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Moist heat treatment on physicochemical change of chitosan salt films

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Abstract

Chitosan salt films were prepared by casting method using acetic, citric, formic, glycolic, lactic, malic and propionic acids as solubilizers. The films were then exposed to moist heat at 60 °C and 75% relative humidity for several time intervals. The influence of moist heat treatment on their physicochemical characteristics was investigated. All freshly prepared films were soluble in deionized water and HCl buffer solution. Chitosan citrate film also dissolved in phosphate buffer solution. After treatment, the percentage of water sorption and dissolution of chitosan films in three media were gradually decreased. Longer alkyl group and less carboxyl and hydroxyl groups in the molecule of organic acid resulted in lower percentage of water sorption and dissolution of treated films. The FT-IR spectra revealed that there was amide formation between chitosan and organic acids after treatment especially in chitosan acetate and propionate films. However, the absorption peaks of ammonium [$'NH_3^+$] and free carboxylate groups were still remained in treated chitosan citrate and malate films. Change in the degree of crystallinity from powder X-ray diffractogram and thermal characteristic from DSC thermogram were also related to the water sorption and dissolution of films.

Keywords: Chitosan salt film; Moist heat treatment; Physicochemical change

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1. Introduction

Chitosan could be used as film former for coated pharmaceuticals (Hall and Wallace, 1998). Since it is soluble in dilute acidic medium, attempt to prepare in insoluble form is to expand its use in controlled release systems. Dry heat treatment to chitosan acetate film was reported to increase its water resistance which was attributed to the crosslinking of chitosan molecules and/or the formation of anhydrous crystalline in the structure (Lim and Wan, 1995). The coloration of chitosan salt film was intensified with the increasing temperature and duration of treatment. Moreover, conversion of chitosan acetate film to chitin film was reported by heating at 80–140 °C undergo-

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ing 'cure' process (Ogawa, 1991; Toffey et al., 1996) whereas stream sterilization increased the rate and extent of the thermal reactions (Rao and Sharma, 1995).

Since coloration affected the elegance and acceptance of pharmaceutical product, and curing process was practiced in film coating, mild condition of moist heat treatment was investigated in this study. Pharmaceutically accepted carboxylic acids of different alkyl, carboxyl and hydroxyl groups were used as solubilizer to prepare chitosan salt films. The physicochemical change of these films after moist heat treatment was investigated.

2. Materials and methods

2.1. Materials

Chitosan having M.W. 40,000 and from chitin of crab shell was purchased from G.T. Chemicals Co., Bangkok, Thailand. The *N*-deacetylation degree was 85.91 ± 0.75%, determined by colloidal titration method (Hayes, 1978). Chitosan flakes were passed through sieve No. 80 mesh before used. Carboxylic acids; acetic, citric, formic, glycolic, lactic, malic and propionic acid and other reagents were of analytical or food grade, and were used as received.

2.2. Methods

2.2.1. Preparation of chitosan films

Chitosan solutions of 5% w/w were prepared by dissolving chitosan powder in acid solutions for 14 h. The molar ratio of glucosamine unit of chitosan: acid was 1:1.2. After filtering the solutions through polyester cloth, the films were obtained by casting technique to have film thickness of about 80 μm. The obtained films were stored in a desiccator at ambient temperature for 48 h prior to further treatment. Dried chitosan films were then exposed to moist heat treatment of 60 °C and with 75% relative humidity at various time intervals.

2.2.2. Physicochemical characterization

2.2.2.1. Water sorption (WS) and dissolution (DS) of film. To determine the amount of water absorbed, 2×2 cm^2 films were carefully cut, weighed (W_1) and then immersed in deionized water, HCl buffer pH 1.2 and phosphate buffer pH 6.8 at 37 °C. After 24 h, the film remnants were wiped off excess surface water using filter paper and weighed (W_2). The swollen films were dried at 60 °C for 24 h and kept in a desiccator for 48 h prior to reweigh (W_3). The water sorption and the extent of dissolution were calculated using the following formula:

$$\mathrm{WS}(\%) = (W_2 - W_3)/W_3 \times 100 \quad (1)$$

$$\mathrm{DS}(\%) = (W_1 - W_3)/W_1 \times 100. \quad (2)$$

2.2.2.2. Fourier transform infrared (FT-IR) studies. The FT-IR spectra were recorded using an IR spectrometer (Perkin–Elmer 1760 ×, USA) by KBr disc method. Prior to measurement, the films were cut and then ground with a small vibrating mill (Shimadzu vibrating mill, Japan) before grinding with KBr.

2.2.2.3. Powder X-ray diffraction studies. The X-ray diffractograms were recorded at room temperature using an X-ray diffractometer (Philips diffractometer, model PW 1830, Netherland). The X-ray source was nickel-filtered Cu Kα radiation generated at 30 kV and 30 mA. The target element was $\mathrm{Cu} - \lambda = 1.54$ Å. The dried film of uniform thickness was carefully placed on glass plate and scanned in the 2θ range of 5–50°.

2.2.2.4. Differential scanning calorimetry studies. The DSC curves were recorded using a differential scanning calorimetry analyzer (NETZSCH DSC 200, Germany). Samples of chitosan powder, untreated chitosan films and films after treatment for 360 h were encapsulated in a pierced lid aluminum pan prior to test. A heating rate of 10 °C min^{-1} and a temperature range of 40–400 °C were selected for scanning under a nitrogen atmosphere with a flow rate of 15 cm^3 min^{-1}.

3. Results

At 1:1.2 molar ratio of glucosamine unit of chitosan: acid, chitosan powder was soluble in all acids to obtain clear solutions. Dried chitosan salt films were easily peeled off from petri dish. The freshly prepared films were slightly yellowish. The color was gradually deepened to yellowish, then brownish after 360 h of moist heat treatment. Most films were non-tacky and transparent, except chitosan formate and glycolate films that were translucent. Chitosan malate and citrate films were slightly brittle.

3.1. Water sorption and dissolution

Freshly prepared chitosan films readily dissolved in deionized water and HCl buffer solution. They swelled, softened and eventually dissolved. Chitosan propionate film took slightly longer time to dissolve than the other films. All films remained their integrity in phosphate buffer solution except chitosan citrate film.

After moist heat treatment, the percentage of WS and DS of most films in three media noticeably decreased with time of treatment as illustrated in Figs. 1 and 2, respectively. The decrement in three media was ranked; phosphate buffer > deionized water > acidic buffer. The water sorption and dissolution of these films were ranked; chitosan citrate > chitosan malate > chitosan glycolate > chitosan lactate ≈ chitosan formate > chitosan acetate > chitosan propionate, respectively. Some films became soft gel or fragmentation. After treatment for 48 h, chitosan citrate and malate films still completely dissolved in deionized water and HCl buffer solution while chitosan acetate and propionate films became very slightly soluble.

3.2. Fourier transform infrared (FT-IR) study

The IR spectra of chitosan powder and chitosan salt films, untreated and treated for 24–360 h are shown in Fig. 3. Both untreated and treated powder exhibited the NH_2 deformation peak at 1588 cm^{-1} overlapped with the amide II band at 1558 cm^{-1}. The 'NH_3^+ band at 1514 and 1615 cm^{-1} and carboxylate band of –COO^- at 1556 cm^{-1} appeared in all freshly prepared films, indicating of electrostatic interaction between chitosan and acids. After treatment, the intensity of these two bands was decreased with time especially in chitosan acetate and propionate films, except the 'NH_3^+ band at 1615 cm^{-1} in chitosan citrate and malate films. Interestingly, the longer time of treatment to chitosan acetate and chitosan propionate films, the stronger was the peak intensity of amide I band at 1655 cm^{-1}. The ratios of their peak intensity at 1655/3200 cm^{-1} were increasing from 0.215 to 0.299, 0.267, 0.275 and 0.242 for chitosan acetate film, and from 0.496 to 0.500, 0.492, 0.529 and 0.522 for chitosan propionate film after treatment for 24, 48, 120 and 360 h, respectively. Chitosan formate film also exhibited an increase in intensity of amide I band with time of treatment.

Carbonyl absorption peaks at higher than 1700 cm^{-1} indicating of carboxylic acids were found in chitosan salts films except chitosan acetate, propionate and formate films. After treatment, the acidic carbonyl absorption peaks at 1710 and 1724 cm^{-1} of chitosan citrate and malate films were remained while those at 1729 and 1718 cm^{-1} from chitosan glycolate and lactate films were less intensified. Other absorption peaks of the latter films were more pronounced with time of treatment.

3.3. Powder X-ray diffraction study

The X-ray diffractograms of chitosan powder, and chitosan films both untreated and treated are shown in Fig. 4. It could be seen that chitosan powder, chitosan acetate, formate and glycolate films had two or more than two reflections while chitosan citrate, lactate, malate and propionate films had only one major peak. After treatment, the two major reflections at 20° 2θ and 10.6° 2θ from chitosan powder were not changed while the reflections at 23° 2θ, 18.3° 2θ, 11.8° 2θ and 8° 2θ from chitosan acetate films were decreased with time. In addition, a reflection at 21.7 ° 2θ appeared in chitosan acetate film. Similar result could be observed from chitosan formate film. Both treated chitosan lactate and propionate films showed more prominent peak height or narrower peak than their untreated films. In addition, the dominant reflection at 18.3° 2θ appeared in treated chitosan lactate films.

Treated chitosan citrate and malate films showed no dominant sharp peak in their diffractograms indicating that they were in amorphous state. Prior to treatment, the former showed no sharp peak while the latter had one prominent peak at 11.2° 2θ. The longer time of treatment to

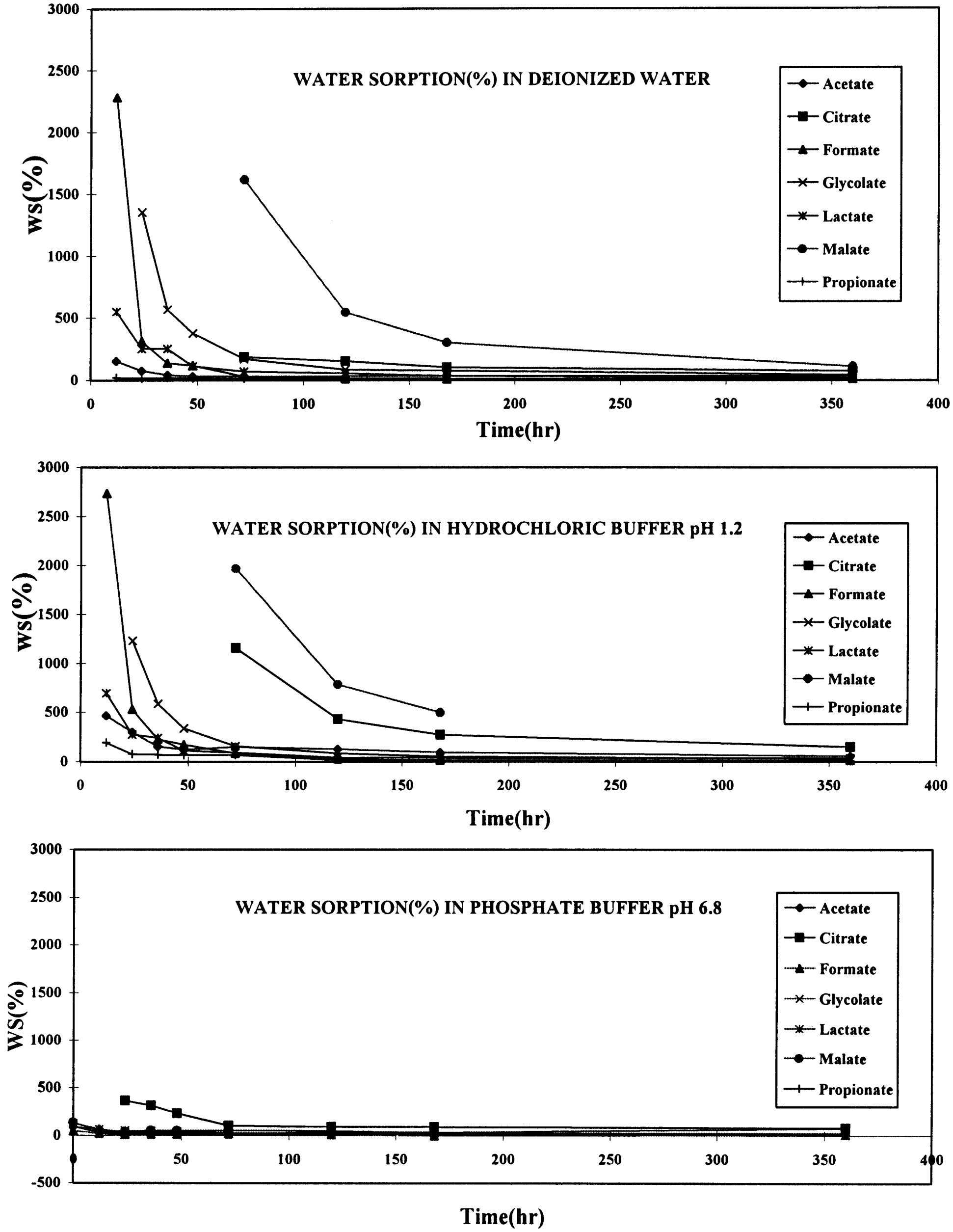


Fig. 1. Water sorption of chitosan salt films, untreated and after moist heat treatment for various time intervals, in different medium.

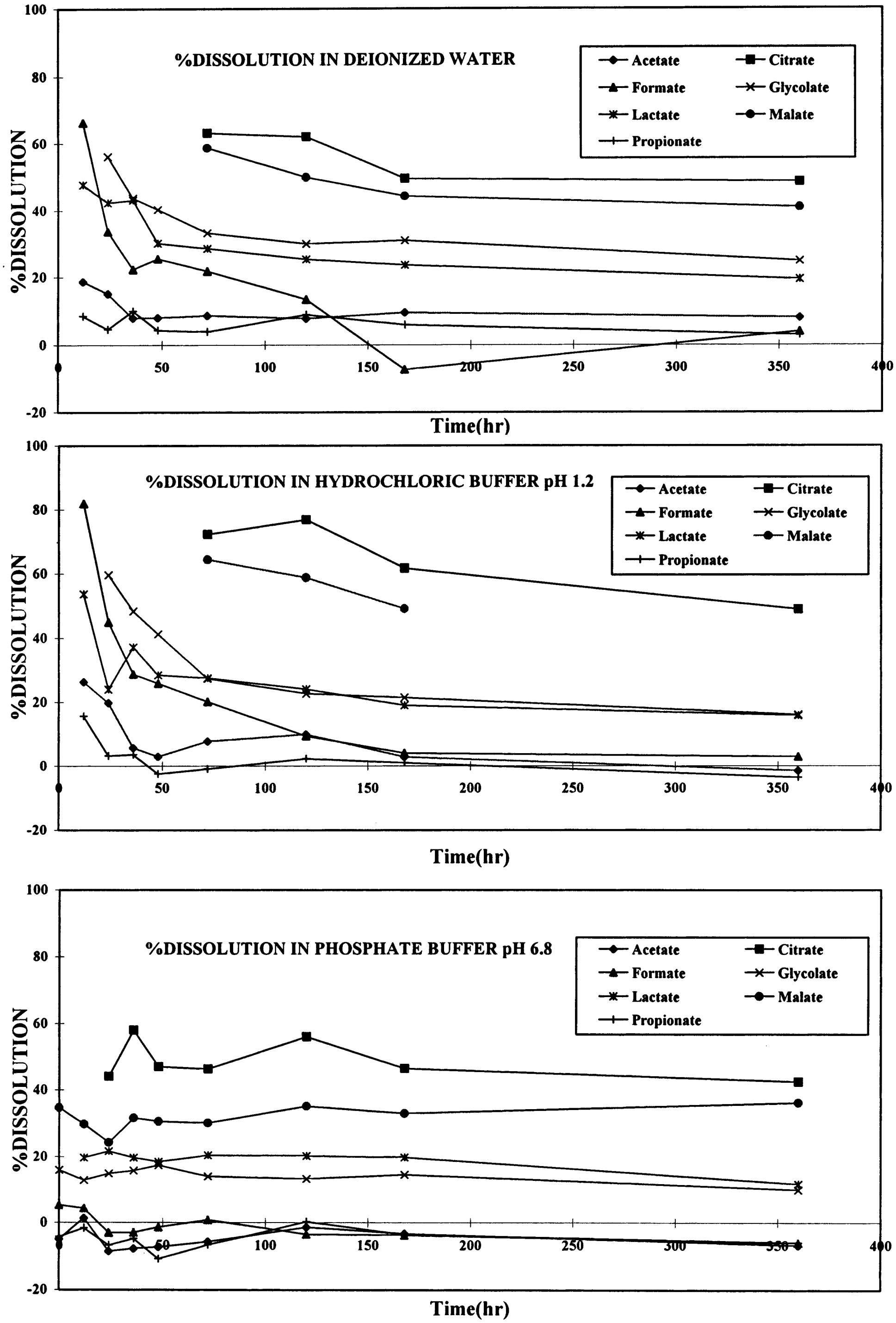
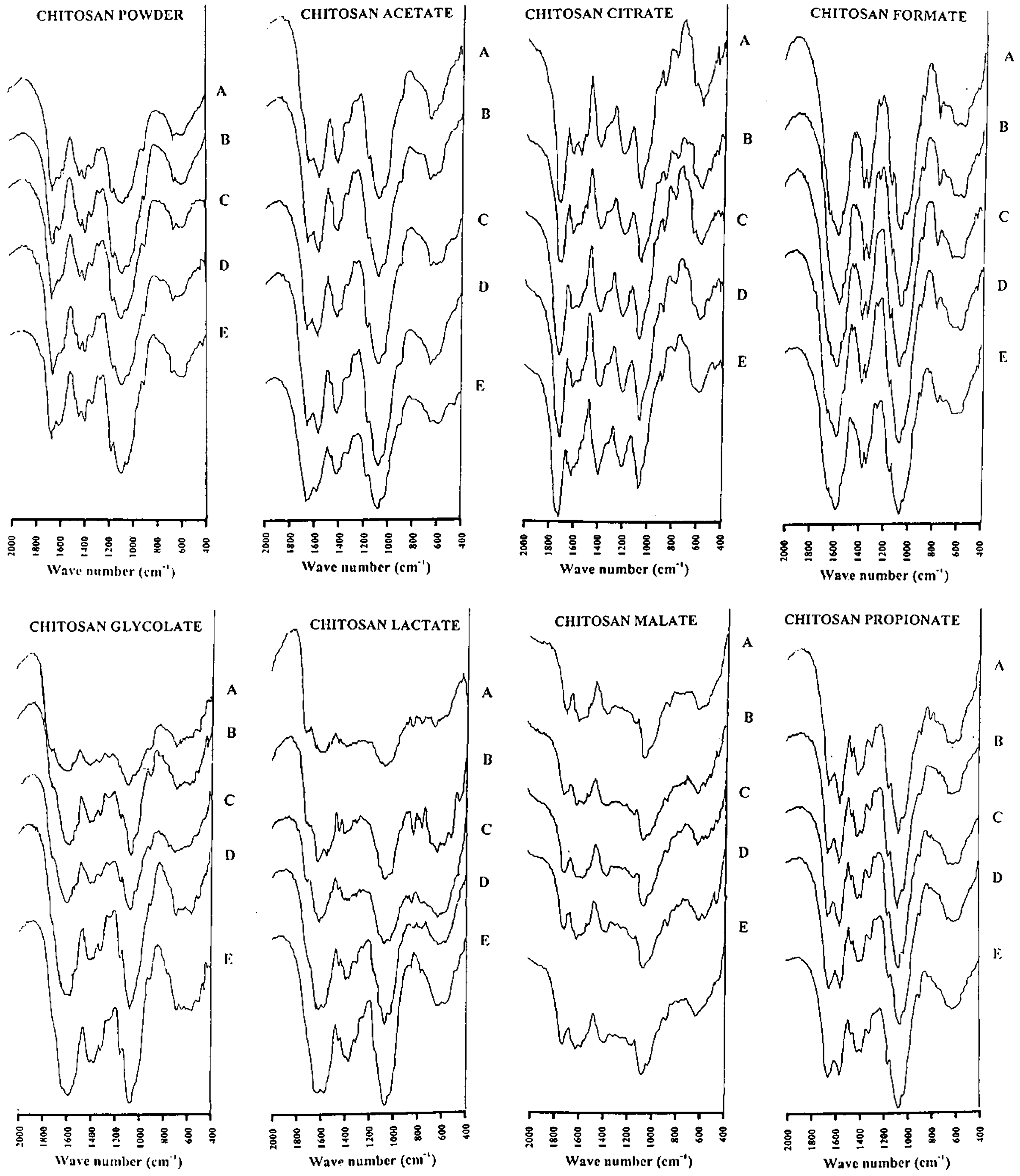


Fig. 2. Dissolution of chitosan salt films, untreated and after moist heat treatment for various time intervals, in different medium.



CHITOSAN POWDER

CHITOSAN ACETATE

CHITOSAN CITRATE

CHITOSAN FORMATE

CHITOSAN GLYCOLATE

CHITOSAN LACTATE

CHITOSAN MALATE

CHITOSAN PROPIONATE

Fig. 3. FT-IR spectra of chitosan powders and chitosan films: (A) untreated; and after moist heat treatment for; (B) 24 h; (C) 48 h; (D) 120 h; and (E) 360 h.

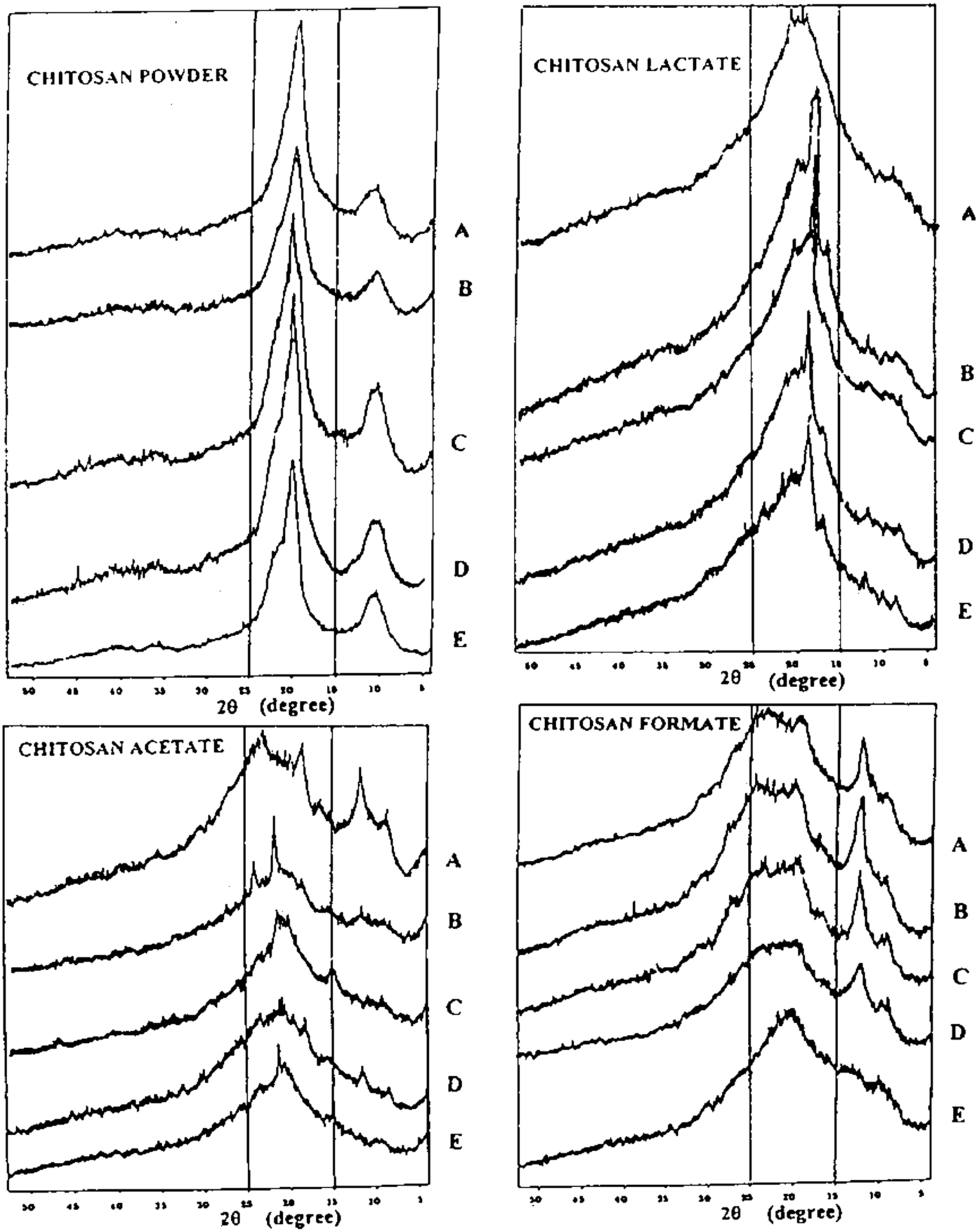


Fig. 4. X-ray diffractograms of chitosan powders and chitosan films: (A) untreated; and after moist heat treatment for; (B) 24 h; (C) 48 h; (D) 120 h; and (E) 360 h.

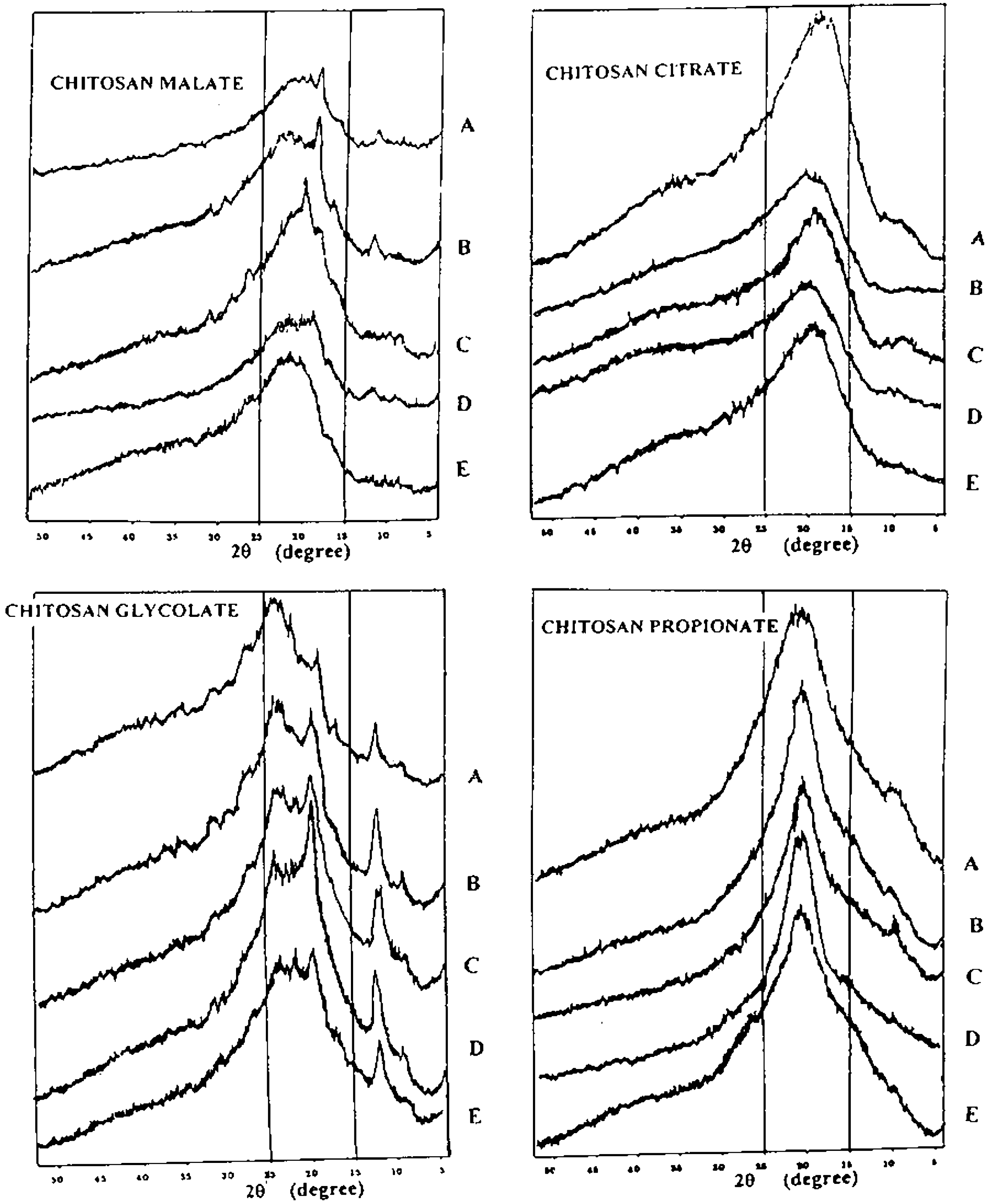


Fig. 4. (*Continued*)

Table 1
The temperature of endothermic and exothermic peaks from DSC curves of chitosan powders and chitosan films, untreated and moist heat treatment

Test specimen	Duration of moist heat treatment (h)	Temperature (°C)	
		Endothermic peak	Exothermic peak
Chitosan powder	0	86.3	306.9
Chitosan powder	360	108.3	305.8
Chitosan acetate	0	99.3	282.8
	360	95.5	296.3
Chitosan citrate	0	172.9	361
	360	71.9, 179.2	360
Chitosan formate	0	84.2, 158.9	305
	360	83.8, 159.8	305.6
Chitosan glycolate	0	92.6, 197.0	277.5
	360	95.7, 196.3	280.2
Chitosan lactate	0	93.5, 191.4	303
	360	87.9, 198.1	303.6
Chitosan malate	0	97.1, 186.3	344.8
	360	186.1	342.5
Chitosan propionate	0	89.7	278.2
	360	83.1	290.6

chitosan glycolate film resulted in more reflection intensity at 20° 2θ and less at 12.4° 2θ.

3.4. Differential scanning calorimetry study

The endothermic and exothermic peaks from DSC curves are summarized in Table 1. Chitosan powder, untreated and treated had exothermic temperatures at 306.9 and 305.8 °C, respectively, corresponding to the decomposition temperature of this polymer.

The exothermic peak of all freshly prepared chitosan films except chitosan citrate and malate films were clearly shifted to lower temperature compared to that of chitosan powder. After moist heat treatment, their exothermic temperatures were slightly higher but still lower than that of chitosan powder. Treated chitosan citrate and malate films showed high exothermic temperatures at 360 and 342.5 °C, respectively, which were slightly lower than those of untreated films.

The endothermic peak appearing nearly to 100 °C was associated with the evaporation of water. Broad endothermic temperature at about 200 °C was also observed in DSC curves of chitosan glycolate and lactate films whereas the endothermic temperatures of chitosan formate, citrate, malate films appeared at 159, 173 and 186 °C, respectively.

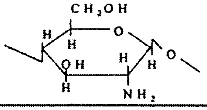
4. Discussion

Chitosan powder could easily be dissolved in acetic, citric, formic, glycolic, lactic, malic and propionic acids because chitosan is a weak polybase. This is considerably advantageous since these acids are classified to be harmless. Some of them are edible or degradation product in human body. The salt film was formed from the electrostatic interaction between protonated amino group in the chitosan backbone and carboxylate ion in the carboxylic acid. This was supported by the FT-IR spectra that all films exhibited ammonium and carboxylate bands at the wavelengths of 1615 and 1556 cm^{-1}, respectively. The polymer exhibited good film forming capability because of its glycosidic linkage like cellulose. However, chitosan citrate and malate films were slightly brittle which was likely due to the solid state of the acids used. The chemical structure and some physicochemical properties of chitosan and the acids used are listed in Table 2.

All films were soluble in deionized water and HCl buffer solution. This was due to the ionization of chitosan salt in the media. The insolubility of film in phosphate buffer solution of pH 6.8 was due to its unionized species since the pK_a of chitosan was reported to be 6.5 (Demarger-Andre and Domard, 1994). However, the prominent unreacted acidic carboxyl group in chitosan citrate film as shown in the FT-IR spectra at the wavelength of 1710 cm^{-1} was responsible for the solubility of this film in phosphate buffer solution.

After moist heat treatment, the increase in peak intensity at 1655 cm^{-1} in the FT-IR spectra of treated chitosan acetate and propionate films indicated that there was amide formation. It was likely that water in atmosphere of 75% RH was absorbed into chitosan film, thus the protonated amino group and carboxylate ion were equilibrated to the free amine nucleophile and free carboxylic acid. At elevated temperature, the carboxylic acid could be self-protonated which was then slowly reacted with the amine nucleophile to form an amide. Water molecule was then eliminated as by-product. Kubota et al. (1993) also claimed that only *N*-acetylation with some crosslinking agents could be formed on chitosan molecule in the presence of water. This amidation decreased the hydrophilic groups, thus decreased the percentage of WS and dissolution of treated films in immersion media. Other chitosan salt films would also have amide formation but their IR absorption characteristic was difficult to detect due to the interfering of the interesting peak.

Table 2
Some physical properties of carboxylic acids and chitosan

Acid	M.W.	bp (°c)	mp (°c)	pK_a	Chemical Structure
Acetic	60.06	118.2		4.76	CH_3COOH
Citric	192.12		153.0	3.15, 4.78, 6.40	$H_2C-COOH$ $\vert$ $HO-C-COOH$ $\vert$ $H_2C-COOH$
Formic	46.03	100.5		3.75	$HCOOH$
Glycolic	76.05		80	3.83	$HO-CH_2-COOH$
Lactic	90.08	122	17	3.86	$COOH$ $\vert$ $HC-OH$ $\vert$ CH_3
Malic	134.09		131-133	3.4, 5.1	$COOH$ $\vert$ $HO-CH$ $\vert$ CH_2 $\vert$ $COOH$
Propionic	74.08	141.1		4.87	CH_3-CH_2-COOH
Chitosan	161.16			6.3 - 7	

The presence of hydrophobic and hydrophilic groups especially the carboxyl group in the molecule of acid profoundly affected the water sorption and dissolution of the treated films. High percentage of WS and dissolution of treated chitosan films of polycarboxylic acids were attributed to the acidic carbonyl absorption peak as shown in the IR spectra at the wavelength about 1700 cm^{-1}. It could be concluded that the more carboxyl and hydroxyl groups and less alkyl group in the molecule of acid would result in the higher water sorption and dissolution of treated chitosan salt films.

The X-ray diffraction study revealed that chitosan films using different acids exhibited different diffractograms indicating of different crystalline structure. After moist heat treatment, amide formation also altered the lattice structure of chitosan salt film. An apparent increase in crystallinity of treated chitosan lactate, glycolate and propionate films was additionally decreased their percentage of WS and dissolution. This was possibly due to the intramolecular and intermolecular condensations of carboxylic acid in chitosan salt film (Roth et al., 1988; Brophy and Deasy, 1988). Treated chitosan citrate and malate films were in amorphous form, therefore, their percentage of WS and dissolution were still substantially high.

The insolubility of treated chitosan films was not from the anhydrous form which reflection at 15° 2θ had to be noted (Ogawa et al., 1984; Ogawa, 1991; Yui et al., 1994; Robert, 1994). Moreover, anhydrous form was difficult to exist due to the difficulty to complete elimination of sorbed water (Demarger-Andre and Domard, 1994).

Chitosan films were less stable than chitosan powder due to their slightly lower degradation temperatures except chitosan citrate and malate films. The degradation of these treated films was shifted to slightly higher temperature. This was attributed to the amide formation and change in the lattice structure or crystallinity. Similar higher exothermic temperature was previously reported on chitosan acetate film (Lim and Wan, 1995). Crosslinking of chitosan molecules with two ester bonds of the polycarboxylic acids might be the reason of higher exothermic temperatures of chitosan citrate and malate films (Yang and Wang, 1997; Rattanawawaleedirojn, 1997). However, these temperatures were lower in treated films due to the increase of amorphism.

5. Conclusions

Interactions between chitosan and carboxylic acids were associated with electrostatic reaction in aqueous solutions and formed salts in cast films. Moist heat treatment at the temperature of 60 °C and 75% RH could change ionic interaction to rather homogeneous amide formation in chitosan films. Consequently, the percentage of WS and dissolution in deionized water, HCl buffer solution and phosphate buffer solution were noticeably decreased. Longer alkyl group or less carboxyl and hydroxyl groups in acid molecule would result in lower percentage of WS and dissolution of treated chitosan salt film. Therefore, the percentage of WS and dissolution of chitosan salt films were depending on the type of carboxylic acid added. No formation of anhydrous crystalline was found in treated chitosan films.

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References

Brophy, M.R., Deasy, P.B., 1988. Biodegradable polyester polymers as drug carrier. In: James, S., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, New York and Basel, pp. 2–3.

Demarger-Andre, S., Domard, A., 1994. Chitosan carboxylic acid salts in solution and in the solid state. Carbohydrate Polymers 23, 211–219.

Hall, S.H., Wallace, J., 1998. Column film coating and processing. In: Hsieh, D.S. (Ed.), Controlled Release Systems: Fabrication Technology, vol. 1. CRS Press, Florida, p. 64.

Hayes, E.R., 1978. Characterization of chitosan II: the determination of the degree of acetylation of chitosan and chitin. Proceed. 1st Intern. Conf. On Chitin/Chitosan, Boston, MIT SG 78-7, pp. 406–420.

Kubota, N., Kikuchi, Y., Mizuhara, Y., Ishihara, T., Takita, Y., 1993. Solid-phase modification of chitosan hydrogel membranes and permeability properties of modified chitosan membranes. J. Appl. Polym. Sci. 50, 1665–1670.

Lim, L.Y., Wan, L.S.C., 1995. Heat treatment of chitosan films. Drug Dev. Ind. Pharm. 21, 839–846.

Ogawa, K., 1991. Effect of heating an aqueous suspension of chitosan on the crystallinity and polymorphs. Agric. Biol. Chem. 55, 2375–2379.

Ogawa, K., Hirano, S., Miyanishi, T., Yui, T., Watanabe, T., 1984. A new polymorph of chitosan. Macromolecules 17, 973–975.

Rao, S.B., Sharma, C.P., 1995. Sterilization of chitosan: implications. J. Biomat. Appl. 10, 136–143.

Rattanawawaleedirojn, P., 1997. Crease resistant finishing of cotton fabric with citric acid. J. Met. Mater. Miner. 7, 38–44.

Robert, G.A.F., 1994. Structure-properties relationship in chitin and its derivatives. Proceeding from Asia-Pacific Chitin and Chitosan Symposium. Bangi, Malaysia, np.

Roth, H.J., Eger, K., Troschutz, R. (Eds.), 1988. Pharmaceutical Chemistry. Ellis Horwood, New York, p. 160.

Toffey, A., Samaranayake, G., Frazier, C.E., Glasser, W.G., 1996. Chitin derivatives. I. Kinetics of the heat-induced conversion of chitosan to chitin. J. Appl. Polym. Sci. 60, 75–85.

Yang, C.Q., Wang, X., 1997. Infrared spectroscopy studies of the cyclic anhydride as the intermediate for the ester crosslinking of cotton cellulose by polycarboxylic acids. III. molecular weight of the crosslinking agent. J. Appl. Polym. Sci. 35, 557–564.

Yui, T., Imada, K., Okuyama, K., Obata, Y., Suzuki, K., Ogawa, K., 1994. Molecular and crystal structure of anhydrous form of chitosan. Macromolecules 27, 7601–7605.