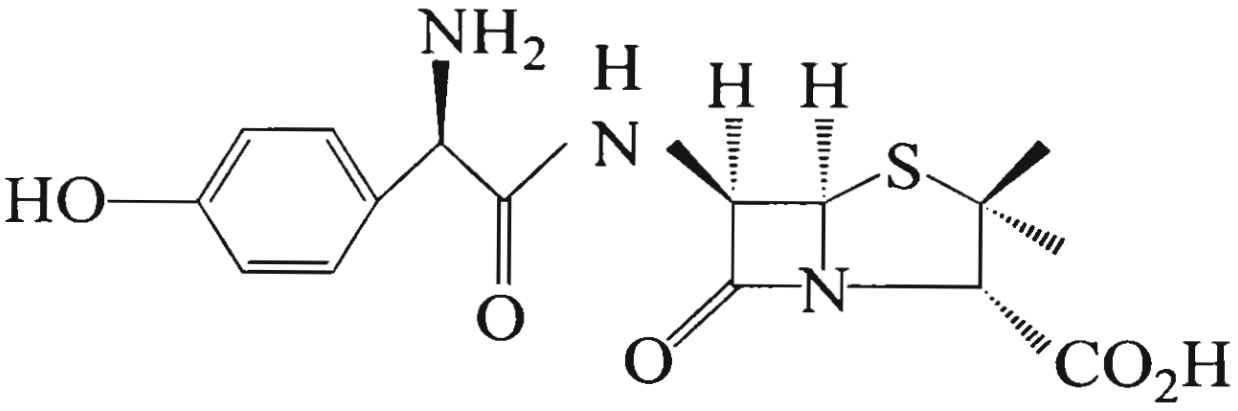

Figure 3.6 Structure of amoxicillin trihydrate.

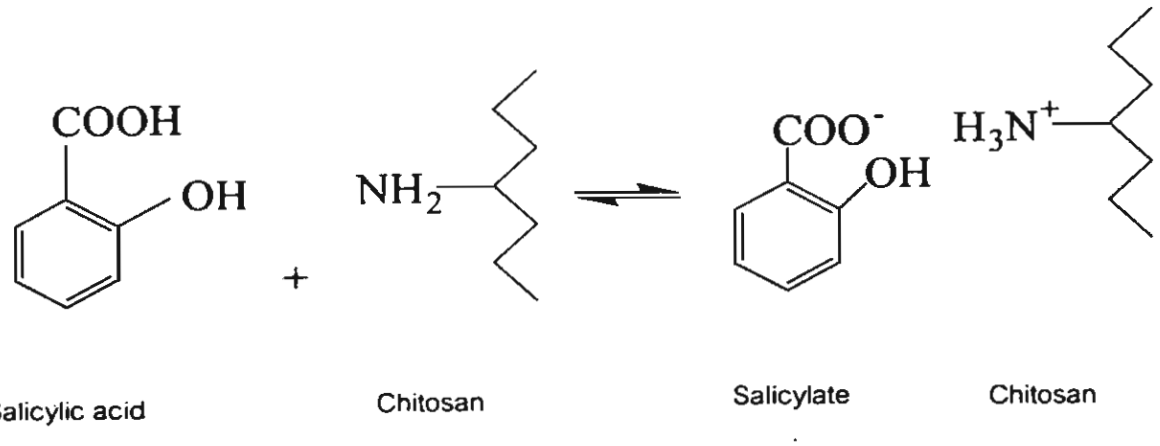

Figure 3.7 Interaction between salicylic acid and chitosan.

Table 3.2 FTIR characteristic absorption bands of chitosan

Frequencies (cm^{-1})	Assignment and remarks
1411	Symmetric COO^- stretch
1561	Amino group of chitosan and amide II of chitin
1257	Amide III of chitin
1153, 898	Saccharide structure

Table 3.3 FTIR characteristic absorption bands of silk fibroin

Frequencies (cm^{-1})	Assignment and remarks
1650	Amide I, C=O stretching
1542	Amide II, N-H bending and C-N stretching

Table3.4 FTIR characteristic absorption bands of theophylline

Frequencies (cm^{-1})	Assignment and remarks
1720	C=O stretching
1676, 1567	C=C stretching
1485	C=N stretching

1446	C-H bending
1314, 1242	C-N, C-O vibration

Table 3.5 FTIR characteristic absorption bands of salicylic acid

Frequencies (cm^{-1})	Assignment and remarks
1656	C=O stretching
1628	Asymmetric NH_3^+
1440	C=C stretching
1384	Asymmetric COO^-
690,760	Aromatic C-H bending

Table 3.6 FTIR characteristic absorption bands of diclofenac sodium

Frequencies (cm^{-1})	Assignment and remarks
3350-3310	N-H stretching
3100-3000	Aromatic C-H stretching vibration
1600-1550	Asymmetrical C=O stretching vibration
1400	Symmetrical C=O stretching vibration

Table 3.7 FTIR characteristic absorption bands of amoxicillin trihydrate

Frequencies (cm^{-1})	Assignment and remarks
1775	β-lactam C=O stretching
1686	amide I, C=O stretching
1482	amide II, N-H bending and C-N stretching
1396	dimethyl C-H deformation and phenol -OH
1250	phenol C=O

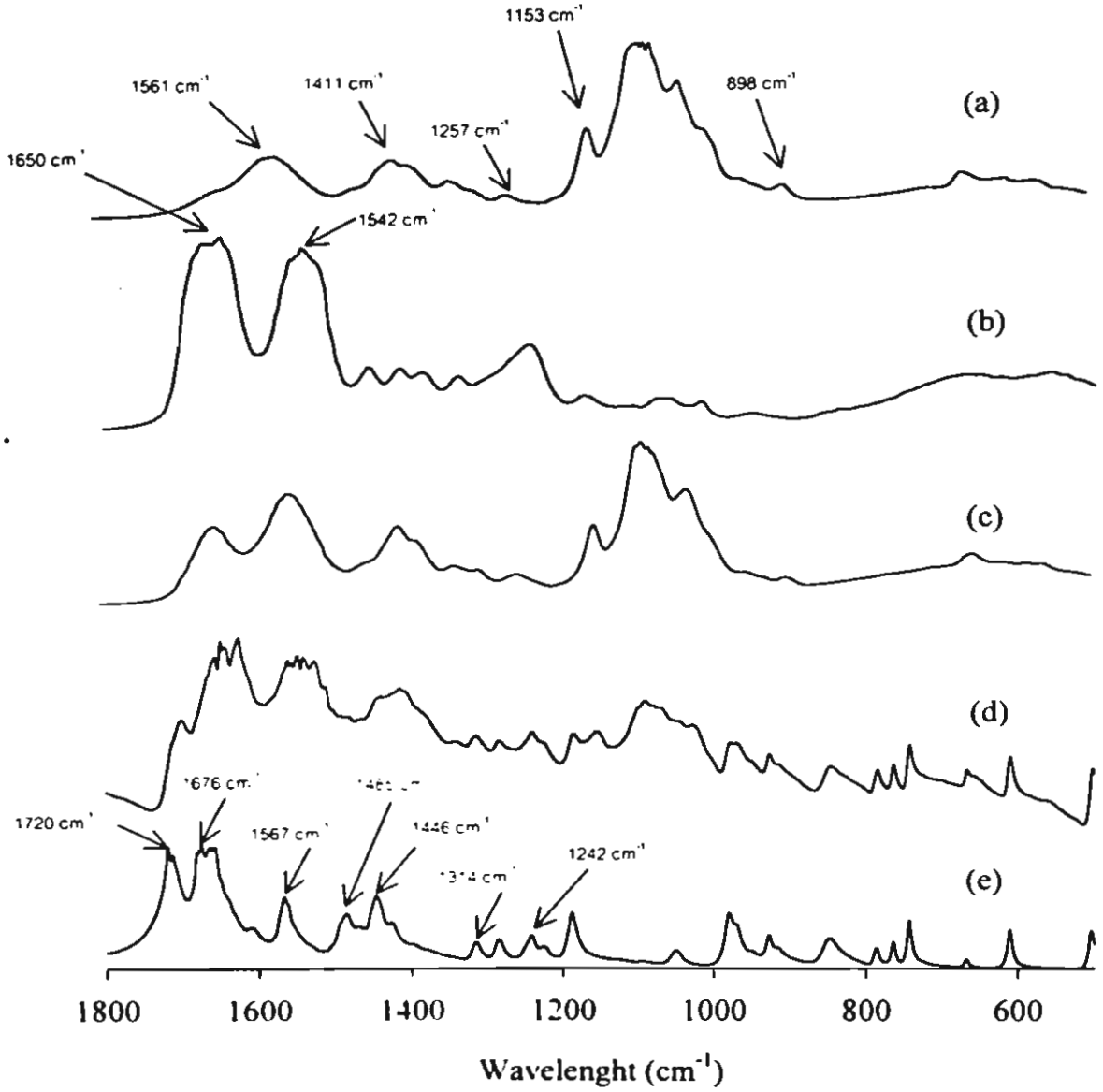

Figure 3.8 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) theophylline-loaded blend films with 80% chitosan content and (e) theophylline.

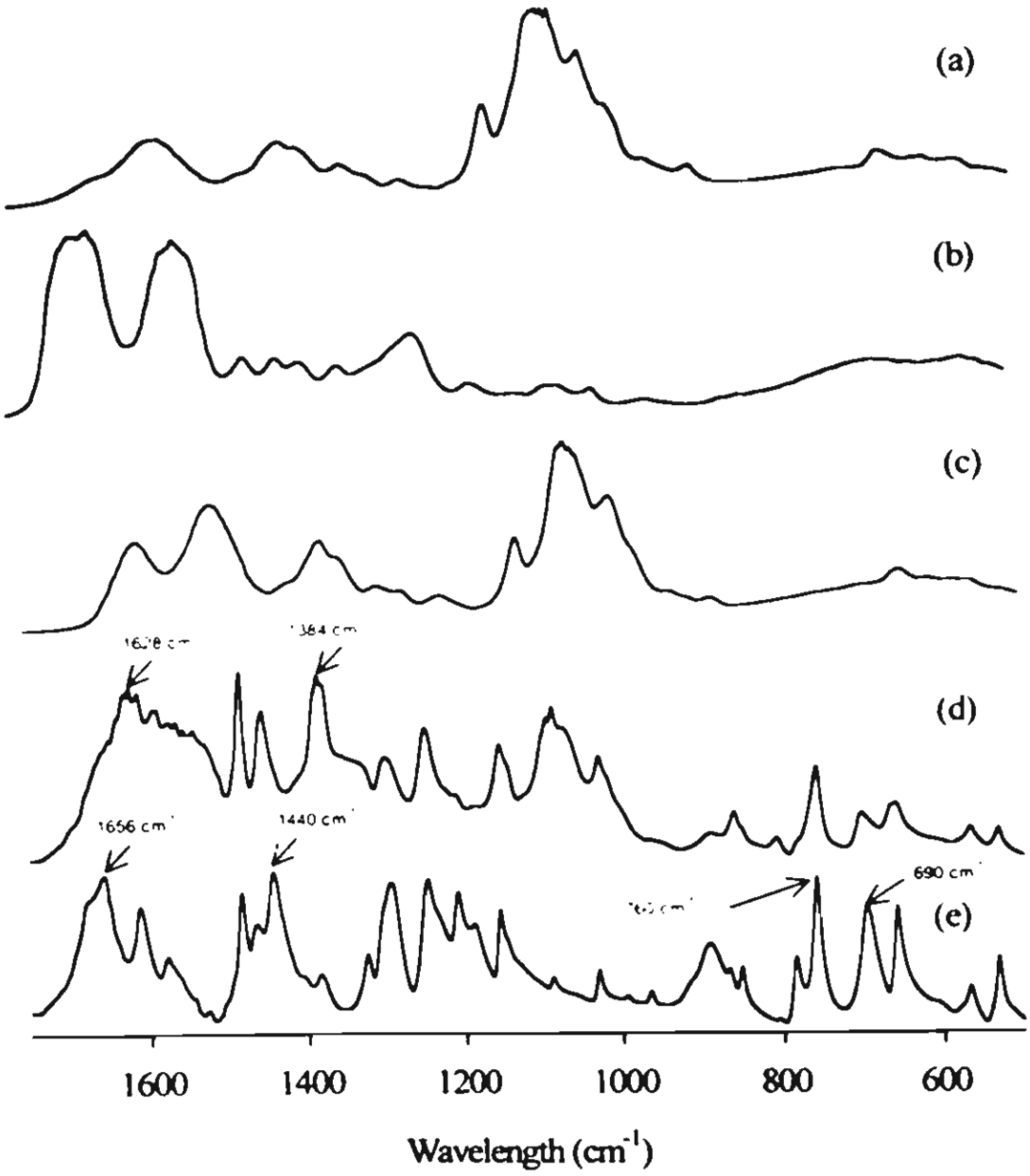

Figure 3.9 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) salicylic acid-loaded blend films with 80% chitosan content and (e) salicylic acid.

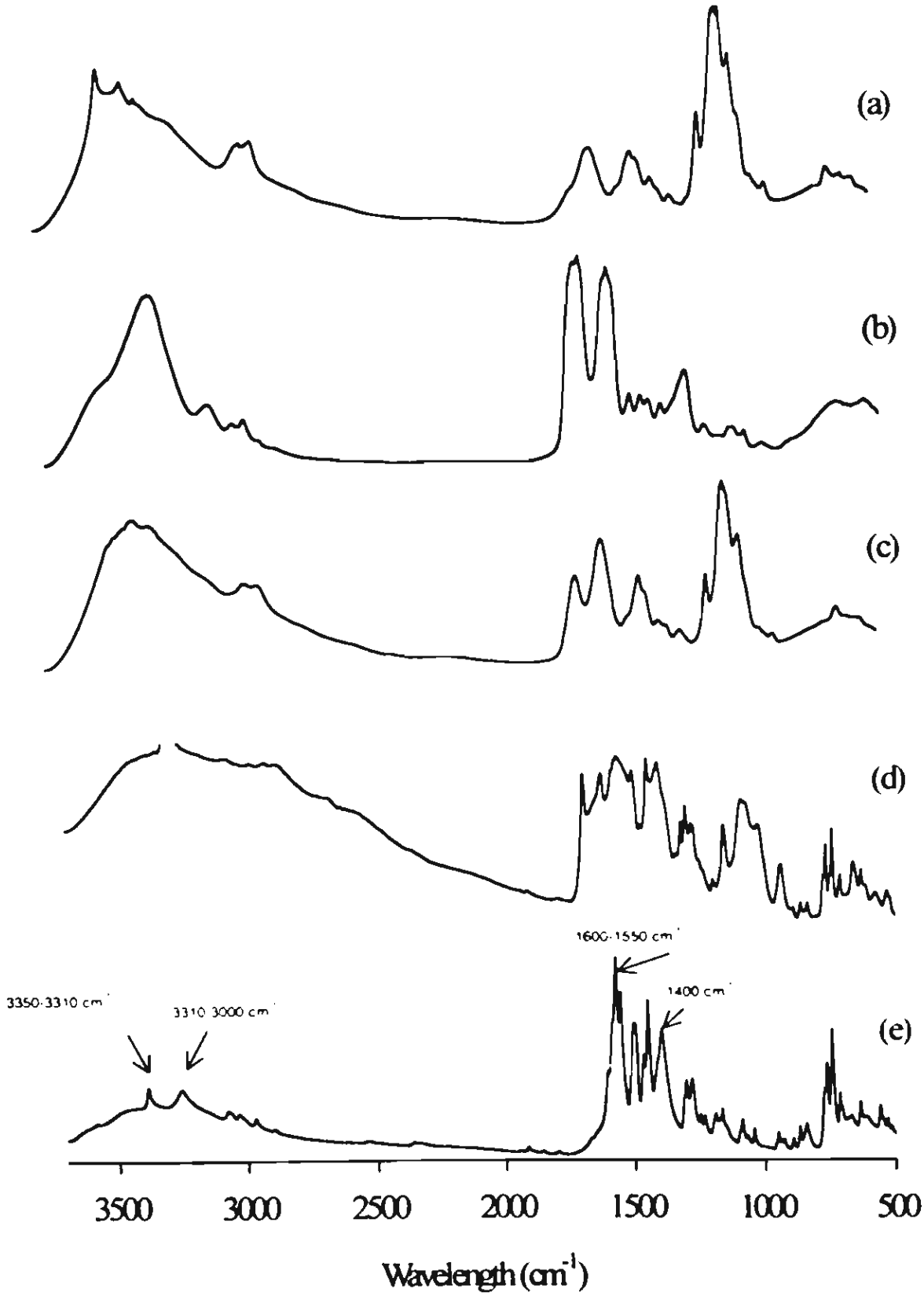

Figure 3.10 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) diclofenac sodium-loaded blend films with 80% chitosan content and (e) diclofenac sodium.

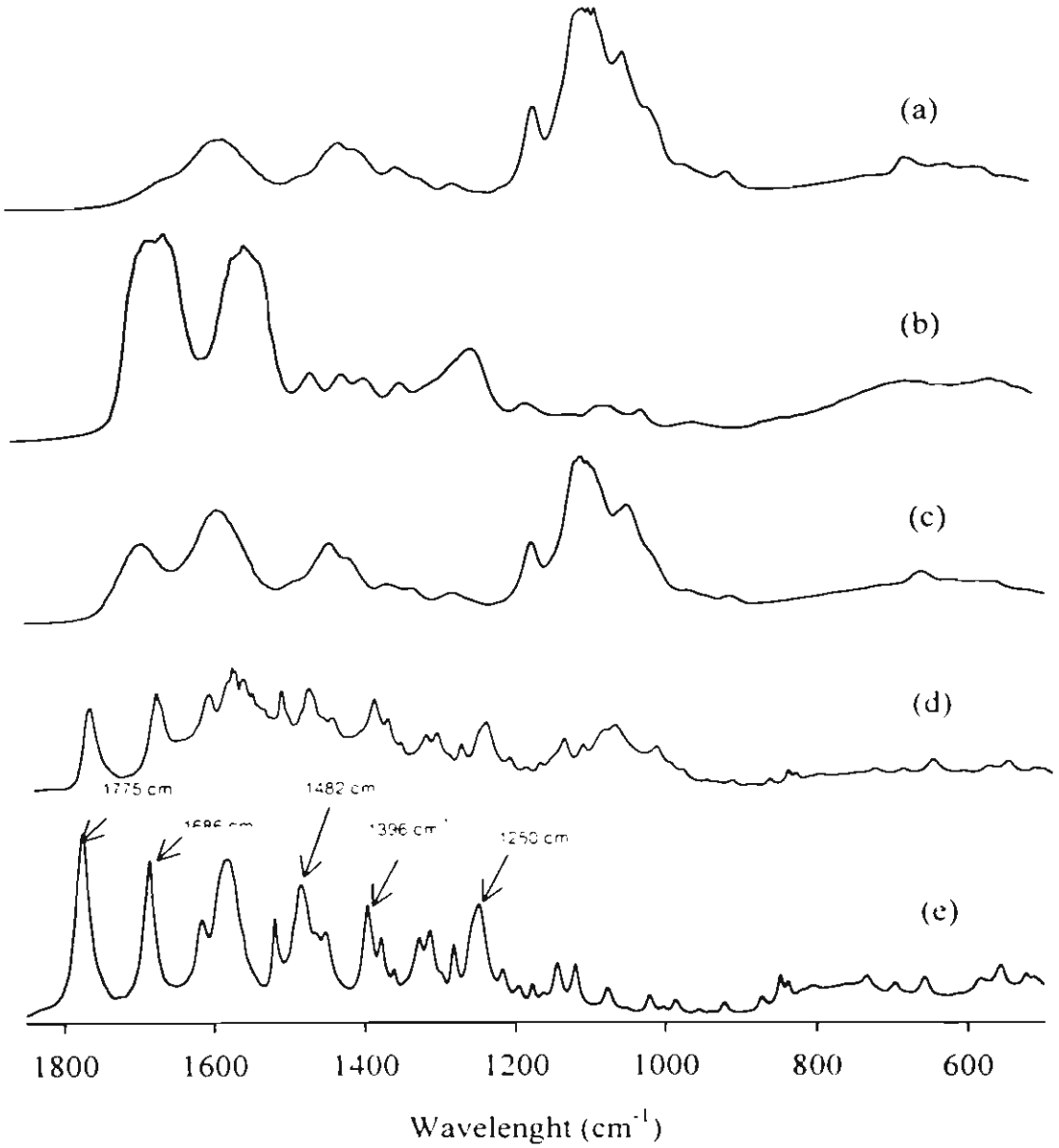

Figure 3.11 FTIR spectra of (a) chitosan film, (b) silk fibroin film, (c) blend film with 80% chitosan content, (d) amoxicillin trihydrate-loaded blend films with 80% chitosan content and (e) amoxicillin trihydrate.

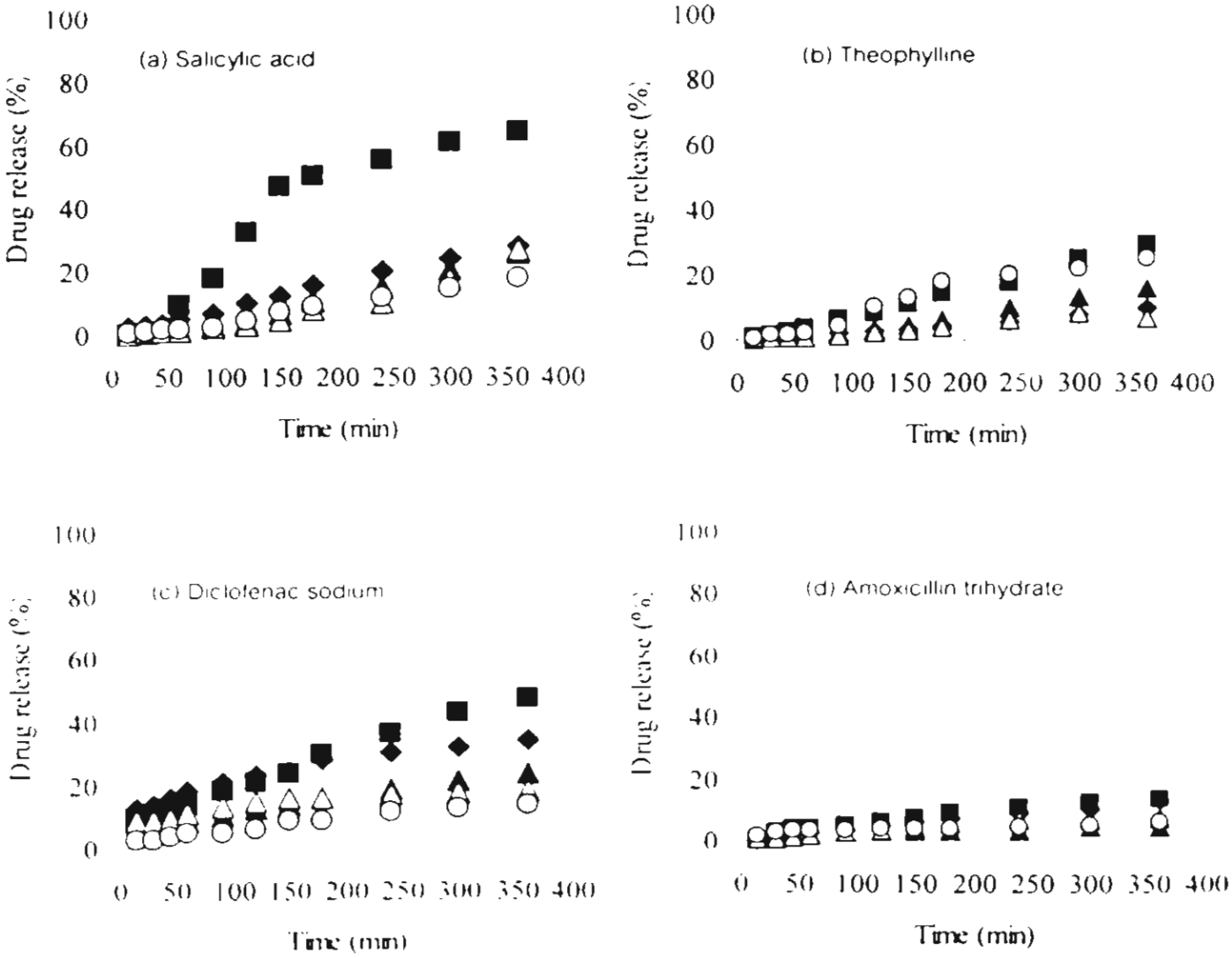

Figure 3.12 Drug release profile for pure chitosan and the blend films. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (○) 40% chitosan content

Effect of Releasing Time on Drug Release

The release profiles of each model drug for chitosan and the blend films are illustrated in Figure 3.13-3.16. The releasing amounts of drug from the films increased as releasing time increased until reached the equilibrium. It is known that the release of drug from hydrogel is controlled by swelling-controlled mechanism. According to this, swelling behavior of chitosan and the blend films as a function of time were also investigated using the diffusion cell. The results on swelling behavior of chitosan and the blend films at 37°C and pH 5.5 are shown in Figure 3.17. The degree of swelling of chitosan and the blend films remarkably increased at the initial stage and finally reached the equilibrium. At the initial stage when the dry films contacted with the pig skin saturated with pH 5.5 at 37°C, the solution from the pig skin diffused into the films leading to swollen stage of hydrogel. At this stage, when the films became swollen, the drug inside the films would penetrate out of the films. The diffusion of water into the films and the diffusion of drugs from the films occurred until the films reached the equilibrium state.

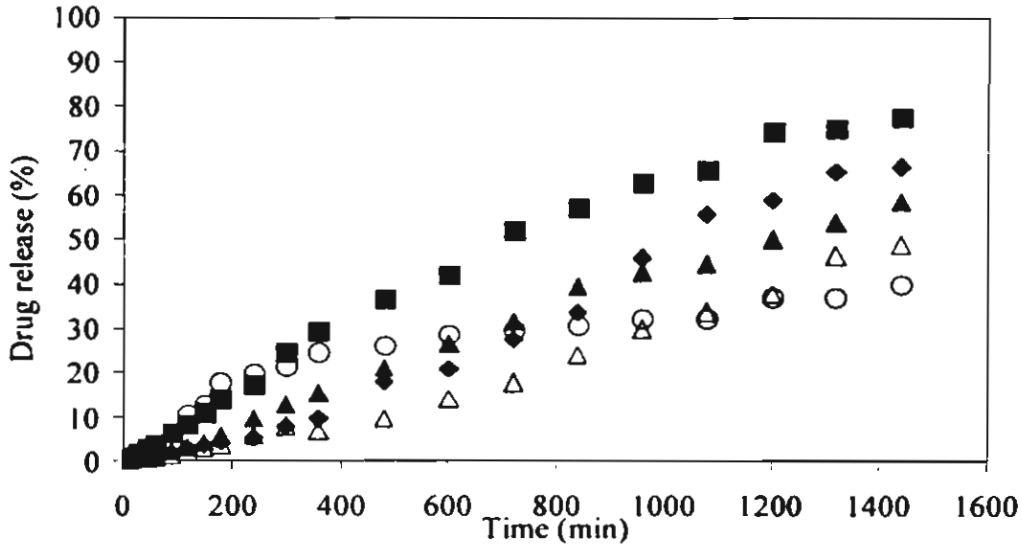

Figure 3.13 Effect of releasing time on releasing of theophylline. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (o) 40% chitosan content.

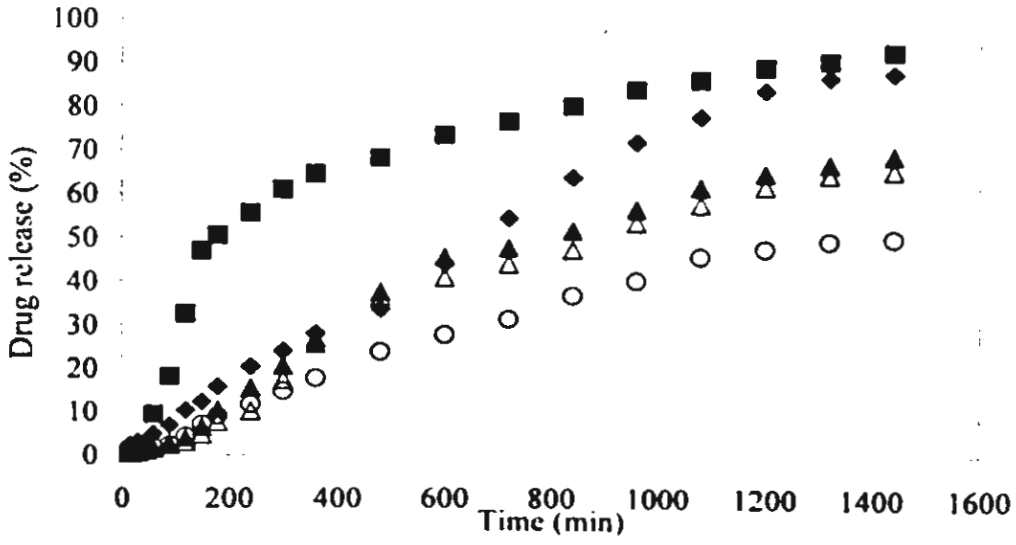

Figure 3.14 Effect of releasing time on releasing of salicylic acid. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (o) 40% chitosan content.

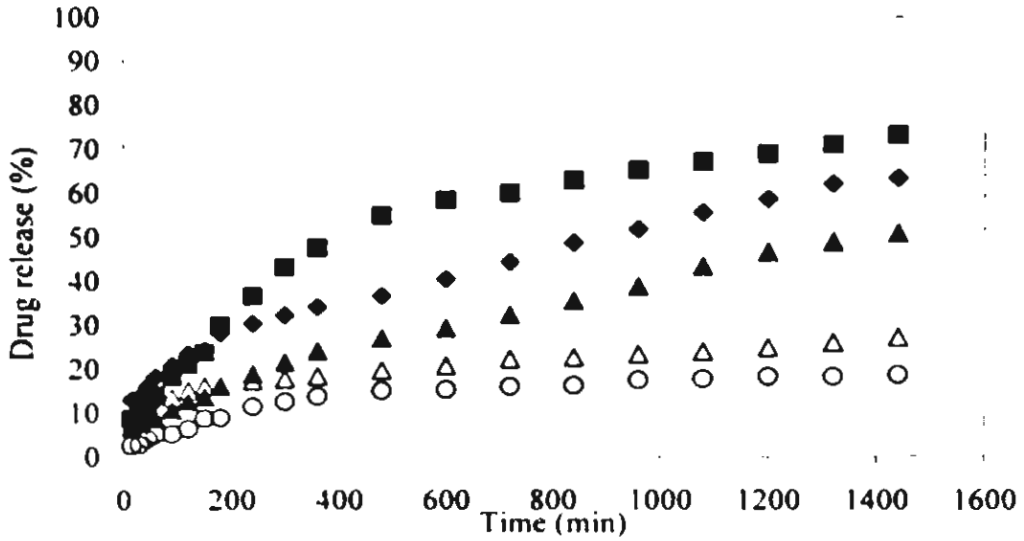

Figure 3.15 Effect of releasing time on releasing of diclofenac sodium. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (o) 40% chitosan content.

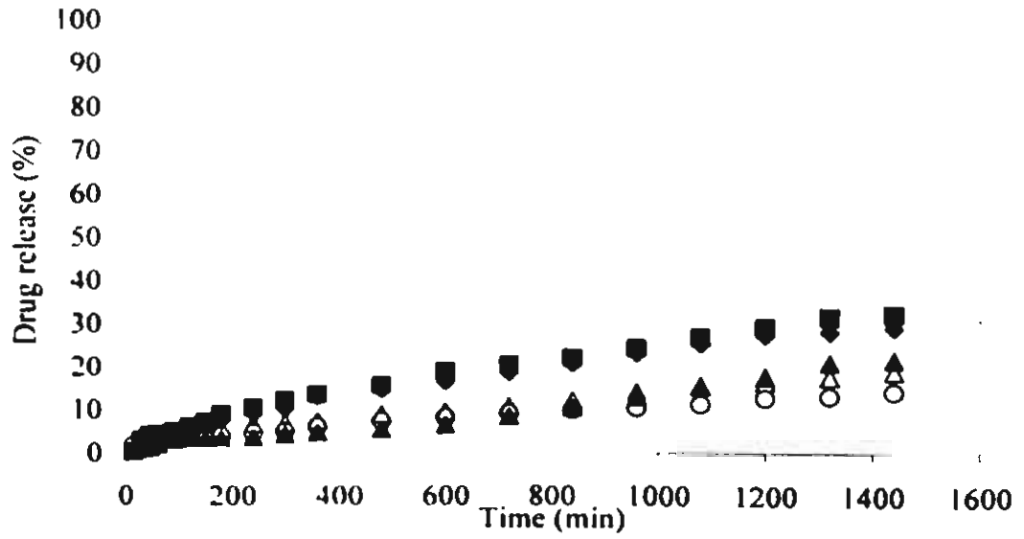

Figure 3.16 Effect of releasing time on releasing of amoxicillin trihydrate. (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (o) 40% chitosan content.

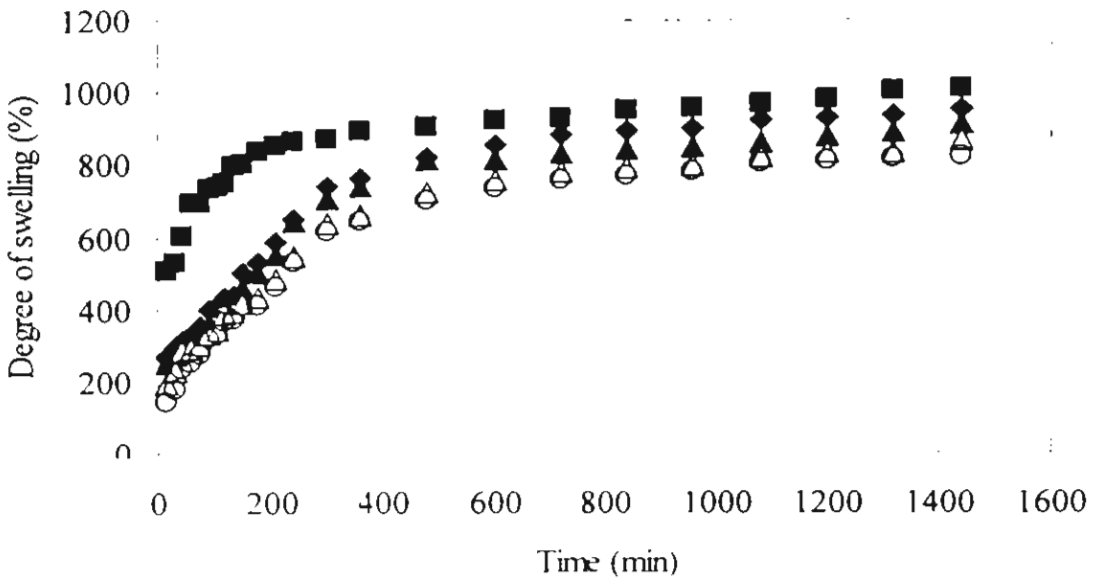

Figure 3.17 Degree of swelling of chitosan/silk fibroin blend films as a function of time (◆) 100% chitosan content, (■) 80% chitosan content, (▲) 60% chitosan content, (△) 50% chitosan content and (o) 40% chitosan content.

Effect of Thickness on Drug Release

Since the length of diffusion distance concerns the thickness of films, the effect of the film thickness on drug release was investigated and the results are shown in Figure 3.18. The experiment was done for the blend film with 80% chitosan content and the model drug used was theophylline. The three ranges of thickness studied were 20-30 μm, 50-60 μm and 100-120 μm. It was found that the amount of theophylline released from the films with the thickness of 20-30 μm, 50-60 μm and 100-120 μm were 77.44%, 14.92% and 10.41%, respectively. The more thickness of the film, the longer diffusion path. This resulted in the lower amounts of drug released. Therefore, the thin film is recommended to obtain high amount of released drug.

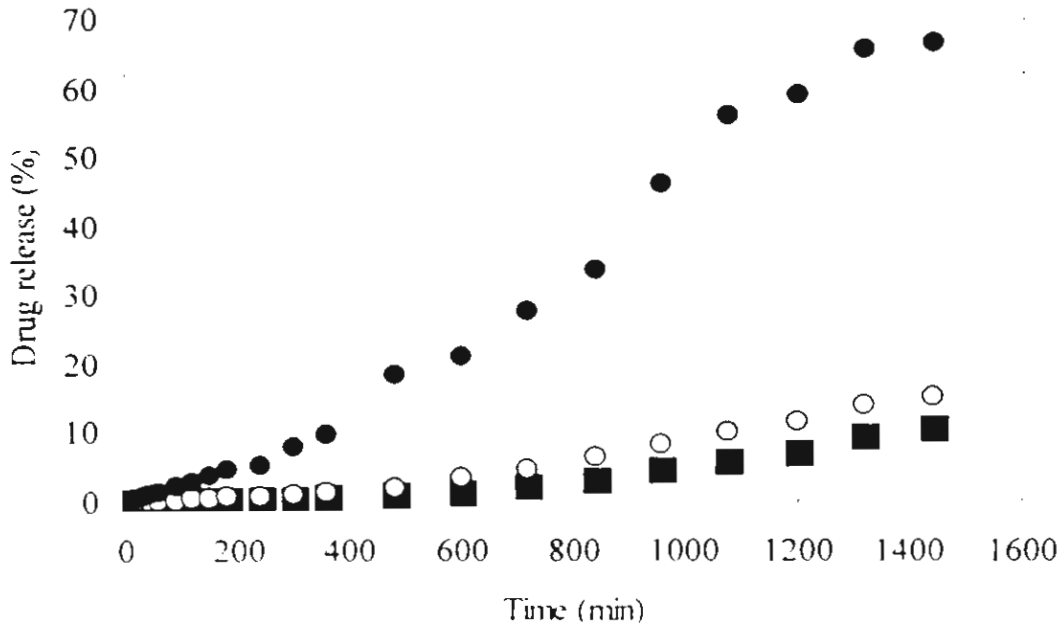

Figure 3.18 Drug release profile for theophylline-loaded blend films with 80% chitosan content. The thickness of the films were (●) 20-30 μm, (o) 50-60 μm and (■) 100-120 μm.

CONCLUSION

The releases of theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate from crosslinked chitosan/silk fibroin blend films were investigated using modified Franz diffusion cell. The blend composition (chitosan and silk fibroin) could affect the degree of swelling and the releases of model drug from chitosan and the blend films. For all model drugs studied, the maximum drug releases were obtained for the blend films with 80% chitosan content. The results of drug releases correlated to the swelling behavior of the blend films. The higher the degrees of swelling, the higher the amounts of drug released. This might be said that the releases of model drugs from the blend films were mainly occurred due to swelling-controlled release mechanism. However, the release of model drugs occurred due to erosion process as well. The orders of drugs from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. Although there are several factors, such as molecular weight of drug, interaction between drug and polymer matrix and solubility of drug, affecting the drug release characteristics, it seemed that molecular weight of drug played an important rule on drug release in this study. In addition, the thickness of the films was another factor that influenced on the amount of drug released. The increase in the thickness of the films resulted in the decreases in the amounts of drug released. In term of kinetics, all the drug release data were either fitted to zero order or Higuchi's model. It could be said that the drug permeation was either rate-controlled or diffusion-controlled release. From this study, it might be concluded that the crosslinked chitosan/silk fibroin blend films were possibly used as the matrix of the transdermal drug delivery system.

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PROJECT OUTPUT

I. International Journal

Rujiravanit, R.*, Kruaykitanon, S., Jameison, A.M., and Tokura, S. "Preparation of crosslinked chitosan/silk fibroin blend films for drug delivery system", Macromolecular Bioscience, in submission.

Peesan, M., **Rujiravanit, R.***, and Supaphol, P. (2003) "Characterization of beta-chitin/poly(vinyl alcohol) blend films", Polymer Testing, 22(4), 381-387.

II. International Conference

Rujiravanit, R., Kuratchatchaval, K., Jamieson, A.M., and Tokura, S. (2003) "Preparation and characterization of CM-chitin/PVA blend films", *in* Advanced in Chitin Science, Vol. VI, Proceedings from the 5th International Conference of the European Chitin Society, K.M. Varum, A. Domard, and O. Smidsrod (Eds.), NTNU Trondheim, Trondheim, Norway, 26-28 June 2002, 275-276.

Kruaykitanon, S., **Rujiravanit, R.**, Jameison, A.M., and Tokura, S. (2002) "Drug-release characteristics of cross-linked chitosan/silk fibroin blend films", *in* Advanced in Chitin Science, Vol. V, Proceedings of the 5th Asia Pacific Chitin and Chiotsan Symposium & Exhibition, K. Suchiva, S. Chandrkrachang, P. Methacanon, and M.G. Peter (Eds.), National Metal and Materials Technology Center, Bangkok, Thailand, 13-15 March 2002, 678-684.

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<u>In future plan</u>

Rujiravanit, R., Limpapat, S., Jamieson, A.M., and Tokura, S. "Sustained release of drug from chitosan and silk fibroin blend films", The 9th International Chitin-Chitosan Conference, Montreal, Canada, 27-30 August 2003.

Rujiravanit, R., Rotchanarak, T., and Tokura, S. "Drug release characteristics of CM-chitin/silk fibroin blend films", The 9th International Chitin-Chitosan Conference, Montreal, Canada, 27-30 August 2003.

APPENDICES

Publications

International journals

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Polymer Testing 22 (2003) 381–387
POLYMER TESTING
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Material Characterisation

Characterisation of beta-chitin/poly(vinyl alcohol) blend films

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Abstract

Blend films of β-chitin (derived from squid pens) and poly(vinyl alcohol) (PVA) were prepared by a solution casting technique from corresponding solutions of β-chitin and PVA in concentrated formic acid. Upon evaporation of the solvent, films prepared from pure β-chitin and pure PVA were found to be transparent, while the film having 50/50 composition was found to be cloudy. Miscibility of the polymers in the amorphous phase of the films at various compositions was assessed using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) techniques. The glass transition temperature of the blend films was found to increase slightly with an increase in the β-chitin content. The effect of blend compositions on apparent degree of crystallinity, mechanical properties, and swelling behavior of the as-prepared blend films was also investigated.

Keywords: β-chitin; Poly(vinyl alcohol); Blend film

1. Introduction

Natural polymers as biotechnological or biomedical resources have been widely investigated because of their unique properties, which include, for example, nontoxicity, degradability, and biological compatibility. Chitin or poly(*N*-acetyl-D-glucosamine) is a polysaccharide which is abundantly available in nature as a component in cell walls of various fungi, as well as in shells of various insects and crustaceans. Chitin is predominantly present as a fibrillar crystalline material. Based on infrared spectroscopy and X-ray diffraction data, chitin can be found in one of the three crystalline forms [1]: α-chitin, β-chitin and γ-chitin, respectively. The molecules in orthorhombic α-chitin are arranged very tightly in an anti-parallel fashion. α-Chitin is mainly present in shells of crabs, lobsters and shrimps. β-Chitin, obtained from squid pens, takes the monoclinic form in which the chains are arranged in a parallel fashion, while γ-chitin is the form in which the molecules are arranged in both parallel and anti-parallel manner. As a result of the molecular packing, intermolecular interactions in β-chitin are weaker than those in α-chitin, making β-chitin being more susceptible to dissolution in a number of solvents. This finally results in β-chitin being more reactive and versatile.

Studies related to film formation of chitin (i.e., α-chitin) have not been so popular as those of its deacetylated derivatives, i.e., chitosan. This is because chitin is insoluble in most common organic solvents, a direct result of the strong intra- and inter-molecular hydrogen bonding [2,3], while chitosan can even be dissolved in dilute organic acids. In certain applications, especially in the biomedical fields, chitin is more favorable than chitosan. This is due to the fact that the acetamide group present in chitin is similar to the amide linkage of protein in living tissues [4], making chitin more biocompatible than chitosan.

Blending is an especially important process for

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developing industrial applications of polymeric materials and compatibility among components has a marked influence on the resulting physical properties of polymer blends [5]. Through a suitable choice of polymer pairs, blends of polymers can often be tailor-made to exhibit specific and desirable properties. Blending a natural polymer with a synthetic one seems to be an alternative way of preparing polymeric alloys to meet specific applications. Studies related to blends of α-chitin with a synthetic polymer, e.g., polycaprolactone [6,7], poly(3-hydroxybutyric acid) [8], and polyamide-6 [3], are available in the open literature.

Due to its good solubility, β-chitin can be solution-cast into films, but, because of its molecular rigidity and high overall apparent degree of crystallinity, the films obtained show rigid character. Blending β-chitin with another flexible, synthetic polymer seems to be an attractive way for improving properties of the films. Poly(vinyl alcohol) (PVA) is a nontoxic, water-soluble, synthetic polymer that is widely used in biomedical applications. With its excellent film-forming ability, PVA is a good candidate for use as membranes and hydrogels [9,10].

In this present contribution, β-chitin/PVA blend films were prepared by solution-casting from solutions of β-chitin and PVA in concentrated formic acid at various compositional ratios. The effect of blend compositions on physical properties, thermal properties, mechanical properties, morphology, and swelling behavior was studied and compared with those of pure components.

2. Experimental details

2.1. Materials

β-Chitin was prepared from squid pens by acid and alkali treatment. β-Chitin was pulverized prior to use into powder, the size of which ranged from 71 to 75 μm. PVA, purchased from Fluka, has the degree of polymerization of ca. 1600 and the degree of hydrolysis of ca. 99.5%. Formic acid (reagent grade, BDH Laboratory) and ethylene glycol (J. T. Baker) were used as-received.

2.2. Preparation of blend films

PVA was first dissolved thoroughly in concentrated formic acid (99%) to prepare 1% by weight (wt%) solution. Later, a known amount of β-chitin powder was suspended in concentrated formic acid (99%) at room temperature to prepare 1 wt% solution and the suspension was frozen overnight at 0 °C. After thawing at room temperature, the solution was filtered with a glass filter. A series of β-chitin/PVA blend films with different blend compositions were then prepared by solution-casting technique. The films obtained were allowed to dry at 60 °C for 12 h. The final thickness of the dried films was in the range of 30–50 μm. All of the as-prepared films were kept under dry conditions before further use.

2.3. Measurements

Infrared spectra of the as-prepared films were recorded using a Bruker vector 3.0 FTIR spectrophotometer (FTIR). A Mettler DSC 822e/400 (DSC) was used to investigate thermal behavior of the films. To set the thermal history for all samples, each sample was first heated to 190 °C and then cooled to 0 °C at the scanning rate of 10 °C×min^{-1}. The thermal properties of the films were measured in the second heating scan at the heating rate of 10 °C×min^{-1}. The glass transition temperature (T_g) and the melting temperature (T_m) were determined as the inflection point of the specific heat increment and the onset of the endothermic melting peak of DSC traces, respectively. Thermal stability of the films was evaluated by a Perkin Elmer TGA7 (TGA) operated under nitrogen atmosphere and at a heating rate of 10 °C×min^{-1} from 30 to 750 °C. A Rigaku D/MAX-2000 wide-angle X-ray diffractometer (WAXD) equipped with a CuKα X-ray source operating at 40 kV and 30 mA was used to obtain diffractograms on the as-prepared films over the 2θ range of 5–40° and the scanning speed of 5 degree×min^{-1}. Morphology of the etched surface of selected samples was observed on a JEOL 5200-2AE scanning electron microscope (SEM). A Lloyd tensile tester was used to assess the mechanical properties of the as-prepared films. The gauge length was 125 mm, and the crosshead speed used was 12.5 mm×min^{-1}.

The swelling behavior of the as-prepared films was carried out by measuring the weight of the films after immersion in distilled water and various salt solutions (i.e., 0.25 M solutions of NaCl, $CaCl_2$, and $FeCl_3$) for 0–8 h in comparison with the dry weight of the films prior to the immersion. The degree of swelling was determined according to the following relationship:

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

where W_s and W_d represent the weight of the films after and prior to immersion. It is important to note that all the experiments were carried at room temperature. Finally, the equilibrium degree of swelling of the as-prepared films was also determined after immersion in water or corresponding solutions for 4 days.

3. Results and discussion

Solutions of pure β-chitin, pure PVA, and their blends appeared to be homogeneous and transparent. The color of the solutions varied from colorless of pure PVA solution to yellowish with increasing β-chitin content. After

evaporation of the solvent, the as-prepared films of pure β-chitin and pure PVA were found to be transparent, while the 50/50 β-chitin/PVA blend film was found to be cloudy. In addition, it was found that the blend films became more brittle with increasing β-chitin content.

3.1. Characteristics of β-Chitin/PVA blend films

Since the molecules of both β-chitin and PVA are capable of forming hydrogen bonds, it is expected that some specific interactions could be formed between the molecules of different species. In this work, observation of the as-prepared blend films using FTIR did not indicate the presence of such intermolecular interactions (results not shown). However, Lee et al. [11] reported, based on their FTIR results, that intermolecular interactions between the molecules of β-chitin and PVA could in fact exist, because they found shifting of both hydroxyl and carbonyl stretching bands upon blending β-chitin with PVA. The difference between our results and their may be due to the difference in the molecular weight characteristics of the constituents studied.

Miscibility of β-chitin and PVA at various weight compositions was investigated by observing the T_g values of the as-prepared pure and blend films. Fig. 1 shows the second heating thermograms for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films. For pure PVA film, the T_g value was found to be ca. 35 °C, which was ca. 25 °C lower than that observed for the neat resin. This could be a result of the oxidative degradation upon dissolution in concentrated formic acid or the plasticizing effect due to the presence of residual solvent molecules in the as-prepared films or both. Based on DSC results, the T_g value for pure β-chitin film could not be observed in this work. However, Kim et al. [12] used a more sensitive DMTA technique to measure the T_g value for pure β-chitin and they reported it to be ca. 170°C. For as-prepared blend films, single T_g shoulder peak was clearly observed for each blend composition. The facts that only single T_g peak was observed for each blend composition and that the resulting T_g value was found to increase slightly with increasing β-chitin content indicated partial miscibility of β-chitin and PVA in the amorphous phase at the molecular level for any given compositional ratio.

The melting endotherms for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films are shown in Fig. 2. Clearly, the T_m value for pure PVA film was found to be ca. 180 °C, and the position of the melting endotherms for β-chitin/PVA blend films at various compositions tended to shift to a lower temperature with increasing β-chitin content. For pure β-chitin film, it is surprising to observe a broad endothermic peak, the onset of which was observed at ca. 120 °C. This could be a result of the relaxation of the acetamide groups attached to the C2 position in β-chitin chains [12]. It is worthy to note that the T_m value for pure β-chitin could not be observed, a direct result of the rigid-rod nature of the β-chitin molecular backbones making them being susceptible to degradation before melting. This phenomenon is, in fact, typical for many other polysaccharides.

Thermal stability of the as-prepared films can be observed by TGA technique. Fig. 3 shows the TGA curves for pure β-chitin, pure PVA, and 50/50 β-chitin/PVA blend films. All of the samples tested showed initial weight loss at ca. 50 °C, likely a result of moisture evaporation upon heating. The amount of moisture content in all of the samples tested was almost similar. According to the derivative TGA curves, pure PVA film was found to degrade at ca. 270 °C (see Fig. 3, curve (a)), while pure β-chitin film showed two degradation peaks at ca. 262 and 349 °C, respectively (see Fig. 3; curve (c)). Apparently, the 50/50 β-chitin/PVA blend film exhibited degradation behavior intermediate to those of the pure components, exhibiting two degradation peaks at ca. 269 and 342 °C, respectively (see Fig. 3; curve (b)). Table 1 lists the degradation peak values

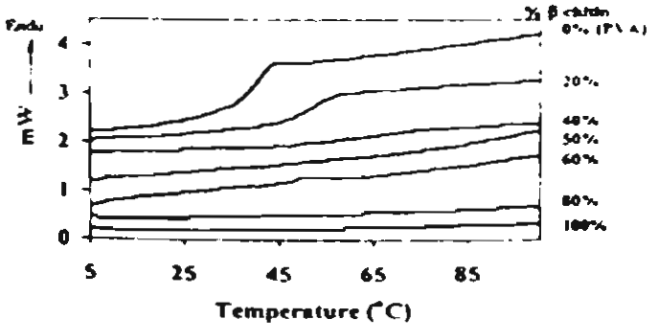

Fig. 1. The second heating thermograms for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films (recorded at 10 °C·min^{-1}) in the temperature range where a glass transition should be observed.

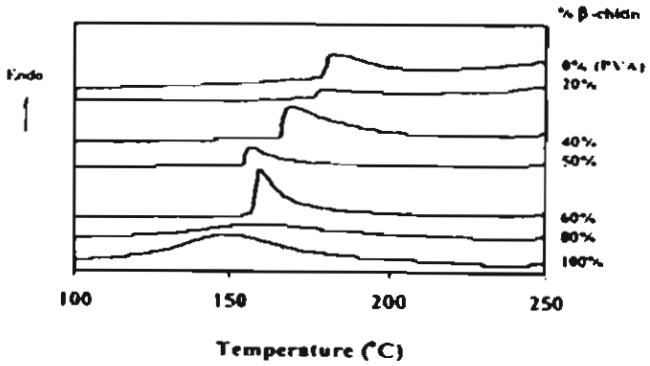

Fig. 2. The second heating thermograms for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films (recorded at 10 °C·min^{-1}) in the temperature range where a melting endotherm should be observed.

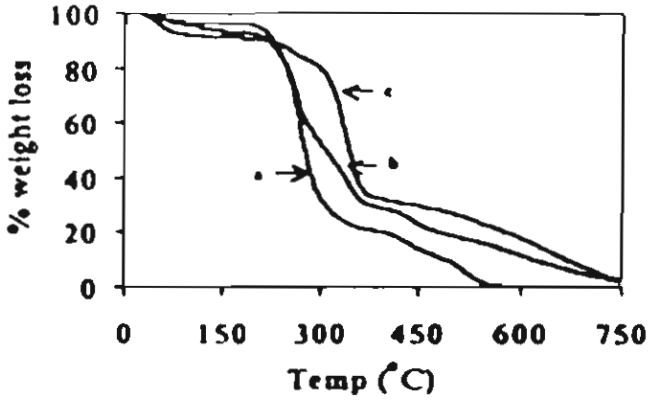

Fig 3 TGA curves for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films (recorded at 10 °C min^{-1})

Table 1
Degradation temperature(s) of pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films

Type of film	1st T_d (°C)	2nd T_d (°C)
β-chitin	262±0	349±1
80/20 β-chitin/PVA	264±2	348±1
60/40 β-chitin/PVA	265±1	346±2
50/50 β-chitin/PVA	269±1	342±2
40/60 β-chitin/PVA	271±2	335±2
20/80 β-chitin/PVA	280±2	-
PVA	270±2	-

(denoted T_d) observed for all of the as-prepared films studied. For most blend compositions, the degradation behavior of the blend films was found to be intermediate to those of the pure components. Interestingly, only 20/80 β-chitin/PVA blend film exhibited only single degradation peak, with the T_d value being much greater than those of the pure components. The reason for such peculiarity will be the matter for further investigation.

Fig. 4 illustrates WAXD patterns for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films. Obviously, the WAXD pattern for pure β-chitin film exhibited two crystalline peaks at the 2θ angles of ca. 7.4 and 19.4°, respectively. This observation is in general accordance with the finding by Ren and Tokura [13], who reported that the two characteristic crystalline peaks of β-chitin were found at 8.50 and 19.98°, corresponding to the (010) and (020) and (1$\bar{1}$0) refraction planes, respectively. When PVA crystallized in a monoclinic unit cell (with the cell characteristics a = 0.781 nm, b = 0.252 nm, and c = 0.511 nm, $\alpha = \beta = 90°$, γ = 97.1°) [14], the main peaks in the WAXD pattern should appear at the 2θ angles of 11.3, 19.7, 22.9, 28, 32.5, and 40.9° [15]. According to Fig. 4, the WAXD pattern for pure as-prepared PVA film only showed a broad crystalline peak at the 2θ angle of ca. 18.7°. For β-chitin/PVA blend films, the diffractograms appeared to be intermediate to those of the pure components. It is evident that, as β-chitin increased, not only did the intensity of β-chitin characteristic crystalline peaks become less pronounced, especially when β-chitin content was lower than 50 wt%, but the crystalline peaks became broader as well, suggesting a decrease in the size of β-chitin crystals as well as in the apparent degree of crystallinity. This might be a result of a dilution effect when PVA was blended with β-chitin.

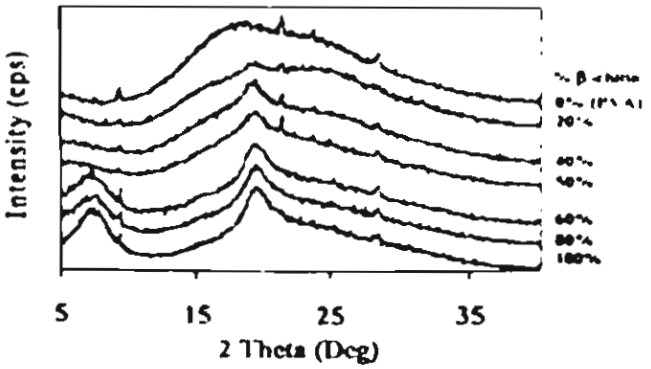

Fig. 4. WAXD patterns for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films.

Even though not shown in this paper, surface morphology of β-chitin/PVA blend films was also observed by scanning electron microscopy. After drying the films at room temperature for 48 h, the films with porous structure were obtained for all of the blend compositions. On the contrary, when the films were instead dried in an oven at 60 °C for 12 h, shrinkage in the films was observed. In order to observe the level of compatibility between β-chitin and PVA in as-prepared β-chitin/PVA blend films which were earlier dried at 60 °C, the films were etched in hot ethylene glycol which is a good solution to PVA and the resulting SEM micrographs are shown in Fig. 5. According to Fig. 5, certain level of phase separation in the micrometer scale is obvious in all of the blend compositions studied.

3.2. Tensile properties

The mechanical properties, in terms of tensile strength and percentage of elongation at break, were determined for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films and the results are reported as a function of β-chitin content in Figs. 6 and 7, respectively. For pure β-chitin film, the tensile strength and the percentage of elongation at break were found to be ca. 5.1 MPa and 2.9%, respectively. This agreed particularly well with the results obtained by Kim et al. [12], who reported that the tensile strength and the percentage of elongation at break for pure β-chitin film, which was solution-casted from its solution in formic acid, were ca.

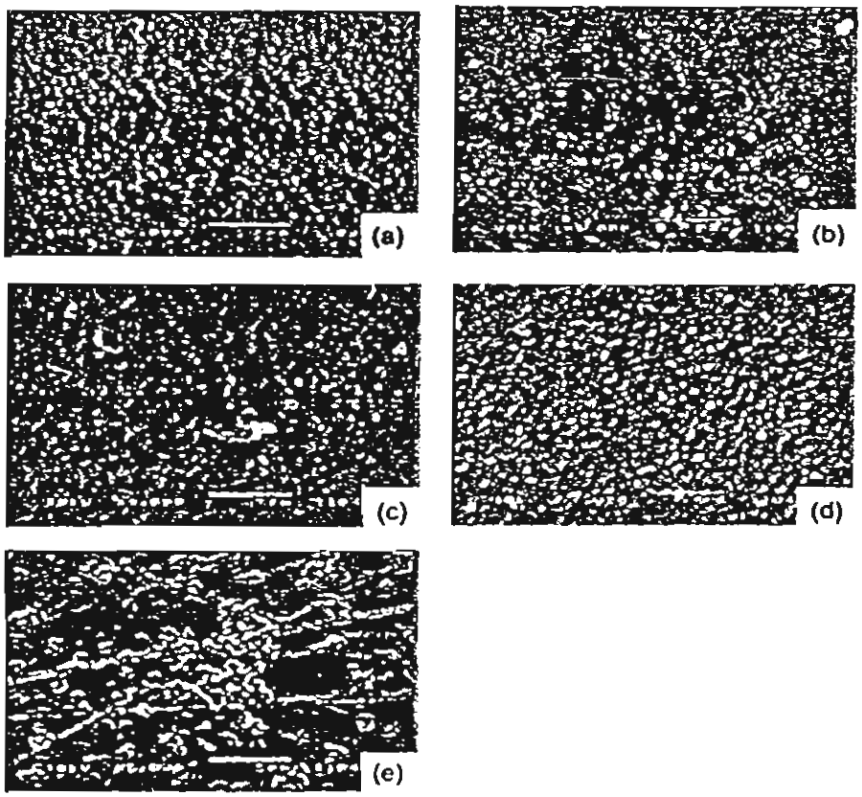

Fig. 5. Scanning electron micrographs of ethylene glycol-etched β-chitin/PVA blend films for (a) 80/20, (b) 60/40, (c) 50/50, (d) 40/60, and (e) 20/80 blend compositions, respectively.

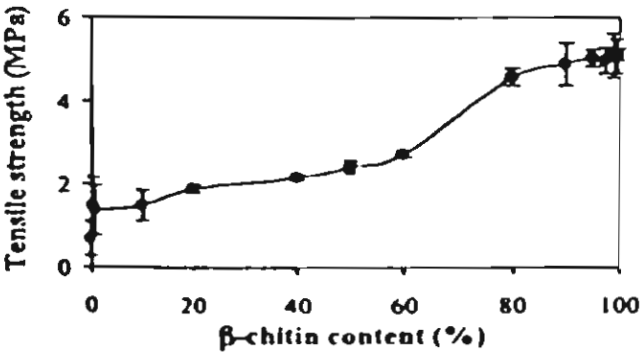

Fig. 6. Tensile strength for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films.

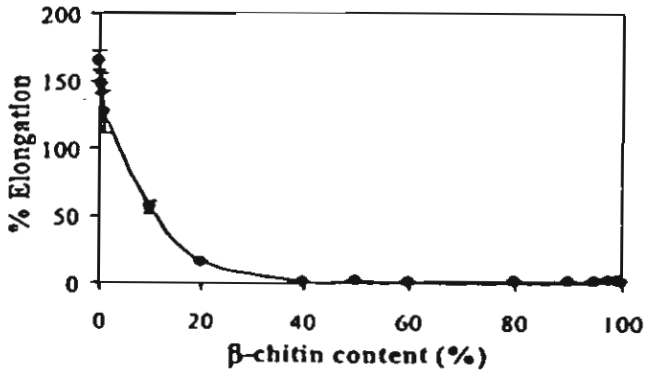

Fig. 7. Percentage of elongation at break for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films.

5.2 MPa and 5%, respectively. On the other hand, PVA exhibited a much softer character, with its tensile strength and the percentage of elongation at break being ca. 0.7 MPa and 165.2%, respectively. For β-chitin/PVA blend films, the tensile strength was found to increase, with increasing β-chitin content, from ca. 0.7 to 5.1 MPa, at the expense of the percentage of elongation at break, which was found to decrease from ca. 165.2 to 2.9%. Physically, the blend films appeared to be more brittle as β-chitin content increased.

3.3. Swelling behavior

The degree of swelling of pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films with different blend compositions is shown in Fig. 8 as a function of immersion time in distilled water. For a given blend composition, the degree of swelling increased with increasing immersion time. After 8 h of immersion time, it is interesting to note that the ultimate degree of swell-

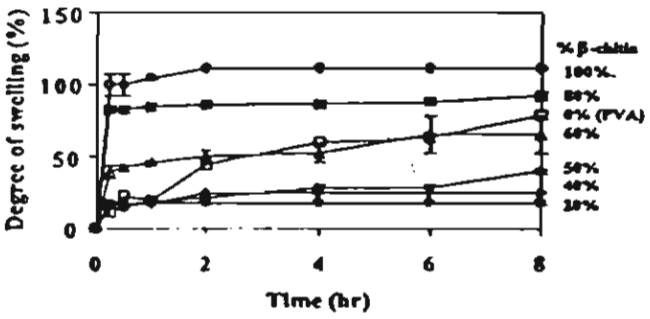

Fig. 8. Dynamic degree of swelling in distilled water for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films as a function of immersion time.

ing of pure β-chitin film was greater than that of pure PVA film (i.e., ca. 100% versus ca. 80%). With increasing β-chitin content, the ultimate degree of swelling after 8 hours of immersion time of β-chitin/PVA blend films was found to increase from ca. 15 to ca. 90%, when β-chitin content increased from 20 to 80 wt%. This behavior is in general agreement with results obtained for IPN hydrogel composed of β-chitin and PEG macromer [16].

The equilibrium degree of swelling (after 4 days of immersion time) for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films as a function of β-chitin content is showed in Fig. 9. Interestingly, the equilibrium degree of swelling of pure PVA film was now greater than that of pure β-chitin film (i.e., ca. 190% versus ca. 110%). Comparison of the results shown in Fig. 8 suggests that pure β-chitin film reached the equilibrium much faster than pure PVA film. For β-chitin/PVA blend films, the equilibrium degree of swelling was found to increase from ca. 30 to ca. 95%, when β-chitin content increased from 20 to 80 wt%. This is in accord with the ultimate degree of swelling after 8 h of immersion time observed earlier. It is rather surprising, however, that both the ultimate and equilibrium degrees of swelling of the 20/80 β-chitin/PVA blend film were found to be the lowest among the films studied, despite the high level of PVA content.

The equilibrium degree of swelling in various media (i.e., water, NaCl, $CaCl_2$, and $FeCl_3$ solutions at the concentration of 0.25 M) of pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films was investigated and the results are shown in Fig. 10. Evidently among of the media studied, $FeCl_3$ solution was the best medium to swell the as-prepared films. According to a known fact that, when being present in water, ferric ion, Fe^{3+}, can exist in an hydrated form, e.g., $Fe(H_2O)_6^{3+}$ [17], and the bulky size of the hydrated ferric ion can thus be responsible for the high degree of swelling of the as-prepared films studied. It could be further deduced from the results obtained that the blend films swell more substantially in trivalent ion solutions than in monovalent and bivalent ion solutions.

4. Conclusions

In this contribution, β-chitin/PVA blend films were prepared by solution-casting from solutions of β-chitin and PVA in concentrated formic acid at various compositional ratios. The effect of blend compositions on physical properties, thermal properties, mechanical properties, morphology, and swelling behavior was investigated and the results were compared with those of pure components. DSC measurements showed that the glass transition temperatures of the blend films increased with increasing β-chitin content, while melting temperatures tended to shift to a lower temperature. Thermal stability of the blend films was found to be intermediate to those of the pure components. WAXD patterns indicated a reduction in the apparent degree of crystallinity of β-chitin with increasing PVA content. Surface morphology of ethylene glycol-etched β-chitin/PVA blend films suggested that a certain level of phase separation in a micrometer scale was found for blend films of all

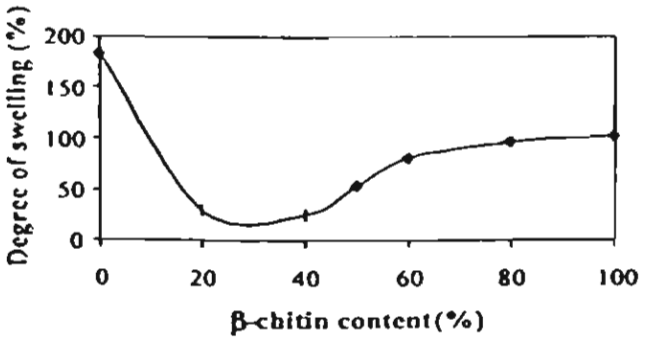

Fig. 9. Equilibrium degree of swelling in distilled water for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films.

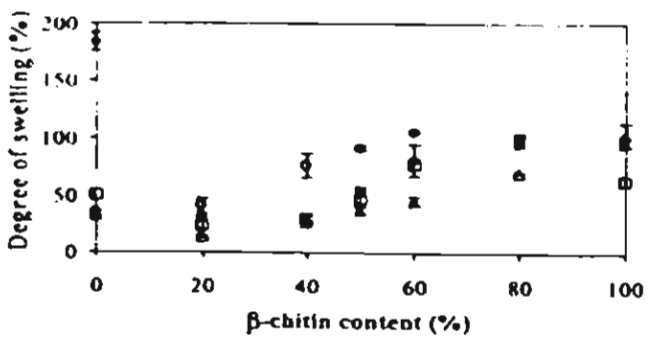

Fig. 10. Equilibrium degree of swelling in various media for pure β-chitin, pure PVA, and a series of β-chitin/PVA blend films. Keys: (◆) water, (□) NaCl, (△) $CaCl_2$, and (○) $FeCl_3$.

blend compositions. The tensile strength of the blend films was found to increase, with increasing β-chitin content, from ca. 0.7 to 5.1 MPa, at the expense of the percentage of elongation at break which was found to decrease from ca. 165.2 to 2.9%. The equilibrium degrees of swelling in distilled water of β-chitin/PVA blend films of all blend compositions were found to be lower than those of the pure constituents, with that of the 20/80 β-chitin/PVA blend film being the lowest. Lastly, 0.25 M $FeCl_3$ solution, among the various swelling media investigated, was the best to swell most of the films studied.

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In submission

Macromolecular Bioscience

Preparation of Crosslinked Chitosan/Silk Fibroin Blend Films for Drug Delivery System

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Summary

Crosslinked chitosan/silk fibroin blend films were prepared by solution casting technique using glutaraldehyde as a crosslinking agent. Drug-released characteristics of the blend films with various blend compositions were investigated. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid were used as model drugs. The release studies were done at 37°C in buffer solutions pH 2.0, pH 5.5 and pH 7.2. It was found that the blend films with 80% chitosan content showed the maximum amounts of model drugs released at pH 2.0 for all types of drugs. This result corresponded to swelling ability of the blend films. From swelling study, the maximum degrees of swelling of the drug-loaded blend films were obtained at this pH and this blend composition. The amounts of drugs released from the films with 80% chitosan content from the highest to the lowest values obtained in the following sequence: salicylic acid > theophylline > diclofenac sodium > amoxicillin. This could be due to the effects of molecular sizes of drugs, solubility of drugs in the blend solutions and interaction between drugs and polymer matrix.

Keyword: chitosan; drug delivery system; silk fibroin

Introduction

A drug delivery system may be a matrix of polymer incorporating a drug. Polymeric drug carrier systems have several advantages in optimizing patient treatment regimes. In particular, swelling-controlled release systems are capable of delivering drugs at constant rates over an extended period of time. In these systems, the rate of drug delivery is controlled by the balance between drug (solute) diffusion across a concentration gradient, the polymer relaxation occurring as the crosslinked polymer imbibes water, and the osmotic pressure occurring during the swelling process. Furthermore, a binary polymer matrix constituted of two polymers of different hydrophilic character is another possibility for controlling the degree of swelling of the system and the solute diffusion rate from the matrix.

Chitosan, poly[β-(1-4)-linked-2-amino-2-deoxy-D-glucose], is an aminopolysaccharide derived from N-deacetylation of chitin. Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-dimensional crosslinked polymeric material with the ability to absorb significant amount of water. Crosslinked chitosan hydrogel can swell extensively due to the positive charges on the network and response to change in pH of medium. Due to the benefits of being non-toxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for the sustained delivery of drug. For example, it was investigated for the delivery of drugs such as prednisolone[1] and diclofenac sodium[2]. There have been many studies on the blends of chitosan with various kinds of polymers[3-7] in order to obtain some improved properties. It

is worth to investigate drug release properties of these chitosan-based blends in order to develop more efficient drug delivery devices.

Silk fibroin is a fibrous protein that is composed of 17 amino acids and its main components are nonpolar ones such as glycine, alanine, and serine. Silk fibroin can exist in two general conformations, random coil and β-sheet form. The conformation transition of silk fibroin can be induced to change from random coil to β-sheet structure by treatments such as heating[8], stretching or immersion in polar solvents[9]. This transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β-sheet structure is resistant to water and has good mechanical properties[10]. Silk fibroin is considered to be an interesting starting material for developing new materials and devices for biotechnological and biomedical utilization. It has been reported that silk fibroin film has good oxygen permeability in wet state[11], which suggests promising applications of silk fibroin as wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures[12] and biocompatible devices with controlled drug release[13]. However, silk fibroin in dry state is very brittle and unsuitable for practical uses[14]. To overcome this limitation, silk fibroin has been reported to blend with other synthetic polymers, such as polyacrylamide[15] and poly(vinyl alcohol)[16], or natural polymers, such as cellulose[14] and sodium alginate[17], to improve mechanical and physical properties. Among these, the blend of silk fibroin and chitosan has been interesting. It has been reported that chitosan could induce the conformational transition of silk fibroin from random coil to β-sheet structure[10] and a polymer blend of these biopolymers could also form a hydrogel having a semi-interpenetrating polymer network by using glutaraldehyde as a crosslinking agent [18].

This research is a preliminary study on using crosslinked chitosan/silk fibroin blend film as a matrix for drug delivery system. The model drugs used were theophylline,

diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effects of blend composition, drug nature, swelling time, degree of crosslinking, and pH of the external swelling media on drugs released from the blend films were investigated.

Experimental Part

Materials

Shrimp shell was kindly provided by Suraphol Food Public Co., Ltd., Thailand. Silk fiber (*Bombyx mori*) was degummed by treatment with 0.5% Na_2CO_3 at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk fibroin was dissolved in triad solvent $CaCl_2$: EtOH: H_2O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was filtered through the sintered glass filter and subsequently diluted to achieve a concentration of 1 wt.-%.

Theophylline was purchased from Shanghai Wandai Pharmaceuticals, China. Diclofenac sodium was purchased from Tangyin Yongqi Chemical Industry Co., Ltd., China. Salicylic acid was purchased from Ajax Chemicals, Australia. Amoxicillin trihydrate was purchased from Antibiotics Co., Ltd., Spain. The chemical structures of these model drugs are shown in Figure 1. All other chemicals and solvents were of analytical grade and were used without further purification.

Preparation of Chitin

Chitin was prepared from shrimp shell by decalcification and deprotenization to remove calcium carbonate and protein, respectively. The shrimp shell was cleaned and dried under sunlight before grinding into small pieces. The shrimp shell chips were treated by immersing in 1 N HCl solution for 2 days with occasional stirring. The decalcified

product was washed with distilled water until neutral. Deprotenization was followed by boiling in 4 wt.-% of NaOH solution at 80-90°C for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C in a convective oven for 24 h.

Preparation of Chitosan

Chitin was deacetylated by heating in NaOH 50 wt.-% solution with sodium borohydride ($NaBH_4$) 0.5 wt.-% based on the weight of chitin to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustedly with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1 wt.-% of acetic acid solution. The chitosan solution was allowed to stand overnight at room temperature to reduce of air bubbles before preparation of films.

Preparation of Crosslinked Drug-loaded Blend Films.

The blend solutions of chitosan and silk fibroin were prepared by mixing various ratios of 1 wt.-% of silk fibroin solution and 1 wt.-% of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added into the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added into the blend solutions to achieve a

concentration of 0.1 wt.-%. The blend solution containing a model drug was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry perti dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use. The thickness of the films were kept between 25-30 μm (measured by Peacock digital thickness gauge model PDN12N)

Drug Release Studies

To study the release characteristics of the model drugs from the films, drug-loaded blend films were immersed in buffer solutions pH 2.0, pH 5.5 and pH 7.2 at 37°C. At a time interval, 1-mL aliquots were withdrawn and assayed for the amount of drug released. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid released in the solutions were determined by a UV-Visible spectrophotometer (Perkin Elmer, Lambda 10) at 272, 275, 272, 299 nm, respectively. The experiments were done in triplicate. The percentages of released drugs were calculated from calibration curves of each model drug.

Results and Discussion

Effect of Blend Composition on Drug Release

The effect of blend composition on drug release is shown in Figure 2-5. Silk fibroin contents of 0, 20, 40, 50 and 60% in drug-loaded blend films were used in this study. The blend films with silk fibroin contents higher than 60% were not reported because the films were brittle and difficult to handle without cracking. It was found that the maximum release of drug was observed for the blend film with 80% chitosan content for all model drugs. This could be explained by the term of swelling behavior of the blend films. Table 1 shows the degrees of swelling of the drug-loaded blend films of different blend

compositions. It was found that the blend film with 80% chitosan content showed the maximum degree of swelling. It is known that for hydrogel delivery system the releasing of drug was controlled by swelling behavior of hydrogel. The swelling of carrier increases the aqueous solvent content within the polymer matrix, enabling the drug to diffuse through the swollen network into the external environment. Several chitosan-based hydrogels have been investigated for a potential application as drug delivery devices. Risbud *et al.* (2000)[20] indicated that the releases of amoxicillin from the air-dried and freeze-dried chitosan/poly(vinyl pyrrolidone) hydrogels were related to the degree of swelling of the hydrogels. Yao *et al.* (1993 and 1994)[21-22] studied the release of chlorhexidini acetas and cimetidine from chitosan/polyether semi-interpenetrating hydrogel. They found that the higher the degrees of swelling the higher the amounts of drug released. In this study, the swelling of chitosan/silk fibroin blend films may be occurred due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan[19].

Effect of pH on Drug Release

Since the swelling of polymeric gels can be triggered by a change in the environmental surrounding such as pH, the effect of pH on drug released from chitosan and the blend films was investigated and the result is shown in Figure 2-5. Drug release properties of the films were studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the highest amounts of drugs released from the systems were observed at pH 2.0 for all model drugs. This is in good agreement with the results of swelling of the films shown in Table 1. It appeared that the highest values of the degrees of swelling were obtained at pH 2.0 and the degrees of swelling of the films tended to decrease as pH of swelling solution was increased. It can be explained by the fact that in an acidic medium the amino groups of

chitosan are protonized, resulting that the hydrogen bonds between chitosan and silk fibroin are broken and the network is dissociated.[20] The blend films exhibited lower degree of swelling in neutral medium. This may be due to the decrease in the number of protonated amino groups of chitosan at this pH. The pK_a of chitosan is 6.3-6.5, indicating that chitosan tends to protonate in acidic solution. Therefore, the degrees of swelling of the films at pH 7.2 were lower than those of the films in acidic solution. Risbud *et al.* (2000) [16] reported that the degrees of swelling of chitosan/poly(vinyl pyrrolidone) hydrogels were high in acidic solutions (pH 1.0, pH 2.0 and pH 3.0) and became lower in neutral and alkaline solutions (pH 7.2 and pH 9.2). The release of amoxicillin from the films was found to be maximum at pH 1.0. Besides the release of drug being controlled by swelling behavior of the carrier, drug release may be concerned with the erosion process. This process is associated with macroscopic changes in the appearance of the device, including changes in the pysicomechanical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions. Table 1 shows the weight losses of chitosan and the blend films in the conditions studied. It was found that the weight losses of the films at pH 2 were higher than the values at pH 5.5 and pH 7.2. This indicated that drugs released by erosion process could also be occurred in this system.

Effect of Model Drug Nature on Drug Release

Comparison of the amounts of model drug released from chitosan and the blend film with 80% chitosan content is shown in Figure 6-8. The amounts of drugs released from the blend film with 80% chitosan content were higher than those released from pure chitosan films for all model drugs. The amounts of salicylic acid released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 92.7%, 83.4% and 73.5%, respectively. The amounts of theophylline released at pH 2.0, pH 5.5 and pH 7.2 from the

blend film with 80% chitosan were 81.1%, 73.6% and 69.0%, respectively. The releasing amounts of salicylic acid at equilibrium were higher than those of theophylline at all pH studied. In addition to drug solubility, another factor that can affect the penetration of a drug from a polymer matrix is the molecular size of drug. The molecule of salicylic acid (MW = 138.12) was smaller than theophylline (MW = 180.16). Due to the better solubility and smaller size, salicylic acid could diffuse from the matrix to the medium outside easier than theophylline. The amounts of diclofenac sodium released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 76.6%, 66.1% and 65.1%, respectively. Since diclofenac sodium did not completely dissolve in the blend solutions and remained in the blend films as solid particles. Therefore, the amounts of diclofenac sodium released to the solution were less as compared to salicylic acid and theophylline.

Among the model drugs investigated in this study, the amounts of amoxicillin released from the blend films with 80% chitosan content were the lowest values at all pH studied. It was found that the amount of amoxicillin released at pH 2.0, pH 5.5 and pH 7.2 were 37.2%, 34.0% and 23.5%, respectively. Risbud *et al.* (2000)[120] has also reported that the releasing of amoxicillin from crosslinked chitosan-poly(vinyl pyrrolidone) air-dried hydrogel was rather low, about 31.68% and 27% at pH 1.0 and pH 2.0, respectively. This may be due to the interaction between the drug molecule and polymer matrix. Amoxicillin has carboxylic group that can interact with amino group of chitosan. In addition, among the model drugs used in this study, the molecular size of amoxicillin (MW = 381.45) is the biggest. Accordingly, the diffusion of amoxicillin through the polymer matrix was low.

Effect of Swelling Time on Drug Release

The release profiles of each model drug in buffer solutions at pH 2.0, 5.5, and 7.2 from the blend films with 80% chitosan content are illustrated in Figure 9-11. It was found

that the release of theophylline from the blend films with 80% chitosan content was very fast at all pH studied. Puttipipatkhachorn (2001)[23] investigated the drug-polymer interaction between theophylline and chitosan by FTIR and solid sate ^{13}C NMR spectroscopy. It was concluded that there was no interaction between theophylline and chitosan. Moreover, the molecular size of theophylline was rather small as compared with the other model drugs used. Then, theophylline could easily penetrate from the blend film to the medium.

The release of salicylic acid from the blend films with 80% chitosan content was also fast but a little slower than theophylline at pH 5.5 and pH 7.2. It was known that the salicylate formation can occur by the interaction between carboxylic group of salicylic acid and amino group of chitosan.[23] Therefore, the releases of salicylic acid at pH 5.5 and pH 7.2 were faster than at pH 2.0 due to more ionized carboxylic groups that can interact with amino groups of chitosan.

Due to the poor solubility of diclofenac sodium in the blend solution, Some diclofenac sodium remained as solid particles in the blend film. Accordingly, at initial stage, a time was needed for dissolving and penetrating of the drug from the blend film to the external medium. Therefore, the release of diclofenac sodium was slower and took longer time to reach the equilibrium as compared with the other model drugs.

Since amoxicillin has several polar groups, including hydroxyl group, amino group and carboxylic group, which can interact with the polymer matrix, the interaction between the drug and the polymer matrix can be formed. As a result, the amount of amoxicillin released from the blend films was only about 20-30%. However the rate of drug release occurred within 10 minutes. This may be due to the sufficient swell of the blend film resulting in the fast release of unbound drug.

Effect of Concentration of Crosslinking Agent on Drug Release

The effect of crosslinking agent concentration on drug release from the blend films is shown in Figure 12. To study the effect of concentration of crosslinking agent on drug release, the salicylic acid-loaded blend films with 80% chitosan content containing glutaraldehyde concentrations of 0.001, 0.01, and 0.5 mole/glucosamine unit were used. It was found that the amount of salicylic acid released from the blend film decreased with the increasing of glutaraldehyde concentration at all pH studied. It could possibly be explained by the term of degree of swelling (Table 2). The result revealed that the degree of swelling of the salicylic acid-loaded blend films decreased with the increasing of glutaraldehyde concentration. This is attributed to the swelling behavior of the crosslink network. At low concentration of crosslinking agent, the density of crosslinking is low that make hydrogel swell extensively. While the mesh size of the network become big resulting in high penetration of drug molecules to external environment. On the other hand, at high concentration of crosslinking agent, the degree of swelling is limited. Therefore, the mesh size of the network is closer to the size of drug, and the drug is more difficult to penetrate to the external environment

Conclusion

Drug-released characteristics of crosslinked chitosan and its blend films with silk fibroin using glutaraldehyde as a crosslinking agent were studied. Theophylline, salicylic acid, diclofenac sodium and amoxicillin were used as model drugs. The maximum amounts of drug released were obtained from the blend film with 80% chitosan content. This corresponded to the highest swelling ability of the blend film with this blend ratio. The drugs releases were high in acidic medium due to the protonation of the amino groups on chitosan at acidic pH, resulting in the dissociation of hydrogen bonds between chitosan

and silk fibroin. The maximum and the minimum releases of model drugs were salicylic acid and amoxicillin, respectively. The differences in the rates and the amounts of drug released from the blend films may be due to the solubility of model drugs in the blend solutions, the molecular sizes of drug molecules and interaction between drugs and polymer matrix. Crosslinking is necessary to retain the gel structures after swelling. However, it will limit swelling ability of gels, resulting in the decreasing of the amounts of drug released. These preliminary results suggested the possibility of using crosslinked chitosan/silk fibroin blend films as a drug delivery carrier. Further investigations are now in progress to evaluate a potential application of crosslinked chitosan/silk fibroin blend films as a transdermal drug delivery device.

Acknowledgement

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List of Figure

Figure 1. Chemical structures of model drugs.

Figure 2. Effect of blend composition on releasing of diclofenac sodium. ⌋ pH 2.0, pH ▨ 5.5, ■ pH 7.2

Figure 3. Effect of blend composition on releasing of salicylic acid. ⌋ pH 2.0, ▨ p H 5.5, ■ pH 7.2

Figure 4. Effect of blend composition on releasing of amoxicillin. ⌉ pH 2.0, ▨ pH 5.5, ■ pH 7.2

Figure 5. Effect of blend composition on releasing of theophylline. ⌉ pH 2.0, ▨ p H 5.5, ■ pH 7.2

Figure 6. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 2.0. ■ chitosan film ⌉ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 7. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 5.5. ■ chitosan film ⌉ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 8. Comparison of the amounts of drug released from chitosan and the blend film with 80% chitosan content at pH 7.2. ■ chitosan film ⌉ blend film with 80% chitosan content (SAL = salicylic acid, THEO = theophylline, DFS = diclofenac sodium, AMX = amoxicillin)

Figure 9. Drug release profiles from the blend films with 80% chitosan content at pH 2.0. (o) Salicylic acid, (◆) theophyline, (⌊) diclofenac sodium, (▲) amoxicillin trihydrate

Figure 10. Drug release profiles from the blend films with 80% chitosan content at pH 5.5. (o) Salicylic acid, (◆) theophyline, (⌊) diclofenac sodium, (▲) amoxicillin trihydrate

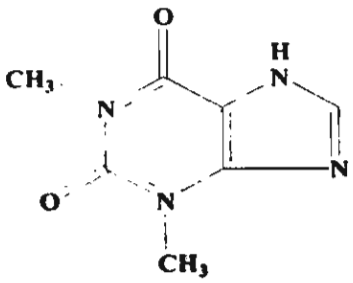
Figure 11. Drug release profiles from the blend films with 80% chitosan content at pH 7.2. (o) Salicylic acid, (◆) theophyline, (⌊) diclofenac sodium, (▲) amoxicillin trihydrate

Figure 12. Salicylic acid released from the blend films with 80% chitosan content containing various glutaraldehyde concentrations at pH 2.0, 5.5 and 7.2. 0.001 mole/glucosamine unit, ▨ 0.01 mole/glucosamine unit, ■ 0.05 mole/glucosamine unit

List of table

Table 1. Degree of swelling and percent weight loss of drug-loaded crosslinked chitosan and blend films

Table 2. Degree of swelling and percent weight loss of salicylic acid-loaded blend films with 80% chitosan containing various glutaraldehyde concentrations



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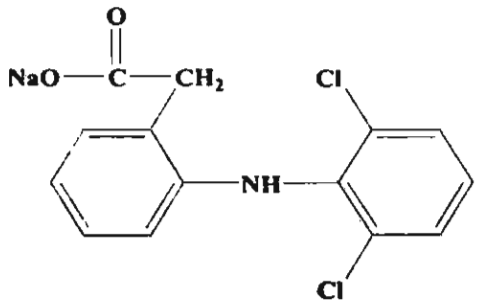

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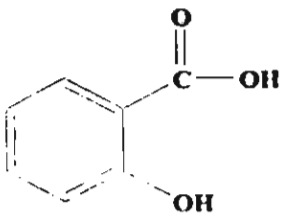

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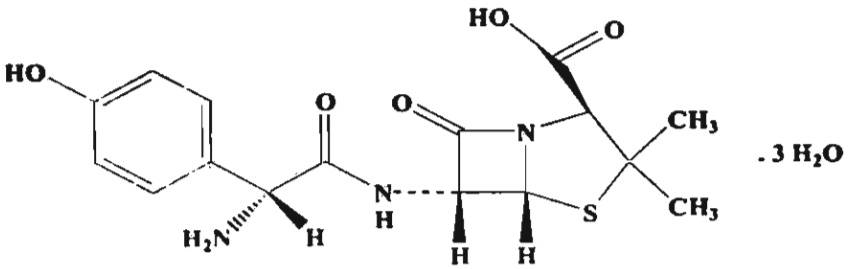
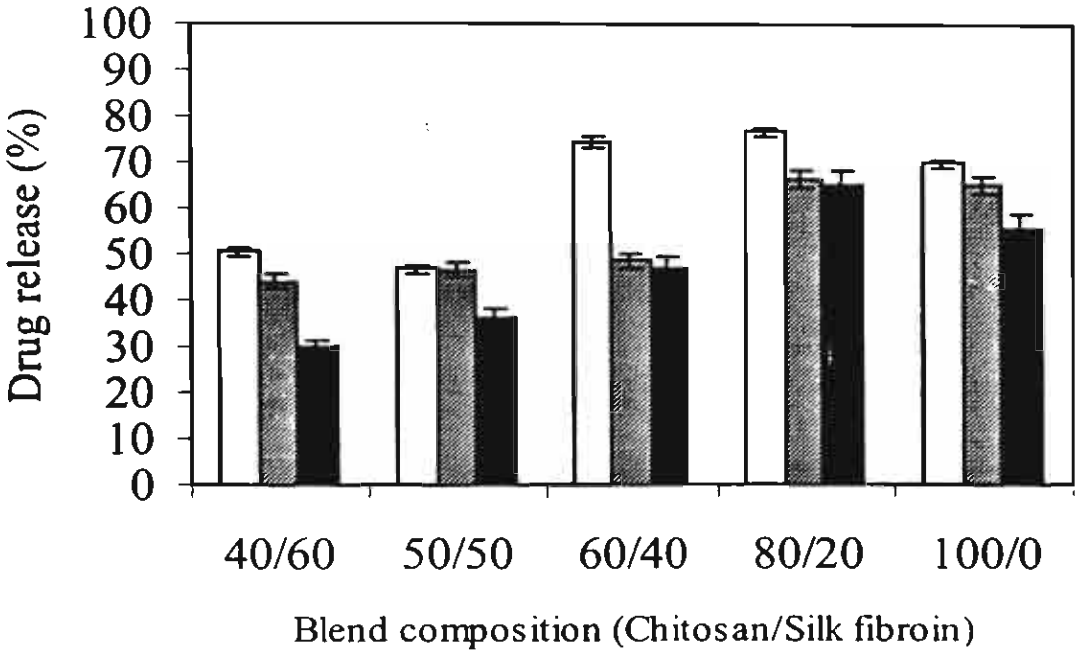


Amoxicilline trihydrate

Figure 1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Blend composition (Chitosan/Silk fibroin)

Figure 2

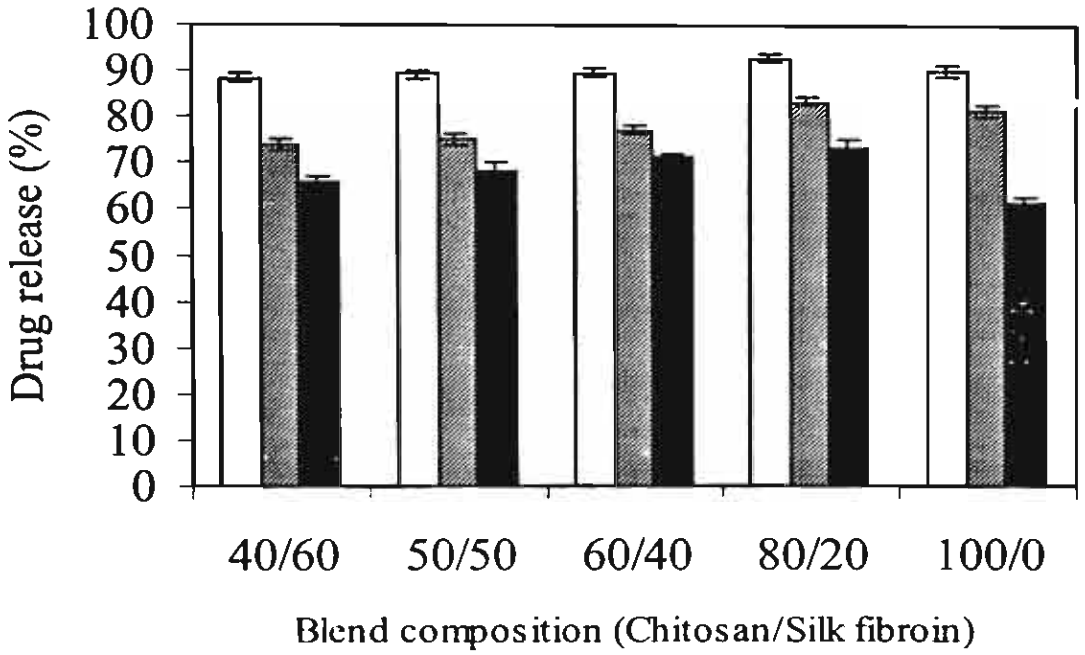
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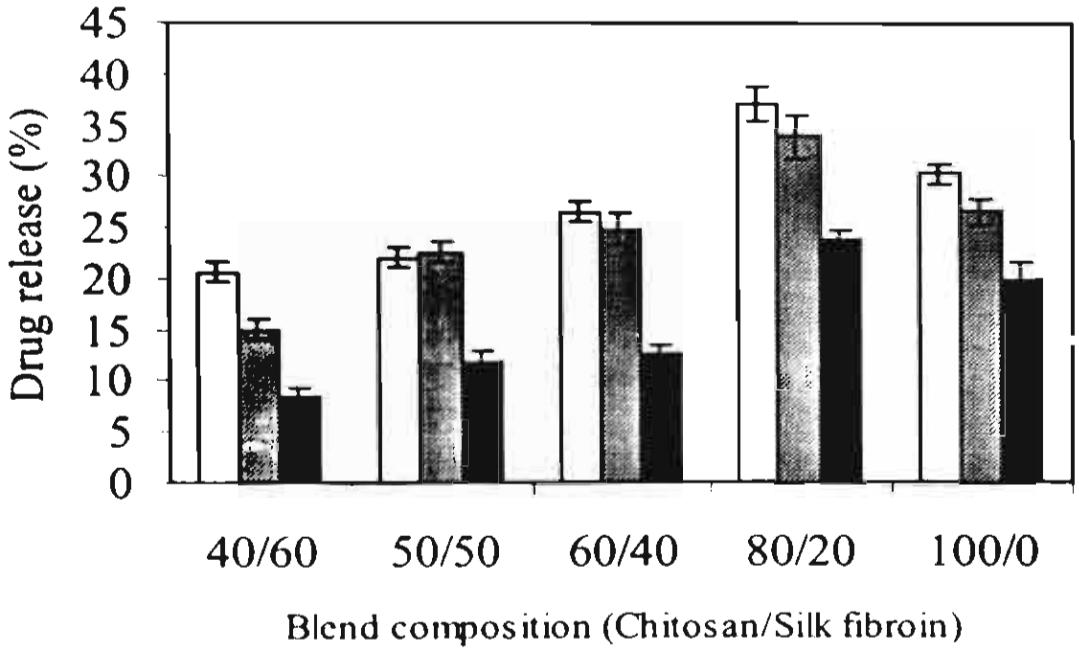
Figure 3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Blend composition (Chitosan/Silk fibroin)

Figure 4

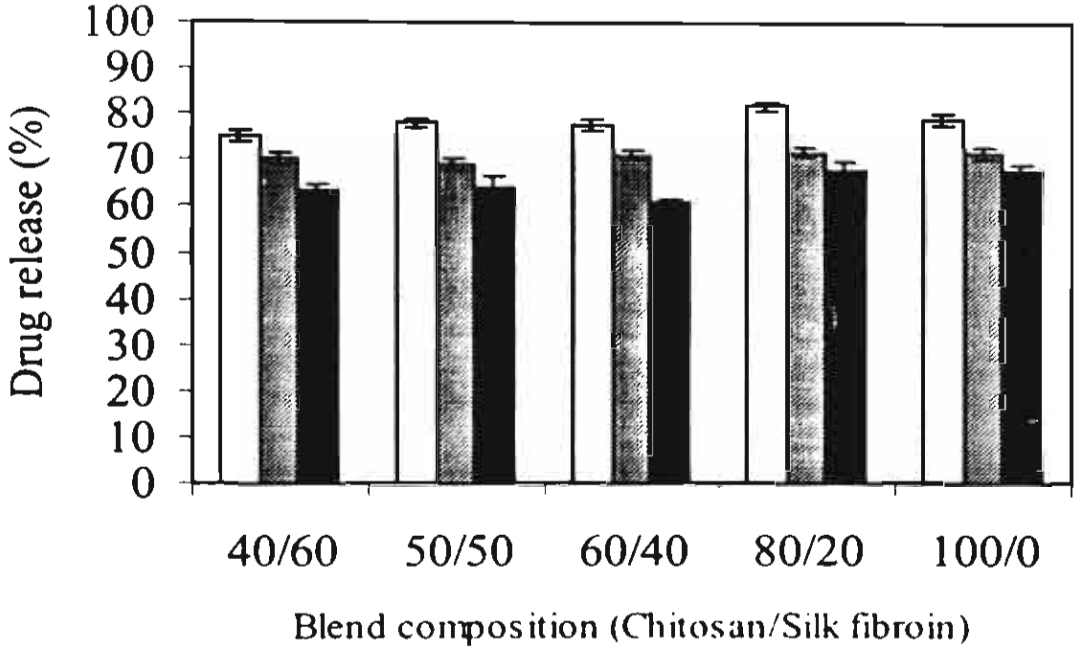
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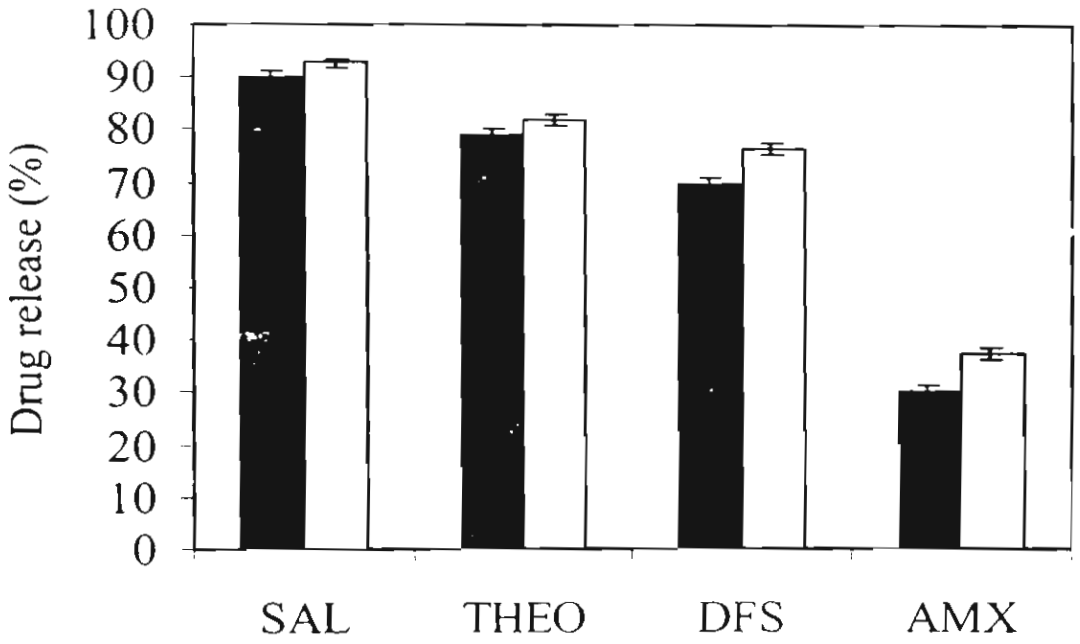
Figure 5



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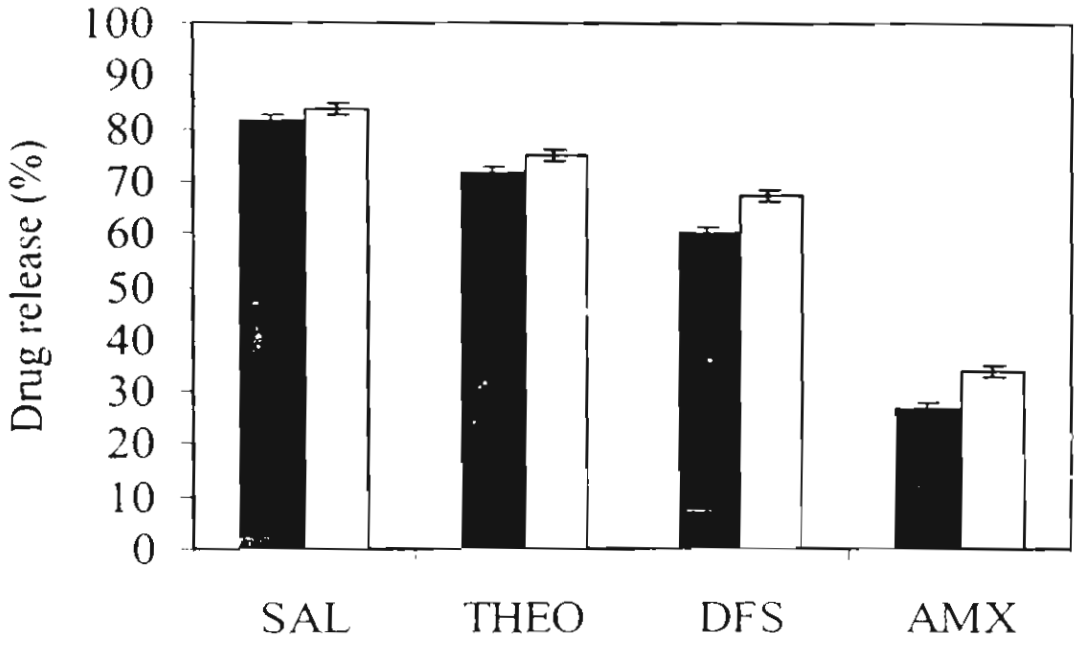

Figure 7

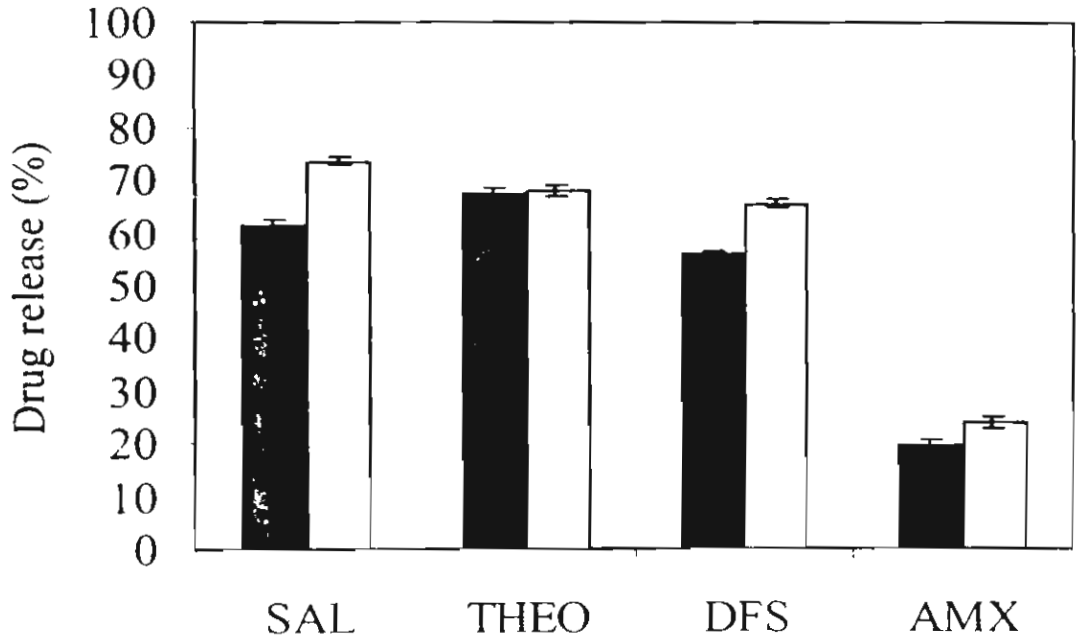

Figure 8

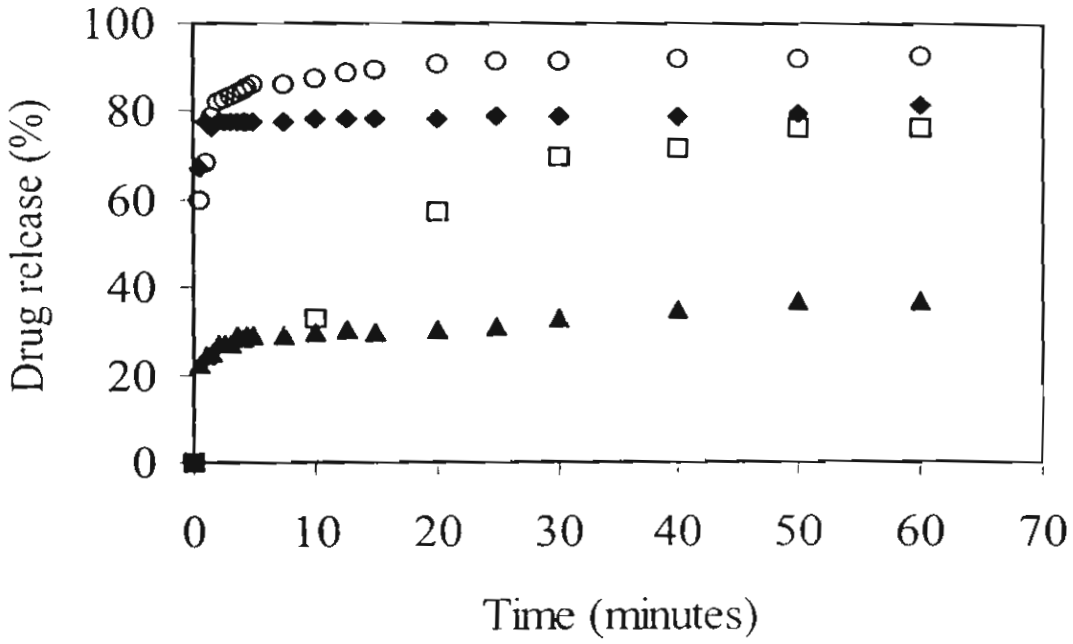

Figure 9

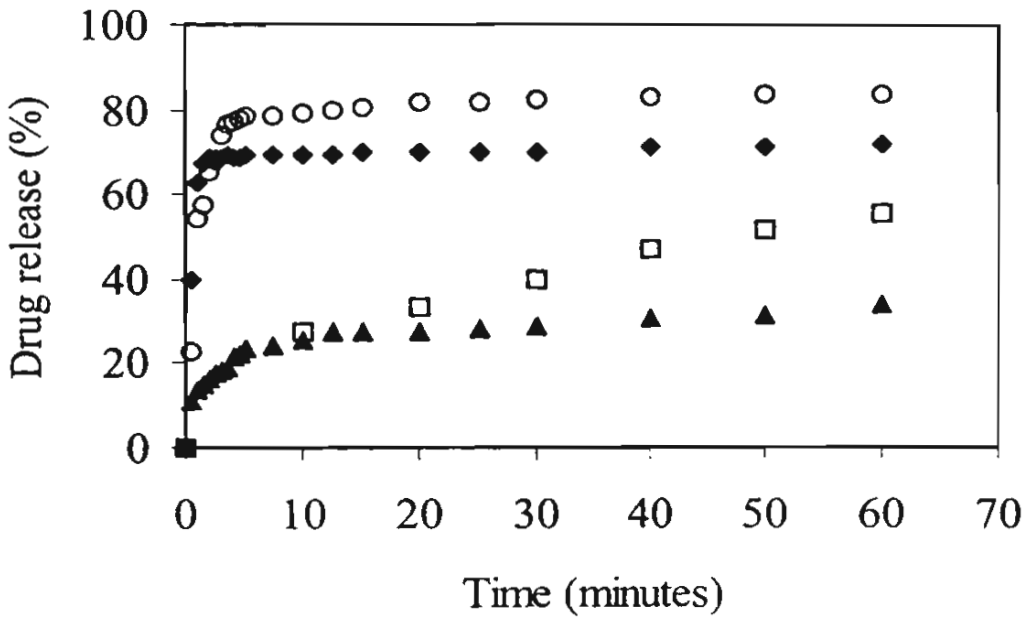

Figure 10

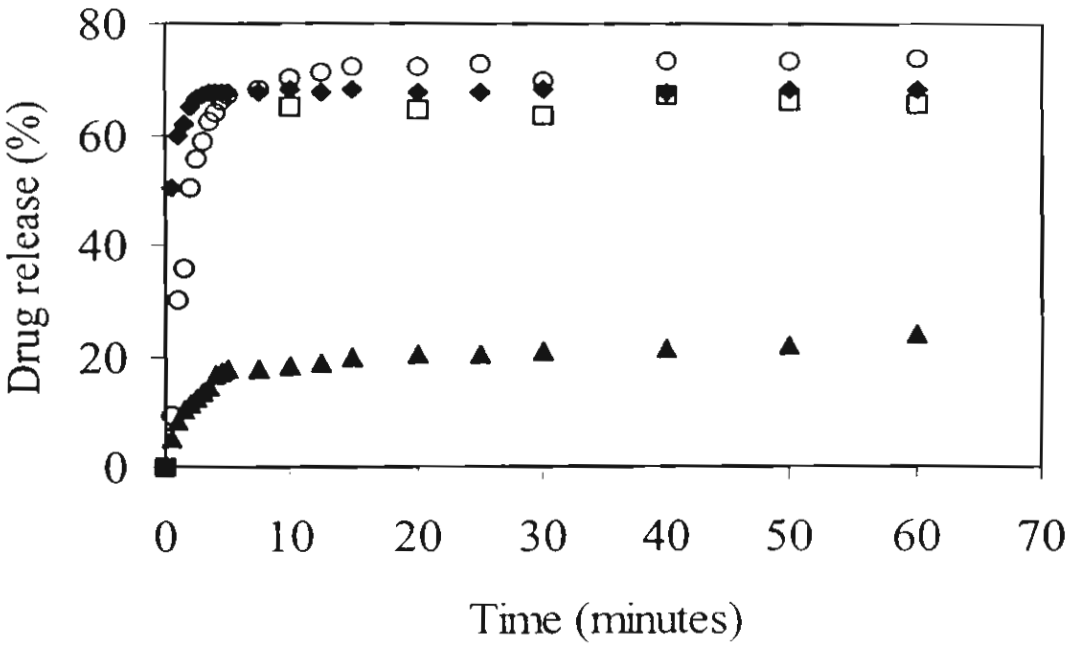

Figure 11

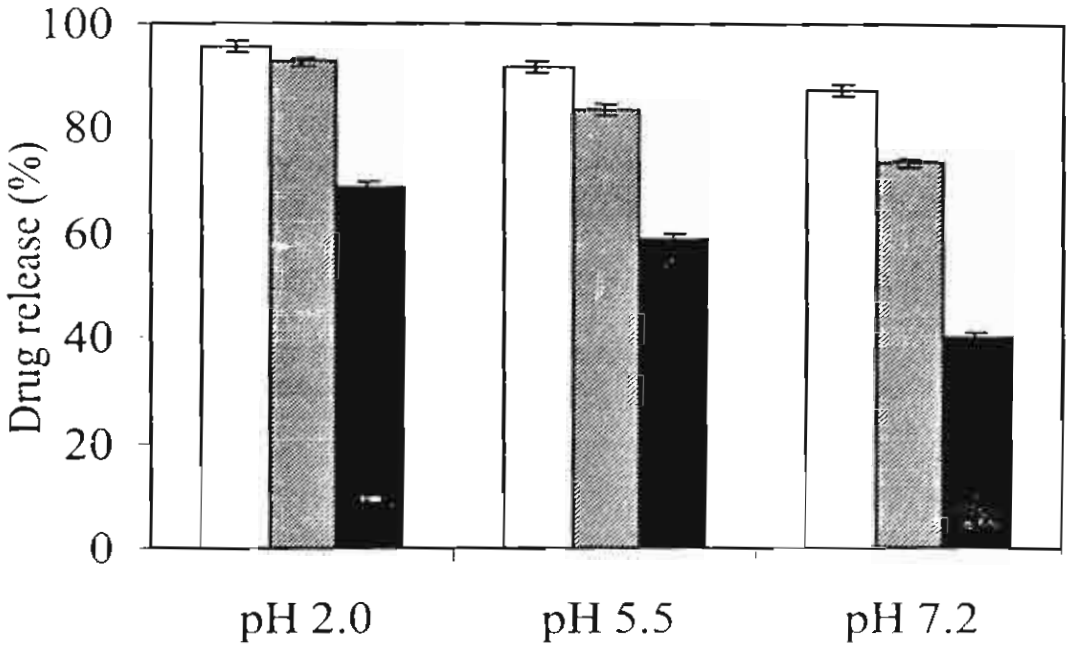

Figure 12

Table 1

Drug	Weight Ratio of Chitosan to Silk Fibroin	Degree of Swelling (%)[a]			Weight Loss (%)[a]		
		pH 2.0	pH 5.5	pH 7.2	pH 2.0	pH 5.5	pH 7.2
Salicylic acid	100:0	840	662	185	34.20	24.27	17.60
	80:20	1152	974	199	39.50	27.81	24.32
	60:40	763	535	179	35.20	24.21	15.40
	50:50	539	465	173	28.10	20.04	14.20
	40:60	582	471	169	23.70	19.86	13.80
Amoxicillin	100:0	643	492	194	20.38	19.98	17.73
	80:20	736	587	197	28.25	27.24	18.67
	60:40	639	472	168	22.30	20.75	18.25
	50:50	553	431	145	19.93	18.50	19.10
	40:60	504	409	129	18.10	17.63	17.98
Diclofenac sodium	100:0	753	653	167	35.43	30.07	28.60
	80:20	809	721	173	37.85	35.18	28.80
	60:40	783	600	125	30.53	30.01	23.40
	50:50	722	534	108	29.93	28.56	20.60
	40:60	687	497	102	21.20	20.01	17.50
Theophylline	100:0	306	252	167	38.87	35.54	26.14
	80:20	376	325	175	40.05	37.75	27.22
	60:40	360	289	159	36.67	32.23	23.44
	50:50	303	277	151	33.32	28.87	20.50
	40:60	232	221	142	30.01	25.34	18.70

[a] the average value from three experiments

Table 2

pH	Degree of Swelling (%)[a]			Weight Loss (%)[a]		
	Glutaraldehyde concentration (mole/glucosamine unit)			Glutaraldehyde concentration (mole/glucosamine unit)		
	0.001	0.01	0.05	0.001	0.01	0.05
2.0	1453	1152	825	42.21	39.50	32.13
5.5	1213	974	672	33.16	27.81	22.81
7.2	320	199	123	25.19	24.32	19.25

[a] the average value from three experiments

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Advances in Chitin Science

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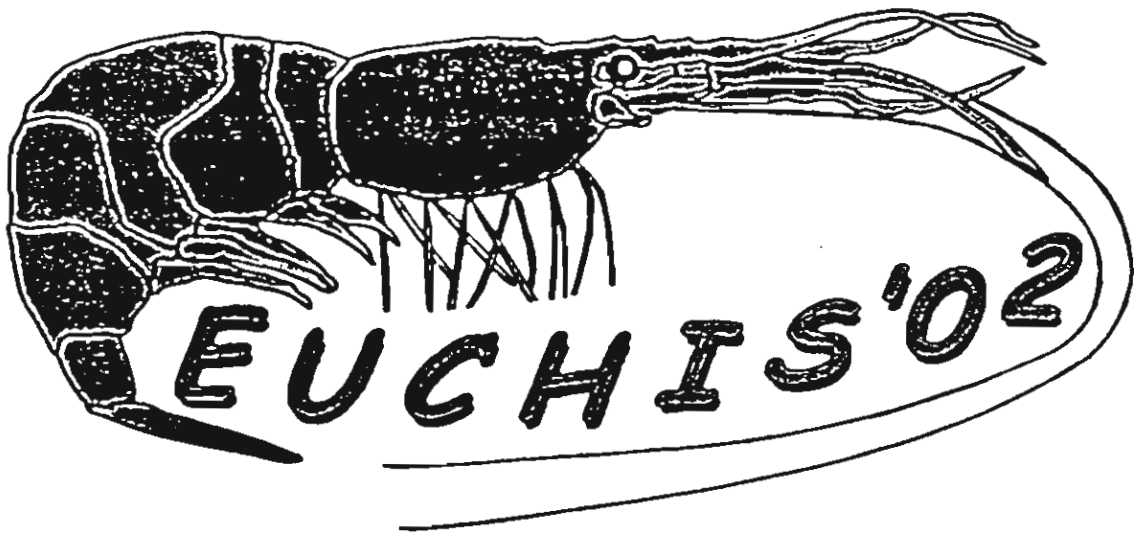

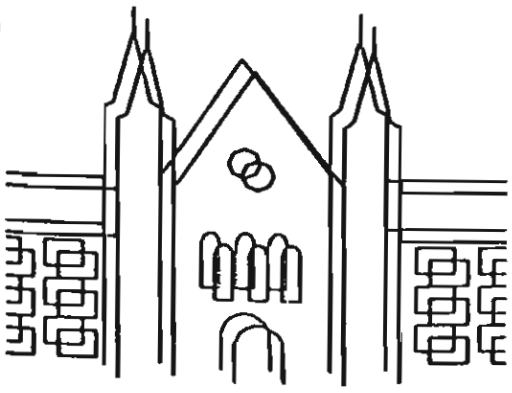

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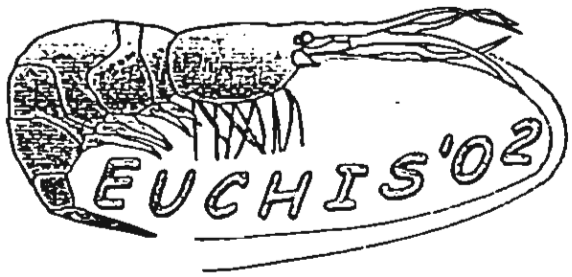

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PREPARATION AND CHARACTERIZATION OF CM-CHITIN/PVA BLEND FILMS

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Keywords: CM-chtin, Poly(vinyl alcohol), Swelling behavior

INTRODUCTION

Carboxymethyl-chitin (CM-chitin) is a water-soluble derivative of chitin. Poly(vinyl alcohol) (PVA) is a nontoxic water-soluble synthetic polymer. In this study, CM-chitin/PVA blend films were prepared by varying blend compositions of CM-chitin and PVA. The effect of blend composition, pH and salt type on the degree of swelling of the blend films were investigated.

EXPERIMENTAL

The blend films of PVA and CM-chitin were prepared by mixing various ratios of 1% by weight of PVA solution and 1% by weight of CM-chitin solution. For crosslinked CM-chitin/PVA blend films, glutaraldehyde was added into the solution to achieve the concentration of 0.01% before casting onto the clean dry plastic plates. The films were allowed to dry at 40°C in an oven for 24 h.

The blend films were cut into the disk form with diameter of 16 mm and 25-30 μm in thickness. The weights of the completely dried samples were measured, and the samples were dipped into a vial filled with different pH buffer solutions and different salt solutions (LiCl, NaCl, $CaCl_2$ and $FeCl_3$) with the concentration of 0.25 M at room temperature. The degrees of swelling of these samples were calculated.

RESULTS AND DISCUSSION

Effect of pH on Swelling Behavior of the Blend Films

The effect of pH on the degree of swelling of CM-chitin/PVA blend films with various blend compositions is shown in Figure 1. The degrees of swelling of PVA films were rather constant for the whole pH range from pH 3 to 11 due to pH stability of PVA. On the other hand, the degree of swelling of CM-chitin films and the blend films increased substantially in both acidic and alkaline pH ranges. The pK_a of the carboxymethyl group and amino group are 3.4 and 6.4, respectively [1]. The reason to explain the effect of pH on the degree of swelling of CM-chitin films and the blend films is that in acidic pH solutions, the amine groups of CM-chitin are ionized leading to the dissociation of the adjacent chains [2]. For alkaline pH solutions, the effect of pH on the degree of swelling of CM-chitin films increased because of the presence of the carboxymethyl groups that are ionizable functional groups of CM-chitin. It could say that the CM-chitin/PVA blend films showed the pH sensitive property.

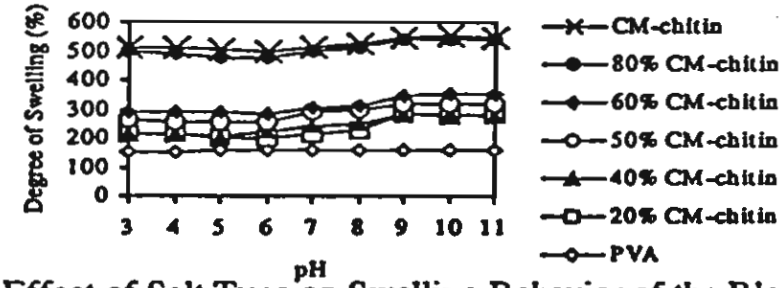

Figure 1
Degree of swelling of CM-chitin/PVA blend films with addition of 0.01% glutaraldehyde as a function of pH.

Effect of Salt Type on Swelling Behavior of the Blend Films

The degrees of swelling of pure and the blend films in various types of salt solutions are shown in Figure 2. It was found that, for all salt solutions, the degrees of swelling of the blend films increased as CM-chitin content increased, especially, when CM-chitin content were higher than 40%. However, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solutions (NaCl and LiCl). For $CaCl_2$ solution, Tokura and coworkers (1983) found that CM-chitin can bind calcium ions even in the presence of monovalent cations [1]. Watanabe and coworkers (1992) reported that the addition of iron (III) chloride into CM-chitin solution induces gel formation. This indicated that CM-chitin can also bind with Fe^{3+} [3].

For PVA films and the blend films with CM-chitin content less than 40%, the effect of different salt types on the change in degree of swelling of the blend films was very small. From Figure 2, it was observed that pure PVA films had the lowest degree of swelling. By the addition of CM-chitin to PVA films, the degree of swelling of PVA in salt soutions could be enhanced.

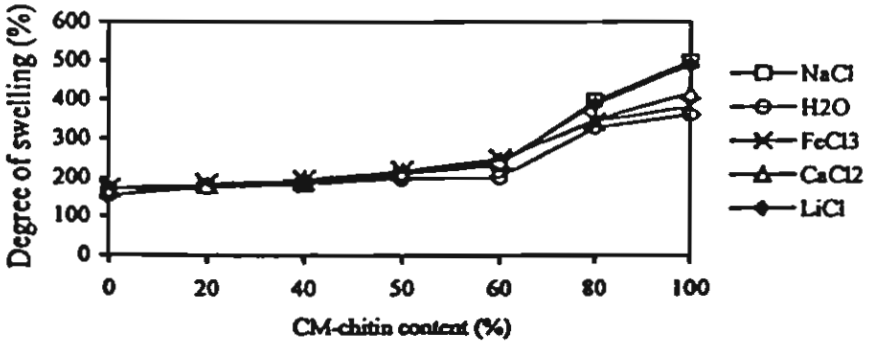

Figure 2
Effect of salt type on degree of swelling of chitin/PVA blend films with the addition of 0.01% glutaraldehyde function of CM-chitin content.

CONCLUSION

The blend compositions of CM-chitin/PVA blend films had a large effect on the swelling behavior of the blend films. The swelling behavior of CM-chitin/PVA blend films varied with the respect to changes in pH and salt type, indicating that the blend films had pH- and salt-responsive properties.

ACKNOWLEDGEMENT

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DRUG-RELEASED CHARACTERISTICS OF CROSS-LINKED CHITOSAN/SILK FIBROIN BLEND FILMS

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ABSTRACT

Crosslinked chitosan/silk fibroin blend films were prepared by solution casting technique using glutaraldehyde as a crosslinking agent. Drug-released characteristics of the blend films with various blend compositions were investigated. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid were used as model drugs. The release studies were carried out at 37°C in buffer solutions pH 2.0, pH 5.5 and pH 7.2. It was found that the blend films with 80% chitosan content showed the maximum amounts of model drugs released at pH 2.0 for all types of drugs. This result corresponded to swelling ability of the blend films. From swelling study, the maximum degrees of swelling of the drug-loaded blend films were obtained at this pH and this blend composition. The amounts of drugs released from the films with 80% chitosan content from the highest to the lowest values obtained in the following sequence: salicylic acid > theophylline> diclofenac sodium > amoxicillin. This could be due to the effects of molecular sizes of drugs, solubility of drugs in the blend solutions and interaction between drugs and polymer matrix.

INTRODUCTION

Nowadays, natural polymers such as protein and polysaccharide have become more and more important for their rich resources and low cost. Especially, they are useful materials in biomedical areas due to their non-toxicity, biodegradability, and biocompatibility. Silk fibroin is a fibrous protein that is composed of 17 amino acids and its main components are nonpolar ones such as glycine, alanine, and serine [1]. Silk fibroin can exist in two general conformations, random coil and β-sheet form. The conformation transition of silk fibroin can be induced to be changed from random coil to β-sheet structure by treatments such as heating, stretching or immersion in polar solvents [2]. This transition makes silk fibroin attractive as a biomaterial because silk fibroin with a β-sheet structure is resistant to water and has good mechanical properties [3]. Silk fibroin is considered to be an interesting starting material for developing new materials and devices for biotechnological and biomedical utilizations. It has been reported that silk fibroin film has good oxygen permeability in wet state, which suggests promising applications of silk fibroin as a wound dressing and artificial skin. In addition, silk fibroin can be utilized as surgical sutures [4], biocompatible devices with controlled drug release [5] and bone binding functions [6]. However, silk fibroin in dry state is very brittle and unsuitable for practical uses [7]. To overcome this limitation, silk fibroin has been reported to be blended with other synthetic polymers, such as polyacrylamide [8] and poly (vinyl alcohol) [9], or natural polymers, such as cellulose [7] and sodium alginate [10], to improve mechanical and physical properties.

Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of β(1→4)-linked 2-acetamido-D-glucose unit and

β(1→4)-linked 2-amino-D-glucose unit with the latter usually greater than 75%. Chitosan is one of a few natural cationic polyelectrolytes. It is known that chitosan can form a hydrogel, which is a three-dimensional crosslinked polymeric material with the ability to absorb significant amount of water. Crosslinked chitosan hydrogels can swell extensively due to the positive charges on the network and response to changes in pH of medium. Due to the benefits of being non-toxic, biocompatible and biodegradable, chitosan is known to be an excellent material for drug preparation. It has been studied as a unique vehicle for the sustained delivery of drug. For example, it was investigated for the delivery of drugs such as prednisolone [12] and diclofenac sodium [13]. Furthermore, it has been reported that chitosan could induce a conformational transition of silk fibroin from random coil to β-sheet structure [3] and a polymer blend of these biopolymers could also form a hydrogel having a semi-interpenetrating polymer network by using glutaraldehyde as a crosslinking agent [14]. Up to now, there are no reports on using crosslinked chitosan/silk fibroin blend film as drug delivery device. This research is a preliminary study on using cross-linked chitosan/silk fibroin blend film as a matrix for drug delivery system. The model drugs used were theophylline, diclofenac sodium, amoxicillin trihydrate, and salicylic acid. The effects of blend composition, degree of crosslinking, and pHs of the external swelling media on model drugs released from the blend films were investigated.

EXPERIMENTAL

Materials

Shrimp shell was kindly provided by Suraphol Food Public Co., Ltd. Silk fiber (*Bombyx mori*) was degummed by treatment with 0.5% Na_2CO_3 at 100°C for 30 min, followed by washing with boiling distilled water. The degummed silk was dried at 60°C for 24 h in an oven. Afterwards, the silk fibroin was dissolved in triad solvent $CaCl_2$: EtOH: H_2O with mole ratio of 1:2:8 at 100°C for 15 min. The silk solution was then dialyzed against distilled water for 7 days. The solution was filtered through the sintered glass filter and subsequently diluted to achieve a concentration of 1% w/w.

Preparation of chitin

Chitin was prepared from shrimp shell by decalcification and deprotenization to remove calcium carbonate and protein, respectively. The shrimp shell was cleaned and dried under sunlight before grinding into small pieces. The shrimp shell chips were treated by immersing in 1 N HCl solution for 2 days with occasional stirring. The decalcified product was washed with distilled water until neutral. Deprotenization was followed by boiling in 4% w/w of NaOH solution at 80-90°C for 4 h. After NaOH solution was decanted, the chips were washed with deionized water until neutral. The product obtained was dried at 60°C for 24 h.

Preparation of chitosan

Chitin was deacetylated by heating in NaOH 50% w/w solution with sodium borohydride ($NaBH_4$) 0.5% w/w based on the weight of chitin to prevent depolymerization. The ratio of chitin to NaOH solution was 1 g of chitin in 10 ml of NaOH solution. The deacetylation was performed in an autoclave at 110°C for 1 h. The deacetylated product obtained was washed exhaustedly with deionized water until neutral. The resulting chitosan flakes was dried in an oven at 60°C for 24 h.

Preparation of Chitosan Solution

Chitosan flake was dried at 110°C for 1 h before use. Chitosan solution was prepared by dissolution of chitosan in 1% w/w of acetic acid solution. The chitosan solution was allowed to stand overnight at room temperature to reduce of air bubbles before preparation of films.

Preparation of crosslinked drug-loaded blend films.

The blend solutions of chitosan and silk fibroin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of chitosan solution. Glutaraldehyde, used as crosslinking agent, was added into the blend solutions at the amount of 0.01 mole/glucosamine unit of chitosan. The model drugs (theophylline, diclofenac sodium, salicylic acid and amoxicillin trihydrate) were added into the blend solutions to achieve a concentration of 0.1% w/w. The blend solution containing a model drug was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry perti dishes in a dust-free atmosphere at room temperature. The films were allowed to dry at ambient temperature for 72 h and then stored over silica in a desiccator before use.

Drug Release Studies

To study the release characteristics of the model drugs from the films, drug-loaded blend films were immersed in buffer solutions at pH 2.0, pH 5.5 and pH 7.2. At a time interval, 1-mL aliquots were withdrawn and assayed for the amount of drug released. Theophylline, diclofenac sodium, amoxicillin trihydrate and salicylic acid released in the solutions were determined using an UV-Visible spectrophotometer (Perkin Elmer, Lambda 10) at 272, 275, 272, and 299 nm, respectively. The experiments were done in triplicate. The percentages of released drugs were calculated from calibration curves.

RESULTS AND DISCUSSION

Effect of blend composition on drug release

The effect of blend composition on drug release is shown in Figure 1. Silk fibroin contents of 0, 20, 40, 50 and 60% in drug-loaded blend films were used in this study. The blend films with silk fibroin contents higher than 60% were not reported because the films were brittle and difficult to handle without cracking. It was found that the maximum release of drug was observed for the blend film with 80% chitosan content for all types of model drugs. This could be explained by the term of swelling behavior of the blend films. It was found that the blend film with 80% chitosan content showed the maximum degree of swelling (Table 1). Peppas *et al.* [15] suggested that hydrogel delivery system was controlled by swelling behavior of hydrogel. Risbud *et al.* [16] concluded that the release of amoxicillin from the air-dried and freeze-dried chitosan/poly(vinyl pyrrolidone) hydrogels was related to the degree of swelling of the hydrogels. Furthermore, Yao *et al.* [17,18] studied the release of chlorhexidini acetas and cimetidine from chitosan/polyether semi-interpenetrating hydrogel. They found that the higher degrees of swelling, the higher amounts of drug released. Chen *et al.* [14] reported that the maximum degree of swelling of the blend films was observed for chitosan/silk fibroin blend film with 80% chitosan content. The swelling of chitosan/silk fibroin blend films may be occurred due to the dissociation between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan. However, the lower amounts of released drug were obtained when silk fibroin content in the drug-loaded blend films was increased. Suesat *et al.* [19] reported that there was no change in the degree of swelling of pure silk fibroin film immersed in buffer solutions for the whole pH range from pH 3 to pH 11. Therefore, swelling ability of the blend films depended on the amounts of chitosan content in the blend films.

Effect of pH on drug release

The effect of pH on drug released from chitosan and blend films is shown in Figure 1. Drug release profile was studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the amount of drug released from the systems was highest at pH 2.0 for all types of model drugs. This is in good agreement with the result of swelling as shown in Table 1. It appeared that the degree of swelling was the highest at pH 2.0 and tended to decrease when

pH of swelling solution was increased. This result corresponded to the previous works [14,19], which also reported that the degree of swelling of the crosslinked chitosan/silk fibroin blend films was maximum at pH 2.0 and decreased when pH of the swelling solution was increased. It can be explained by the fact that in an acidic medium the amino groups of chitosan were protonized, resulting that the hydrogen bonds between chitosan and silk fibroin were broken and the network was dissociated [14]. The blend films exhibited lower degree of swelling when pH was higher than 5. This may be due to the number of protonated amino groups of chitosan become lower at neutral and alkaline pH. The pK_a of chitosan is 6.3-6.5, which indicates that chitosan tends to protonate in acidic solution[20]. Therefore, the degree of swelling of the blend films in alkaline solution was very low as compared to that of the blend films in acidic solution. Risbud *et al.* [16] reported that the degrees of swelling of chitosan/poly(vinyl pyrrolidone) hydrogels were high in acidic solutions (pH 1.0, pH 2.0 and pH 3.0) and became lower in neutral and alkaline solutions (pH 7.2 and pH 9.2). The release of amoxicillin was found to be maximum at pH 1.0. Besides the release of drug is controlled by swelling condition of the carrier, drug release may be concerned with the erosion process. This process is associated with macroscopic changes in the appearance of the device, changes in the pysicomechanical properties of the polymeric material, deformation or structural disintegration, weight loss, and the eventual loss of functions [21]. Table 1 shows the weight loss of chitosan and blend films. It was found that the weight loss of the films was highest at pH 2. This indicated that drug release by erosion process could be occurred in this system.

Effect of drug types on drug release

The effect of drug molecules on drug release is shown in Figure 2. The releases of model drugs, theophylline, salicylic acid, diclofenac sodium and amoxicillin, were studied at pH 2.0, pH 5.5 and pH 7.2. It was found that the blend film at 80% chitosan content gave the highest amount of released drugs. The amounts of released salicylic acid at pH 2.0, pH 5.5 and pH 7.2 from blend film with 80% chitosan content were 92.7%, 83.4% and 73.5%, respectively. The amounts of theophylline released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan were 81.1%, 73.6% and 69.0%, respectively. The maximum amount of released salicylic acid at equilibrium was higher than that of theophylline. One factor that can affect the penetration of a drug from a polymer matrix is the molecular size of the drug. The molecule of salicylic acid (MW = 138.12) was smaller than theophylline (MW = 180.16). Thus, the penetration of salicylic acid from the matrix was easier than theophylline. Diclofenac sodium released at pH 2.0, pH 5.5 and pH 7.2 from the blend film with 80% chitosan content were 76.6%, 66.1% and 65.1%, respectively. The amount of diclofenac sodium released was less than those of theophylline and salicylic acid because diclofenac sodium did not dissolve in the blend solutions and appeared in the blend films as solid particles. Therefore, the diffusion of diclofenac sodium to the solution took longer time than salicylic acid and theophylline.

Among the drugs investigated in this study, the amounts of amoxicillin released from the blend films was the least values for all pH studied. It was found that the amount of amoxicillin released at pH 2.0, pH 5.5 and pH 7.2 were 37.2%, 34.0% and 23.5%, respectively. This may be due to the interaction between the drug molecule and polymer matrix. Risbud *et al.* [16] reported the amoxicillin released from crosslinked chitosan-poly (vinyl pyrrolidone) air-dried hydrogel was about 31.68% and 27% at pH 1.0 and pH 2.0, respectively. They explained that the low amounts of drug released might be due to non-porous nature of the air-dried films.

CONCLUSION

Drug -released characteristics from crosslinked chitosan and its blend films with silk fibroin by using glutaraldehyde as a crosslinking agent was studied. Theophylline, salicylic acid, diclofenac sodium and amoxicillin were used as model drugs. The amount of drug released from the blend film with 80% chitosan content was the highest value corresponding to the highest swelling ability of the blend film with this blend ratio. The drug released in acidic pH media was higher than neutral or alkaline pH media because the protonation of the amino groups on chitosan at acidic pH resulting in the dissociation of hydrogen bond between chitosan and silk fibroin network. The maximum and the minimum of released drug were saliclylic acid and amoxicillin, respectively. The difference in the amounts of drug released may be due to the effect of the molecular size of drug molecules and interaction between drugs and polymer matrix. Finally, theophylline was the fastest release because no drug polymer interaction between theophylline and chitosan was observed.

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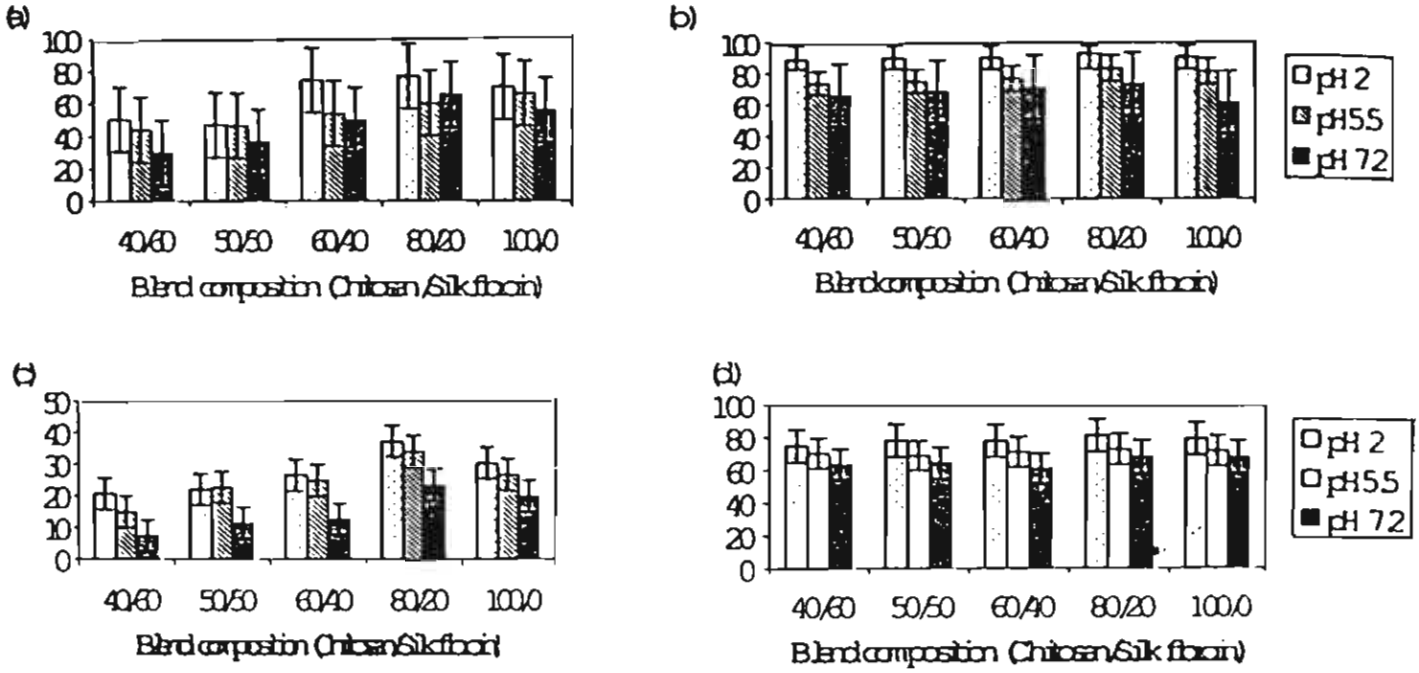

Figure 1 Effect of blend compositions on releasing of (a) diclofenac sodium, (b) salicylic acid, (c) amoxicillin and (d) theophylline at pH 2.0, pH 5.5 and pH 7.2 for 60 min.

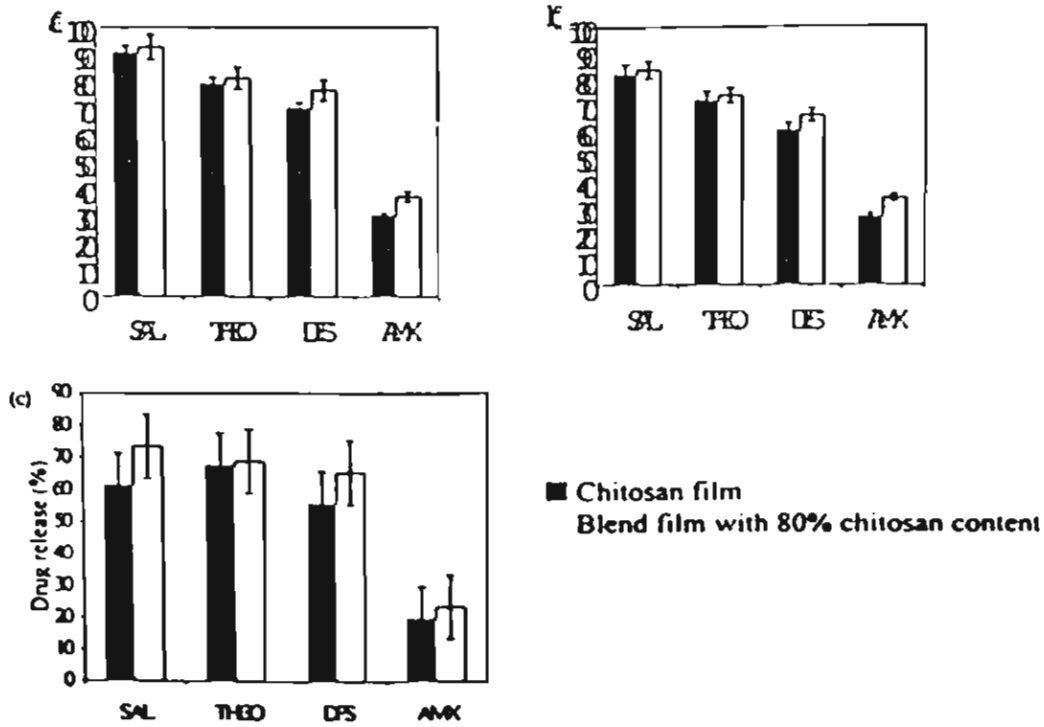

Figure 2 Comparison of the amounts of drugs released from chitosan and the blend film with 80% chitosan at (a) pH 2.0, (b) pH 5.5 and (c) pH 7.2. SAL= salicylic acid; THEO =theophylline; DFS = diclofenac sodium; and AMX = amoxicillin.

Table 1 Degree of swelling and % weight loss of drug- loaded crosslinked chitosan and blend films

Drug	Weight Ratio of Chitosan to Silk Fibroin	Degree of Swelling (%)[a]			Weight Loss (%)[a]		
		pH 2	pH5.5	pH 7.2	pH 2	pH5.5	pH 7.2
Salicylic acid	100:0	840	662	185	34.20	24.27	17.60
	80 : 20	1152	974	199	39.50	27.81	24.32
	60 : 40	763	535	179	35.20	24.21	15.4
	50 : 50	539	495	173	28.10	20.04	14.2
	40 : 60	582	471	169	23.70	19.86	13.8
Amoxicillin	100:0	643	492	194	20.38	19.98	17.73
	80 : 20	736	587	197	28.25	27.24	18.67
	60 : 40	639	472	168	22.30	20.75	18.25
	50 : 50	553	431	145	19.93	18.50	19.10
	40 : 60	504	409	129	18.10	17.63	17.98
Diclofenac sodium	100:0	753	653	167	35.43	30.07	28.6
	80 : 20	809	721	173	37.85	35.18	28.8
	60 : 40	783	600	125	30.53	30.01	23.4
	50 : 50	722	534	108	29.93	28.56	20.6
	40 : 60	687	497	102	21.20	20.01	17.5
Theophylline	100:0	306	252	167	38.87	35.54	26.14
	80 : 20	376	325	175	40.05	37.75	27.22
	60 : 40	360	289	159	36.67	32.23	23.44
	50 : 50	303	277	151	33.32	28.87	20.5
	40 : 60	232	221	142	30.01	25.34	18.7

[a] The average values from three experiments

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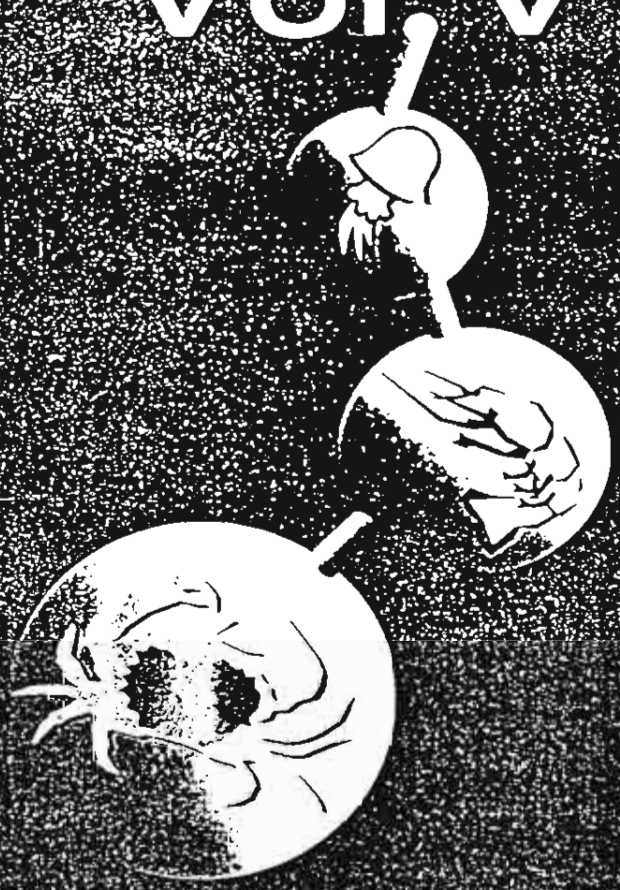
Vol. V



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PREPARATION AND CHARACTERIZATION OF CM-CHITIN/SILK FIBROIN BLEND FILMS

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ABSTRACT

Films of CM-chitin and silk fibroin were prepared with various ratios of CM-chitin to silk fibroin, with and without glutaraldehyde as a cross-linking agent. The effects of the ratios of CM-chitin to silk fibroin and cross-linking agent on swelling behavior of the blend films were studied. The structures and properties of the blends were characterized by FT-IR, X-ray diffraction, differential thermal analysis, and thermogravimetric analysis.

INTRODUCTION

Silk fibroin is a fibrous protein that is composed of 17 amino acids. The highly repetitive sections consist mainly of glycine, alanine and serine [1]. The sum of these repetitive amino acids is greater than 80 mol% of the total amino acid composition. The primary structure arising from this characteristic amino acid composition contains many $-(\text{-Gly-Ala-})_n-$ repeating unit. Silk fibroin can be prepared in the form of powder, gel and film from either silk fiber, after dissolution with concentrated salt solution, or liquid silk taken directly from the nature silk gland. Silk fibroin has become more important because of it properties, such as non-toxicity, biodegradability and good biological compatibility. It has been investigated for biomaterials in biotechnological and biomedical fields. For example, the glucose sensor prepared by using silk fibroin as enzyme substrate to immobilize glucose oxydase showed high stability against pH and temperature changes [2]. Silk fibroin has been studied for biomedical applications such as surgical sutures [3], wound covering materials [4], wound-repairing, and bone binding function [5]. However, silk fibroin is very brittle and almost unsuitable for practical use in the dry state [6]. Some inferior physical and mechanical properties of silk fibroin membranes can be improved by blending with other natural or synthetic polymers. Water absorption, mechanical properties, and thermal stability of silk fibroin films were improved by blending with sodium alginate [7]. The addition of cellulose to silk fibroin permitted preparation of films with excellent elastic behavior [8]. Silk fibroin/PVA blend films showed increased permeability to neutral salts [9].

Chitin is a natural abundant polysaccharide that is widely distributed in crustaceans, insects, mushrooms and in the cell walls of bacteria. Chitin consists of 2-acetamido-2-deoxy-D-glucose through a β $(1\rightarrow 4)$ linkage. Chitin has become attractive because of its rich resources and some interesting properties such as biocompatibility, biodegradability and non-toxicity. Chitin has been found to be useful as a biodegradable pharmaceutical carrier [10], a blood anticoagulant [11], and a wound-healing accelerator [12]. Chitin has also proved to be a highly effective antigen [13]. However, chitin has a limitation. It is know that chitin is insoluble in most common solvents except for strong acids such as methanesulfonic, sulfuric and formic acids. The insolubility of chitin has been suggested to be due to its rigid crystalline structure through intra-and inter-molecular hydrogen bonds [14]. This property can be improved by chemical modification of chitin. Chitin could be modified by introducing carboxymethyl groups to enhance its solubility in water.

Carboxymethyl-chitin (CM-chitin) is a water soluble chitin derivative. CM-chitin is soluble not only in acidic media but at any pH. CM-chitin has been investigated as polymeric drug [15], wound healing [16], cosmetic ingredients for hair and skin cares [17], and chelating agent [18]. However, silk fibroin and CM-chitin blend films still have not been investigated. In this study the effect of blend compositions on physical properties, as well as swelling behavior, of the blend films were investigated.

EXPERIMENTAL

Materials: Shrimp shell was kindly supplied from Surapol Food Public Co., Ltd. Chitin was prepared by the method of Shimahara and Takigushi [19]. The degree of deacetylation of chitin, determined by infrared spectroscopic measurement according to the method of Sannan *et al.* [20], was 31.30%. Chitin was powdered to size in the range of 71-75 μm before use.

Preparation of CM-chitin: CM-chitin was prepared by the method of Hirano [21]. Alkaline chitin was prepared by suspending powered chitin in 42% NaOH solution. After the suspension was allowed in desiccator for 30 min under reduced pressure, crushed ice was added and the mixture was mechanically stirred for 30 min in an ice bath to dissolve chitin. A viscous alkaline chitin solution was obtained. For successful synthesis of CM-chitin, the concentration of NaOH solution should not less than 14%. Monochloroacetic acid solution was prepared by dissolving in 14% NaOH solution in an ice bath and was added dropwise into the alkaline chitin solution with stirring over 30 min. After standing overnight at room temperature, the mixture was neutralized with acetic acid under cooling in an ice bath and dialyzed against running water for 2 days, followed by dialysis against distilled water for 1 day. The dialysate was centrifuged at 5000 rpm for 20 min, in order to remove insoluble material, and the supernatant was added to 3 volumes of acetone. After standing overnight, the precipitate was collected by centrifugation and washed with acetone. The product was resuspended in ethanol and collected by filtration. After drying at room temperature, CM-chitin Na salt was obtained. The degree of substitution of CM-chitin was 0.44 as estimated by elemental analysis (Perkin Elmer PE2400 Series II). The viscosity average molecular weight of CM-chitin was 7.70×10^4.

Preparation of silk fibroin solution: Raw silk fiber (*Bombyx mori*) was degummed by heating in 0.5% Na_2CO_3 solution at 100°C for 1 h followed by washing with boiling water and drying at 60°C for 24 h in an oven. Degummed silk fibroin 6 g was then dissolved in 94 g of 1:2:8 by mole of $CaCl_2$:EtOH:H_2O solvent system at 100°C for 15 min [22]. The resulting silk fibroin solution was filtered through the sintered glass filter and subsequently dialyzed against distilled water for 7 days. The dialyzed silk fibroin solution was filtered and diluted to achieve a concentration of 1% w/w.

Preparation of blend films: The blend films of silk fibroin and CM-chitin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of CM-chitin solution. The blend solution was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. For the crosslinked silk fibroin/CM-chitin blend films, glutaraldehyde used as crosslinking agent was added into the blend solution to achieve the concentration of 0.005%, 0.01%, and 0.05%.

Measurements: Infrared spectra of pure and blend films were measured by a Bruker FTIR spectrophotometer, model Vector 3.0, with 32 scans at a resolution of 4 cm^{-1}. Wide-angle X-ray diffraction patterns were recorded with an X-ray diffractometer (D/MAX-2000 series of Rigaku X-ray Diffractometer system). The X-ray source was Ni-filtered Cu Kα radiation (40 kV/30 mA). The films were scanned from 5 to 30 degree 2θ at speed of 5 degree/min. Differential scanning calorimetry (DSC) measurements were performed on a

Perkin Elmer DSC 7. A 5 mg of cut film was compressed and sealed in an aluminum pan. DSC curves of each film were obtained from the second heating run at a rate of 10°C/min, after the first run of heating up to 190°C and cooling to 25°C at the same rate of 10°C /min, under nitrogen atmosphere. A thermogravimetric analyzer (Dupont, model TGA 2950) was used to investigate the thermal stability of the blends with a heating rate of 10° C/min from 30°C to 600° C. The swelling behavior of the blend films was carried out by measuring the weight of the films after immersion in distilled water, buffer pH solution, and various salt solutions (i.e., 0.25 M solutions of LiCl, NaCl, $CaCl_2$ and $FeCl_3$) for 24 hours in comparison with the dry weight of the film before immersion. The blend films samples were 16 mm in diameter and 30-40 μm in thickness. The degree of swelling was determined according to the following equation:

$$\text{Degree of swelling (\%)} = [(Ws-Wd)/Ws] \times 100,$$

where *Ws* and *Wd* denote the weights of swollen and dry films, respectively.

RESULTS AND DISCUSSION

Characterization of CM-chitin/silk fibroin blend films

FTIR Analysis: The conformation characterization of pure and blend films, as well as the study of specific interactions between CM-chitin and silk fibroin were carried out. The FTIR spectrum of silk fibroin [Fig.1(a)] showed the characteristic absorption bands at 1654 cm^{-1} (amide I), 1542 cm^{-1} (amide II), 1243 cm^{-1} (amide III), and 669 cm^{-1}, (amide V), assigned to random coil form. The FTIR spectrum of CM-chitin [Fig.1(c)] showed the characteristic absorption bands at 1647 cm^{-1} (amide I), 1559 cm^{-1} (amide II). The spectrum of the blend film with 50% silk [Fig.1(b)] showed amide I band and amide II band at 1651 $cm^{-1}$and 1558 cm^{-1}, respectively. The peak shift between amide I and amide II of silk fibroin and CM-chitin was observed. This might suggest that there was some inter-molecular interactions between silk fibroin and CM-chitin.

X-ray Diffraction Patterns: Wide-angle X-ray diffraction (WAXD) patterns of the films are shown in Fig.2. CM-chitin exhibited crystalline peak at $2\theta = 9.4°$ and $2\theta = 19.3°$ [Fig.2 (a)]. The silk fibroin films showed a non-crystalline structure. According to Freddi *et al.* [8], the dissolution of silk fibroin was caused by reagent penetrating into the adjacent chains and breaking hydrogen bonds between polymer chains. This led to the decrease in crystallinity of silk fibroin films as compared to the original silk fibroin fiber. The pattern of the blend films exhibited a gradual transformation from characteristic crystalline peaks of CM-chitin to the completely amorphous pattern of silk fibroin with the increasing of silk fibroin content in the blend films. Chen *et al.* [22] studied the crystallinity of pure silk fibroin membrane and found that the diffraction pattern of pure silk fibroin membrane showed no clear 2θ peak. Our result showed that the diffraction patterns of pure and blend films did not give clear information about the crystallinity because the crystalline structure of CM-chitin and silk fibroin were remarkably frustrated during dissolution process. Therefore, the CM-chitin/silk fibroin blend films were mainly amorphous.

Thermal Property: Thermal property of pure and blend films were characterized by differential scanning calorimeter (DSC). The DSC thermograms of pure and blend films are shown in Fig.3. The pure silk fibroin film showed endothermic peak at 284.86°C [Fig.3(a)]. According to Tsukada *et al.* [9] who studied thermal property of silk fibroin/PVA blend films, it was demonstrated that silk fibroin with low degree of crystallinity thermally decomposed at around 280-290°C. Therefore, it suggested that the endotherms observed at 284.86°C should be attributed to the thermal decomposition of silk fibroin film. The pure CM-chitin film showed the glass transition temperature at 244.70°C [Fig.3(g)]. According to Sakurai *et al.* [23], the glass transition temperature of chitosan

was reported to be 203°C. The thermograms of blend films are showed in Fig.3(b-f). The DSC thermograms of the blend films with 40% and 50% CM-chitin content [Fig.3(c-d)] showed the broad peak of decomposition endotherm in lower temperature range and observed an upward shift of glass transition temperature of CM-chitin. The 60/40 and 80/20 CM-chitin/silk fibroin blends [Fig.3(e-f)] showed only the shift of glass transition temperature of CM-chitin. While at 20/80 CM-chitin/silk fibroin showed only the shift of decomposition endotherm. It could be suggested that there was some inter-molecular interactions occurred between CM-chitin/silk fibroin films.

Thermal stability: The decomposition temperatures of pure and blend films (Fig.4) were characterized by themogravimetric analysis (TGA). The TGA curves of pure silk fibroin and CM-chitin films showed the decomposition temperatures at 299.34°C and 265.70°C, respectively. The decomposition temperatures of the blend films were in the range of the decomposition temperatures of pure silk fibroin and pure CM-chitin. The result showed that the decomposition temperatures of the blend films decreased with increasing of CM-chitin content.

Swelling Study

Equilibrium Water Content (EWC)

The effect of immersion time on the water content of the blend films is shown in Fig.5. All samples in water reached an equilibrium state within 3 h. Fig.6 shows EWC of the films as a function of CM-chitin content. The EWC of pure CM-chitin film was approximately 545%. Pure silk fibroin could not detected because it was very brittle and cracked after water was blotted out from the film surface. The EWC of blend films increased with increasing CM-chitin content. Khor *et al.* [24] suggested that the ability to absorb water of CM-chitin films is attributed to the introduction of carboxymethyl group on the glucose residue. The presence of carboxymethyl groups distributing along chitin chains disrupts the H-bonging interactions between adjacent chitin chains.

Effect of pH

The effect of pH on the degree of swelling of CM-chitin and the blend films is shown in Fig.7. It was found that pure CM-chitin and blend films could swell in both acidic and alkaline pH. Tokura *et al.* [25] reported the pK_a values of the carboxyl group and amino group of CM-chitin are 3.40 and 6.40, respectively. At pH less than 7 (acidic pH solution), the degrees of swelling of CM-chitin and blend films slightly increased, suggesting that the amino groups of CM-chitin molecules ionized leading to the dissociation of the adjacent chains. At pH higher than 7 (alkaline pH solution), the degrees of swelling of CM-chitin and blend films increased, suggesting that the carboxymethyl groups ionized leading to the dissociation of the adjacent chains. The effect of glutaraldehyde concentration on the swelling property of the blend film at 50/50 blend ratio as a function of pH is shown in Fig.8. The degree of swelling decreased with increasing of glutaraldehyde concentration in the films. When the concentrations of cross-linking agent increased more hydroxyl groups in CM-chitin was consumed due to the cross-linking reaction. The response of the blend films with 50% CM-chitin content to a step change in pH is shown in Fig.9. In this experiment, the films were brought into a buffer solution at pH 6 and then transferred to a buffer solution at pH 10 so that an abrupt swelling was ensured. Later, the films were placed back into a buffer solution at pH 6. The result showed that swelling behavior of the blend films was reversible when the environmental pH changed. The blend films showed a pH-sensitive swelling characteristic that may be applicable to a controlled-release system.

Effect of salt type

The degree of swelling of CM-chitin/silk fibroin blend films in various types of salt solutions is shown in Fig.10. The salt solutions used in this study were NaCl, LiCl, $CaCl_2$

and $FeCl_3$ solution. The concentration of salt solution was 0.25M. It was found that the blend film with 60% CM-chitin content showed the highest degree of swelling in NaCl, LiCl, and $CaCl_2$ solution. However, the most increases in degree of swelling of the films were obtained for the films immersed in monovalent salt solution (NaCl and LiCl) and divalent salt solution ($CaCl_2$). For pure CM-chitin, the degrees of swelling in salt solutions from the highest to the lowest were in the following order: NaCl> LiCl> $CaCl_2$> $FeCl_3$. Tokura *et al.* (1983b) studied the specific binding site of calcium ions on CM-chitin (insoluble water typed, DS=0.28). It was found that CM-chitin could bind with calcium ions even in the presence of monovalent cations such as sodium or potassium. The IR spectrum between the CM-chitin and Ca^{2+}-loaded CM-chitin appeared that the tetrahedral chelation of CM-chitin toward Ca^{2+} is assisted by the acetamide and hydroxyl groups in addition to carboxyl groups.

CONCLUSIONS

Silk fibroin could enhance the thermal stability of CM-chitin. When CM-chitin content in the blend films increased, equilibrium water content and degree of swelling of the blend films in pH buffer and salt solution, especially in NaCl and LiCl, increased. The cross-linking was very important for the swelling behavior. The cross-linking agent concentration had influence to the swelling behavior of the blend film. When the concentrations of cross-linking increased, the degrees of swelling of the blend films decreased.

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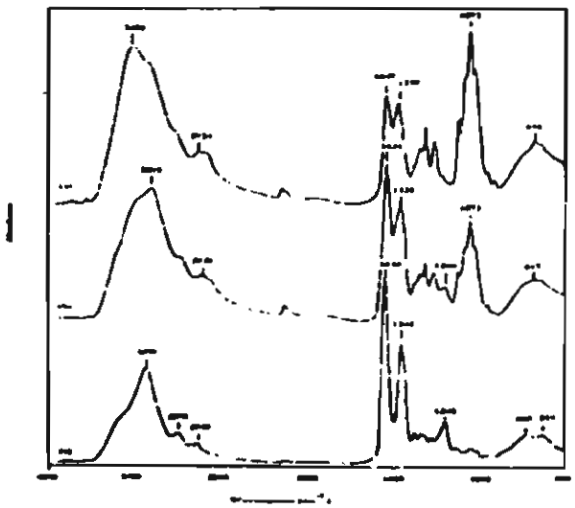

Fig.1. FTIR spectra of pure and blend films of CM-chitin/silk fibroin: (a) 100/0 (CM-chitin); (b) 50/50; and (c) 0/100 (silk fibroin).

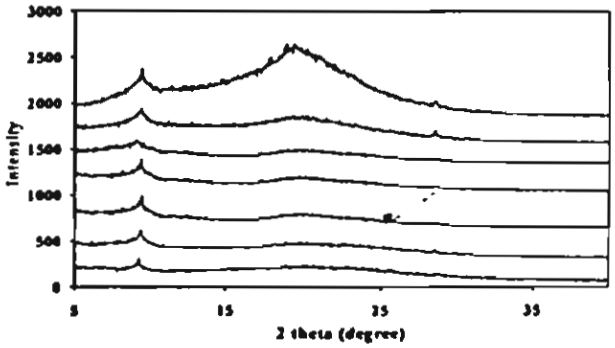

Fig.2. WAXD patterns of CM-chitin/silk fibroin blend films.

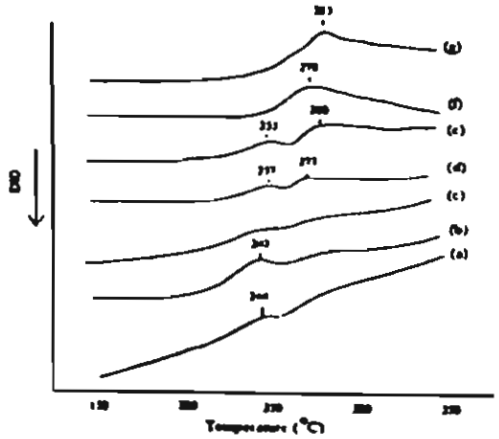

Fig.3. DSC thermograms of CM-chitin/silk fibroin blend films: (a) 100/0 (CM-chitin); (b) 80/20; (c) 60/40; (d) 50/50; (e) 40/60; (f) 20/80; and (g) 0/100 (silk fibroin).

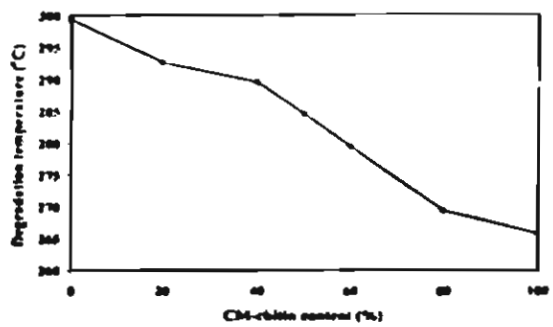

Fig.4. Decomposition temperature of CM-chitin/silk fibroin blend films.

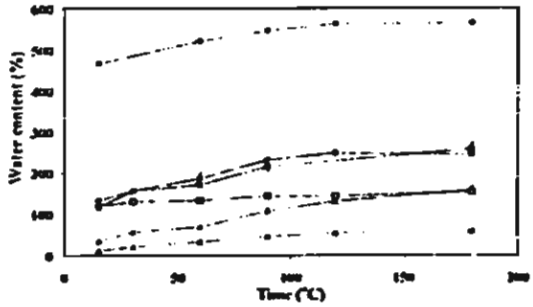

Fig.5. Effect of immersion time on water content of CM-chitin/silk fibroin blend films (containing 0.01% glutaraldehyde): ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; △ 20/80.Decomposition temperature of CM-chitin/silk fibroin blend films.

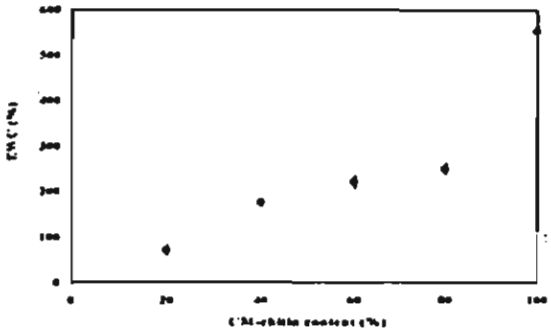

Fig.6. Equilibrium water content of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde.

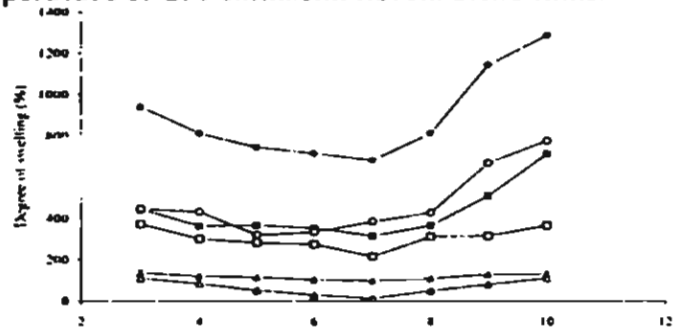

Fig.7. Degree of swelling of CM-chitin/silk fibroin blend films (containing 0.01% glutaraldehyde) as a function of pH: ● 100/0 (CM-chitin); ○ 80/20; ■ 60/40; □ 50/50; ▲ 40/60; △ 20/80.

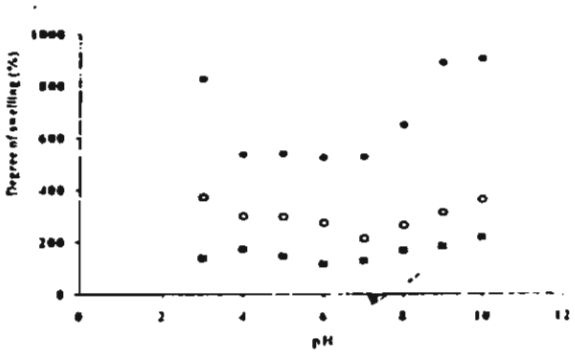

Fig.8. Effect of glutaraldehyde concentration on degree of swelling of CM-chitin/silk fibroin blends films as a function of pH. ● 0.005% glutaraldehyde; ○ 0.01% glutaraldehyde; ■ 0.05% glutaraldehyde.

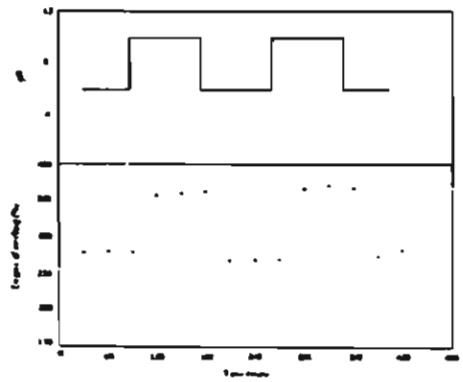

Fig.9. Degree of swelling of CM-chitn/silk fibroin blend films with 50/50 blend ratio containing 0.01% glutaraldehyde on a step change in pH.

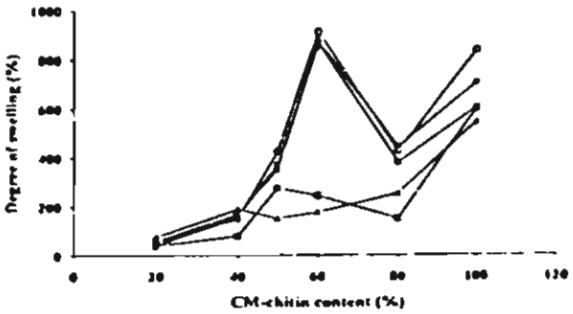

Fig.10. Effect of salt types on degree of swelling of CM-chitin/silk fibroin blend films containing 0.01% glutaraldehyde as a function of CM-chitin content. ● LiCl; ○ NaCl; ■ $CaCl_2$; □ $FeCl_3$; ▲ H_2O.

Preliminary Registration

Please send me further information regarding the 9th International Chitin-Chitosan Conference to be held in Montreal, (Quebec), Canada, August 27-30, 2003.

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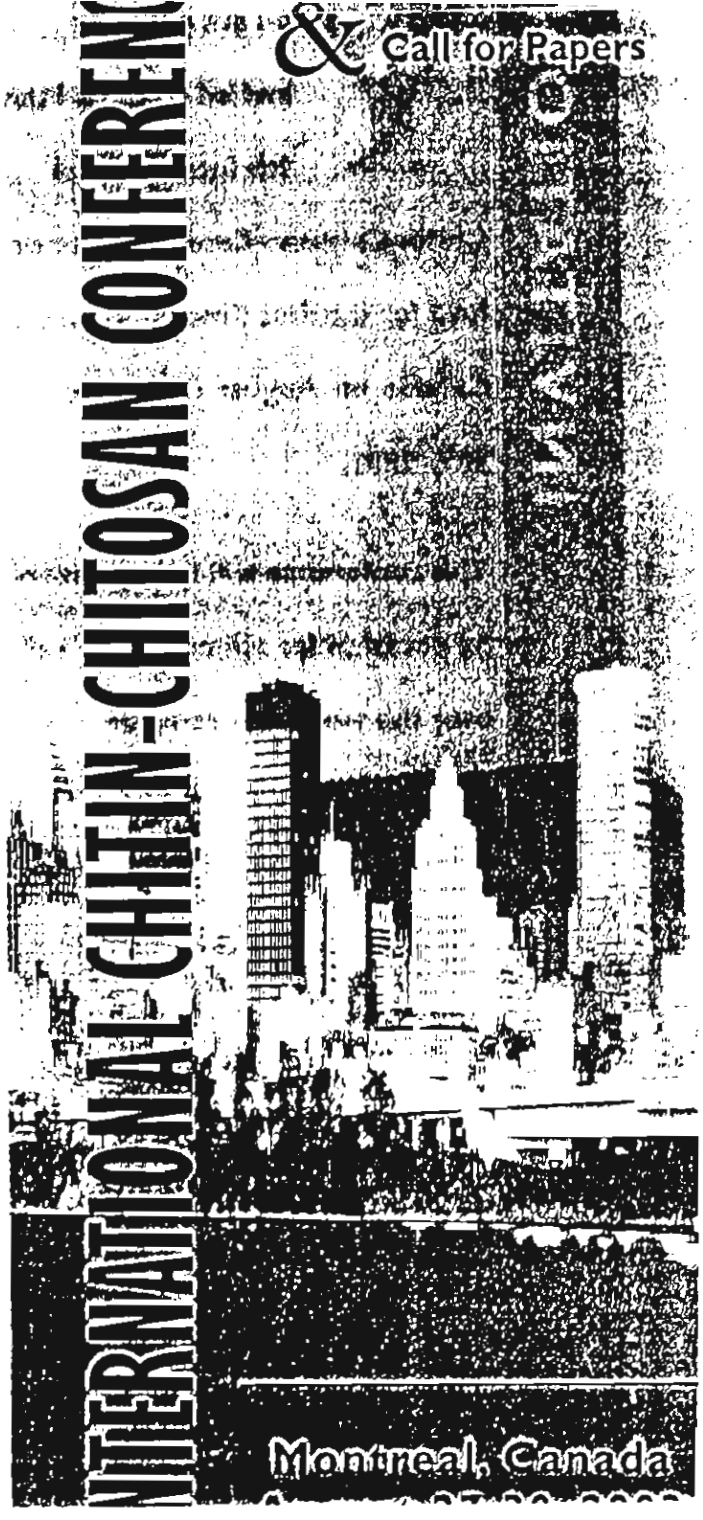

INVITATION

You are cordially invited to attend the 9th International Chitin-Chitosan Conference to be held for the first time in Montreal, Canada, on August 27th to the 30th, 2003.

This conference will be a wonderful occasion for scientists from all over the world to exchange information on the science and technology of Chitin and Chitosan.

THE OFFICIAL LANGUAGE OF THE CONFERENCE IS ENGLISH

Location and Accommodations

The conference will be held at the Renaissance Hotel, located in downtown Montreal. For your convenience, the Organizing Committee has also reserved a block of rooms for conference participants at the same location.

Scientific Program

The scientific program will cover the following tentative topics:

- Sources, production and biological aspects
- Chemical structure and modifications
- Enzymatic modifications
- Functional properties
- Applications in technology, food, medicine, cosmetics, nutraceutics and biotechnology

Social Programs

Social programs for participants, spouses and accompanying persons - during and after conferences - are planned in the beautiful city of Montreal.

Second Circular and Request for information

A second circular containing all the details concerning the scientific program, registration fees, hotel registration and social programming will be distributed to all interested participants by January 2003.

Conference Chairman

Prof. Ryszard Brzezinski

Heads of the Organizing Committee

Dr. Isabelle Boucher

Dr. Arthur Retnakaran

9th I.C.C.C.

Montreal 2003

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CONTACT FOR ADDITIONAL INFORMATION

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Dr. Arthur Rennakaran

9th I.C.C.C.

Sustained Release of Drug from Chitosan

R. Rujiravanit [a*], S. Limpapat [a], A. M. Jam

[a] Petroleum and Petrochemical College, Ch
[b] Department of Macromolecular Science,
[c] Faculty of Engineering, Kansai University
* **Corresponding and presenting author r**

Chitosan/silk fibroin blend films wer crosslinking agent. Drug release prope compositions were investigated *in vitro* usi Pig skin was used as material representing sodium and amoxicillin trihydrate were use release to the lowest release was as follow amoxicillin trihydrate. For all model drugs, release. In addition, an increase in thickne released. All model drug release data were the releases of model drug from chitosan a controlled releases. It was expected that th matrix for sustained release of a drug for a t

Key words: Chitosan; Silk Fibroin; Blend F
(Oral presentation; Application section)

Sustained Release of Drug from Chitosan and Silk Fibroin Blend Films

R. Rujiravanit [a*], S. Limpapat [a], A. M. Jamieson [b] and S. Tokura [c]

[a] Petroleum and Petrochemical College, Chulalongkorn University
[b] Department of Macromolecular Science, Case Western Reserve University
[c] Faculty of Engineering, Kansai University
[*] **Corresponding and presenting author ratana.r@chula.ac.th**

Chitosan/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. Drug release properties of chitosan and blend films of various blend compositions were investigated *in vitro* using a modified Franz Diffusion Cell at 37°C and pH 5.5. Pig skin was used as material representing human skin. Theophylline, salicylic acid, diclofenac sodium and amoxicillin trihydrate were used as model drugs. The order of drugs from the highest release to the lowest release was as follows: salicylic acid > theophylline > diclofenac sodium > amoxicillin trihydrate. For all model drugs, the blend films with 80% chitosan gave the higher drug release. In addition, an increase in thickness of the films resulted in a decrease in amount of drug released. All model drug release data were fitted to zero order or Higuchi's model indicating that the releases of model drug from chitosan and blend films were either rate-controlling or diffusion-controlled releases. It was expected that the chitosan/silk fibroin blend films could be used as the matrix for sustained release of a drug for a transdermal drug delivery system.

Key words: Chitosan; Silk Fibroin; Blend Films; Sustained Release; Drug Delivery System
(Oral presentation; Application section)

Drug Release Characteristics of CM-Chitin/Silk Fibroin Blend Films

R. Rujiravanit [a*], T. Rotchanarak [a] and S. Tokura [b]

[a] Petroleum and Petrochemical College, Chulalongkorn University
[b] Faculty of Engineering, Kansai University
[*] **Corresponding and presenting author ratana.r@chula.ac.th**

CM-chitin/silk fibroin blend films were prepared by solution casting using glutaraldehyde as crosslinking agent. The effects of pH and blend composition on swelling behavior of the blend films were investigated. CM-chitin and the blend films exhibited the minimum degree of swelling at pH 4 and showed pH-sensitive character for every blend composition studied. The degree of swelling of the blend films increased as the CM-chitin content increased. Drug release characteristics of CM-chitin and the blend films at 37 °C and simulated physiological pHs, i.e. pH 2.0, 5.5 and 7.2, were investigated using theophylline, diclofenac sodium, amoxicillin and salicylic acid as model drugs. It was found that the releases of all model drugs from CM-chitin and the blend films at pH 7.2 were higher than at pH 2.0 and pH 5.5. The amounts of model drugs released from the films from the highest to the lowest were in the order: salicylic acid > theophylline > diclofenac sodium > amoxicillin. The drug releasing property of CM-chitin/silk fibroin blend films was compared to that of CM-chitin/PVA blend films using salicylic acid as model drug. Both of them showed similar drug release characteristic. However, the percentages of salicylic acid released from CM-chitin/silk fibroin blend films were a little bit lower than those obtained from CM-chitin/PVA blend films.

Key words: CM-Chitin; Silk Fibroin; Blend Films; Drug Release
(Poster presentation; Application section)