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Kim, Ki Myong; Son, Jeong Hwa; Kim, Sung-Koo; Weller, Curtis L.; and Hanna, Milford, "Properties of Chitosan Films as a Function of pH and Solvent Type" (2006). *Biological Systems Engineering: Papers and Publications.* Paper 111.
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Published in *Journal of Food Science E: Food Engineering and Physical Properties* 71:3 (2006), pp. 119–124;
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Submitted May 8, 2005; revised July 28,2005; accepted December 7, 2005; published online March 15, 2006.

Properties of Chitosan Films as a Function of pH and Solvent Type

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Abstract
Two different deacetylated chitosans were dissolved in formic, acetic, lactic, or propionic acid to prepare chitosan films. The pH values of the film-forming solutions were adjusted to 3, 4, and 5. Water vapor permeability (WVP), tensile strength (TS), elongation (E), and total soluble matter (TSM) were significantly ($P < 0.05$) affected by acid type, pH, and degree of deacetylation (DA). Low DA (LDA) chitosan films had lower WVP and TSM, higher TS compared with high DA (HDA) chitosan films. The E values were not affected by DA. As pH increased, WVP and TSM of chitosan films tended to increase while TS decreased significantly ($P < 0.05$). Chitosan films with acetic and propionic acid solvents had low WVP and TSM and high TS, while films with lactic acid solvent had high E and TSM and the lowest TS. Fourier-transform infrared showed peak shifting in the spectra with different solvents and at different pH values. Chitosan films with lactic acid solvent showed a peak shift to a lower frequency range. The NH_3^+ band was absent in the pH 5.0 chitosan film spectra.

Keywords: chitosan films, deacetylation degree, organic acids, water vapor permeability, total soluble matter

Introduction

The impetus for developments in edible and degradable films comes from various sources. Consumers and processors alike are committed to reducing the environmental problems associated with packaging. In some cases, edible and degradable films may be able to replace synthetic packaging films. Furthermore, food scientists and engineers have isolated new materials that present new opportunities in the formation and properties of edible and degradable films. In many cases, these materials are quite abundant in nature and have previously been regarded as surplus or waste.

Chitosan is obtained by alkaline N-deacetylation of chitin, the 2nd most abundant polysaccharide. Chitosan is commercially available from plentiful, renewable sources, primarily waste from the shellfish industry. The use of chitosan in food applications is particularly promising because of its biocompatibility and nontoxicity. Uses include (1) separator agents in the form of coagulant or adsorbent aids, a beverage-clarifying agent, an immobilizing and permeabilizing matrix for microorganisms, and membranes for reverse osmosis and pervaporation applications (Knorr 1991; Pinotti and others 1997); (2) a matrix in culturing plant cells, artificial skin, tablet binders, and surgical sutures (Thanoo and others 1992; Upadrashta and others 1992); (3) a dietary fiber (Knorr 1991); and (4) biodegradable films and coating materials (Rhim and others 1998; Park and others 2001).

Chitosan is not soluble in pure water or organic solvents but is soluble in aqueous solutions of organic or mineral acids under specific conditions. Due to their unique property of increased viscosity upon hydration, chitosan-based materials may be used as edible and degradable films or coatings. Uses for chitosan films have been limited because of their high moisture permeability and mechanical problems. However, the properties of chitosan films can be modified to improve barrier and mechanical properties by changing pH or solvent. Kienzle-Sterzer and others (1982) evaluated the effect of chitosan concentration and type of solvent on the mechanical properties of chitosan films.

Chitosan possesses great potential for use in films, coatings, and encapsulation. Application in edible and degradable films or coatings and encapsulation may be possible only after the properties of chitosan-based materials are formed using different aqueous solvent systems. Dissolving chitosan in solvent systems containing organic acids as an initial formation step has received limited research attention because of its strong acidity. The pH-adjusting step should be followed to guarantee safety when chitosan-based materials are used for food package encapsulation. Therefore, the main objective of this study was to determine the effects of the chitosan-dissolving solvent system and the deacetylation degree of the chitosan on water vapor permeability, tensile strength, elongation, total soluble matter, and absorbance of chitosan films. This will provide basic data for use in determining proper chitosan and chitosan-solvent systems to use in preparing chitosan coatings and films.

Materials and Methods

Preparation of films

Commercial-grade chitosans were purchased from Vanson-Halosource, Inc. (Redmond, Wash., U.S.A.). Two different levels of deacetylated chitosan, 78.9% (low deacetylated [LDA]) and 92.3% (high deacetylated [HDA]) were used. Chitosan solutions (1%, w/ v) were prepared by dispersing chitosan in 50% (w/w chitosan) of glycerin (USP grade, Mallinckrodt, Paris, Ky., U.S.A.) in aqueous solutions of formic, acetic, lactic, or propionic acids (2%, v/v). The pH values of film solutions were adjusted to 3, 4, or 5 by addition of 2 *N* sodium hydroxide and hydrochloride. A total of 150 mL of each film-forming solution was magnetically stirred for 1 h and cast on a flat, level Teflon-coated glass plate. Films were peeled from the plates after drying at ambient temperature for about 48 h. Dried films were conditioned at 50% RH and 25 °C for 48 h before testing.

Table 1. Mean water vapor permeability (WVP) values for chitosan films for each deacetylation degree (DA), acid type, and pH combination. (unit: × 10–5 g·m/m^2·h·Pa) [a]

	pH	Formic acid	Acetic acid	Lactic acid	Propionic acid	Mean of DA
LDA	3	0.68 ± 0.02d	0.72 ± 0.07d	0.96 ± 0.045cd	0.73 ± 0.055d	1.15 ± 0.51 A
	4	2.22 ± 0.02b	0.82 ± 0.01d	1.67 ± 0.27c	0.76 ± 0.2d	
	5	1.79 ± 0.001cd	0.99 ± 0.002cd	1.56 ± 0.005cd	0.92 ± 0.02cd	
HDA	3	1.11 ± 0.47cd	0.64 ± 0.001d	0.93 ± 0.055cd	0.64 ± 0.019d	2.36 ± 2.69 B
	4	8.17 ± 0.97a	0.79 ± 0.001d	1.91 ± 1.11cd	1.10 ± 0.043cd	
	5	4.24 ± 0.34b	1.10 ± 0.029cd	7.05 ± 3.12a	0.62 ± 0.19d	
Mean of acids		3.04 ± 2.69x	0.85 ± 2.45z	2.34 ± 2.45y	0.79 ± 0.20z	
Mean pH value		3	4	5		
		0.80 ± 0.21 q	2.18 ± 2.40 p	2.28 ± 2.3 p		

[a] Any 2 means followed by the same letter (a-d, A-B, x-z, p-q) are not significantly different by Duncan's multiple range test. LDA = low degree of deacetylated; had = high degree of deacetylated.

Thickness

Film thickness was measured to the nearest 2.54 µm (0.1 mil) with a hand-held micrometer (B.C. Ames Co., Waltham, Mass., U.S.A.). Five thickness measurements were taken on each water vapor permeability (WVP) specimen, 1 at the center and 4 around the perimeter, and the mean was used in the WVP calculation. For the tensile strength (TS) calculations, 5 thickness measurements were taken along the length of each specimen and the mean was used.

Water vapor permeability

Five film specimens were tested for each type of film. WVP (g·m/m^2·h·Pa) was calculated as follows:

$$WVP = (WVTR \cdot l)/\Delta p$$

where WVTR was measured water vapor transmission rate (g/m^2·h) through a film specimen, l was mean film specimen thickness (m), and Δp was partial water vapor pressure difference (Pa) between the 2 sides of the film specimen. WVTR was determined gravimetrically using a modification of ASTM Method E 96-95 (ASTM 1995) as described by Gennadios and others (1994). Film specimens were mounted on polymethylmethacrylate cups filled with 16 mL of distilled water to within 1.03 cm of the film underside. Cups were placed in an environmental chamber set at 25 °C and 50% RH. A fan was operated in the chamber moving the air with velocity of 196 m/min over the surface of film specimens to remove the permeating water vapor. The weights of the cups were recorded 6 times at 1-h intervals. Linear regression derived slopes of the steady state (linear) portion of the mass loss versus time curves were used to estimate WVTR. WVP was replicated 3 times for each type of film.

Tensile strength and percentage elongation at break

TS and elongation (E) were determined with an Instron Universal Testing Machine (Model 5566, Instron Corp., Canton, Mass., U.S.A.) following the guidelines of ASTM Standard Method D 88291 (ASTM 1995). Initial grip separation was set at 50 mm and crosshead speed was set at 500 mm/min. TS was expressed in MPa and calculated by dividing the maximum load (N) by the initial cross- sectional area (m^2) of the specimen. E was calculated as the ratio of the final specimen length at the point of rupture to the initial length of a specimen (50 mm), as a percentage. TS and E measurements were replicated 5 times for each type of film.

Total soluble matter

Total soluble matter (TSM) was expressed as the percentage of film dry matter dissolved during immersion in distilled water for 24 h. Film pieces (20 × 20 mm) were placed in 50-mL beakers containing 30 mL of distilled water. Beakers were covered with Parafilm 'M' wrap (American Natl. Can, Chicago, Ill., U.S.A.) and stored at 25 °C for 24 h. The water remaining in the beakers were discarded and the residual film pieces were rinsed gently with distilled water. The film pieces were then dried in an air-circulating oven (105 °C) for 24 h. The weight of dissolved dry matter was calculated by subtracting the weight of insoluble solid matter from the initial weight of solid matter.

FTIR absorbance

The samples used for Fourier transform infrared (FTIR) measurements were cast to be thin enough to ensure that the observed absorption was within the linearity range of the detector. FTIR spectra of the films were recorded using attenuated total reflection (ATR) in an IR spectrometer (Nicolet Avatar 360, Madison, Wis., U.S.A.). The thin films were applied directly onto the ZnSe ATR cell. The transmission infrared spectra of all samples exhibited broad peaks in a range from 500/cm to 4000/cm. For each spectrum, 128 consecutive scans at 4/cm resolution were averaged for duplicate measurements.

Statistical analysis

The experimental design used was a completely randomized design (CRD). All data were analyzed by the general linear models procedure (GLM) in SAS analysis program (SAS Inst., Cary, N.C., U.S.A. 1999). The analysis of variance (ANOVA) of data estimated the significant ($P < 0.05$) interaction between 3 factors and significant effect of each factor ($P < 0.05$). Duncan's multiple range tests were conducted to compare significant ($P < 0.05$) differences between treatment groups.

Results and Discussion

Water vapor permeability

WVP was significantly ($P < 0.05$) affected by degree of deacetylation of chitosan, solvent pH, and type of acid, which interacted significantly ($P < 0.05$) with each other (Table 1). LDA chitosan films showed lower WVP of 1.15 ± 0.51 × 10^{-5} g·m/m^2·h·Pa than that of 2.36 × 10^{-5} g·m/m^2·h·Pa for HDA chitosan films. Mean WVP of chitosan films with formic and lactic acid solvents were 3.04 and 2.34 × 10^{-5} g·m/m^2·h·Pa, respectively, and were significantly ($P < 0.05$) higher than the WVP of chitosan films with acetic or propionic acid solvents. WVP of chitosan films increased from 0.8 × 10^{-5} g·m/ m^2·h·Pa at pH 3 up to 2.18 and 2.28 × 10^{-5} g·m/m^2·h·Pa at pH 4 and 5, respectively. LDA chitosan films showed lower values of WVP compared with HDA across the pHs and acid types. That may have been due to the fact that there was relatively little protonation of the

Table 2. Mean of tensile strengths for chitosan films for each deacetylation degree (DA), acid type, and pH combination (unit: MPa) [a]

	pH	Formic acid	Acetic acid	Lactic acid	Propionic acid	Mean of DA
LDA	3	15.3 ± 4.65cd	15.9 ± 1.86bcd	1.2 ± 0.057h	15.6 ± 1.66cd	9.8 ± 7.97A
	4	0.98 ± 0.053h	18.2 ± 0.79abc	1.3 ± 0.21h	19.2 ± 1.96a	
	5	0.0072 ± 0.0034h	10.5 ± 0.64ef	1.7 ± 0.083gh	18.2 ± 1.78abc	
HDA	3	10.5 ± 1.21ef	18.9 ± 7.84ab	0.56 ± 0.16h	19.1 ± 2.03a	6.9 ± 7.13B
	4	1.4 ± 0.096h	13.2 ± 1.14de	2.0 ± 0.29gh	8.7 ± 0.82f	
	5	0.81 ± 0.045h	4.5 ± 0.47g	1.96 ± 0.36gh	0.86 ± 0.16h	
Mean of acids		4.8 ± 6.28y	13.6 ± 5.83x	1.5 ± 0.55z	13.6 ± 7.75x	
Mean pH value		3	4	5		
		12.1 ± 7.66 p	8.1 ± 7.54 q	4.8 ± 6.12 r		

[a] Any 2 means with same letter (a-h, A-B, x-z, p-r) are not significantly different by Duncan's multiple range test. LDA = low degree of deacetylated; had = high degree of deacetylated.

Table 3. Mean of elongations for chitosan films for each deacetylation degree (DA), acid type, and pH combination (unit: %) [a]

	pH	Formic acid	Acetic acid	Lactic acid	Propionic acid	Mean of DA
LDA	3	66.6 ± 0.88gh	47.8 ± 6.22hijk	494.8 ± 32.53a	45.0 ± 4.56ijkl	107.7 ± 129.13
	4	34.3 ± 2.48klm	33.0 ± 0.70klm	189.2 ± 25.74c	39.12 ± 5.20jklm	
	5	28.6 ± 2.14lm	66.76 ± 6.03gh	165.6 ± 5.76d	82.2 ± 1.42g	
HDA	3	22.0 ± 0.98m	62.28 ± 1.88hi	181.5 ± 22.82cd	58.8 ± 5.23hi	107.0 ± 75.30
	4	54.01 ± 1.56hij	61.38 ± 3.01hi	250.9 ± 27.59b	104.5 ± 2.27f	
	5	37.7 ± 1.81jklm	83.6 ± 14.01g	232.7 ± 20.74b	135.3 ± 17.24e	
Mean of acids		40.5 ± 15.75D	59.1 ± 17.20C	252.4 ± 117.20A	77.5 ± 39.00B	
Mean pH value		3 4	5			
		122.3 ± 151.38 x	95.8 ± 78.89 z	104.0 ± 66.92 y		

[a] Any 2 means with same letter (a-m, A-D, x-z) are not significantly different by Duncan's multiple range test. LDA = low degree of deacetylated; had = high degree of deacetylated.

amide group in the LDA chitosan. WVP of HDA chitosan films increased more than for the LDA films as pH increased. With increasing pH and with decreasing hydrogen ion concentration more protonation sites in HDA films were neutralized and total ionic charges were changed much more than in LDA chitosan film solutions.

Mean WVP values of chitosan films were the highest values across all samples for formic and lactic acids at pH 4 and pH 5, while WVP of films were lower for acetic and propionic and unaffected by pH. The WVP values of chitosan films observed in this study were not directly comparable to those of Rhim and others (1998) because chitosan solutions can be affected by various factors such as deacetylated degree, molecular weight, and measuring conditions such as RH gradient, temperature. and film thickness. However, relative WVP changes with acid type corresponded to the changes reported by Rhim and others. Rhim and others (1998) used citric, malic, succinic, tartaric, phosphoric and hydrochloric acids, in addition to the ones used in this study. Formic acid gave the highest WVP values, followed by lactic acid, acetic acid, and then propionic acid.

Tensile strength and elongation

Deacetylation (DA), acid type, and pH significantly ($P < 0.05$) affected the TS values of chitosan films. Chitosan films with formic and lactic acids had low TS values, 4.8 and 1.5 MPa, respectively (Table 2). The TS values significantly ($P < 0.05$) decreased as pH increased. HDA chitosan film was very sensitive to pH change in comparison to LDA chitosan film. With increasing pH value, the degree of dissociation of chitosan decreased. Yao and others (1997) studied novel polyelectrolyte complex of 2 natural polyelectrolyte ligands, that is, chitosan and pectin. Their results revealed that the chitosan-pectin polyelectrolyte complex was affected by its composition as well as by the degree of deacetylation of chitosan. They showed the final composition of polyelectrolyte complex was not a function of the initial composition of the reactants. Instead, it was affected greatly by the pH value of the reaction mixture. HDA chitosan has more amino groups that can be dissociated in an acid solvent than does LDA chitosan. Higher TS values were expected for HDA films than LDA chitosan films because they had more active ionic sites in their molecules. However, there was not much difference in the TS values, and the TS value of HDA was dramatically decreased with increasing pH. TS of chitosan film with lactic acid solvent showed significantly ($P < 0.05$) lower values of TS than with formic, acetic, and propionic acids across all pH ranges. That can be explained by the fact that lactic acid had 1 hydroxyl group instead of hydrogen in the structure compared with formic, acetic, and propionic acids, which induced electrolyte instability in the solutions. Results showed no general trend among molecular weight of solvents within the various TS values and acid or pH values. TS of films with formic acid dropped as pH value increased. E values of chitosan films were significantly ($P > 0.05$) affected by acid type and pH but not by the DA of chitosan (Table 3). Kienzle-Sterzer and others (1982) reported changes in mechanical properties of chitosan films with the type of acid solvent. They estimated that acid type and concentration of chitosan used in preparing films may have affected both junction density and topological limitations in the films. This may have been due to the interactions between chitosan and acid solution. Bégin and Calsteren (1999) made antimicrobial films from chitosan with hydrochloric, acetic, lactic, and citric acids. Although the data they presented are not comparable with our study because they used considerably higher chitosan concentrations in their film solutions, they reported that lactic and citric acid solutions formed softer films than did acetic acid solution with Young's moduli of 683 and 183 MPa, respectively, and stress at yield of 22.2 and 2.9 MPa, respectively. They concluded that when the counter ion, such as lactate, was larger, the film lost its strength.

Table 4. Mean of total soluble matter values for chitosan films for each deacetylation degree (DA), acid type, and pH combination (unit: %) [a]

	pH	Formic acid	Acetic acid	Lactic acid	Propionic acid	Mean of DA
LDA	3	33.8 ± 1.31f	17.4 ± 0.17l	100a	23.2 ± 4.37h	59.0 ± 33.87Y
	4	73.3 ± 1.15b	29.5 ± 1.05g	100a	16.2 ± 0.53ij	
	5	100a	64.4 ± 0.43d	100a	51.2 ± 2.14d	
HDA	3	29.4 ± 0.50g	12.5 ± 9.43j	100a	21.1 ± 4.68h	62.5 ± 32.84X
	4	69.4 ± 0.51c	27.6 ± 2.77g	100a	50.8 ± 1.58e	
	5	75.5 ± 0.71b	64.6 ± 2.49d	100a	100a	
Mean of acids		63.5 ± 25.38B	36.0 ± 21.83D	100 ± 0.00A	43.7 ± 30.97C	
Mean pH value		3	4	5		
		42.1 ± 34.83 r	58.3 ± 31.15 q	81.9 ± 19.47 p		

[a] Any 2 means with same letter (a-j, X-Y, A-D, p-r) are not significantly different by Duncan's multiple range test. LDA = low degree of deacetylated; had = high degree of deacetylated.

Total soluble matter

TSM of chitosan films increased as solution pH increased (Table 4). Chitosan film made with lactic acid had the highest TSM followed by formic, propionic, and then acetic acid. Chitosan films with lactic acid solvent dissolved completely in water within 24 h. The high solubility of chitosan films with lactic acid was not shown in other reports. This can be explained by the use of high glycerin contents as compared with the study of Rhim and others (1998). Their formulation was 2% (w/v) chitosan, 25% glycerol (w/w chitosan), and 1% acid. The reported water solubilities of chitosan films varied from 15% to 22%. Formic, acetic, and propionic acid films were found to be more water resistant than lactic acid films, and their results were in agreement with our study.

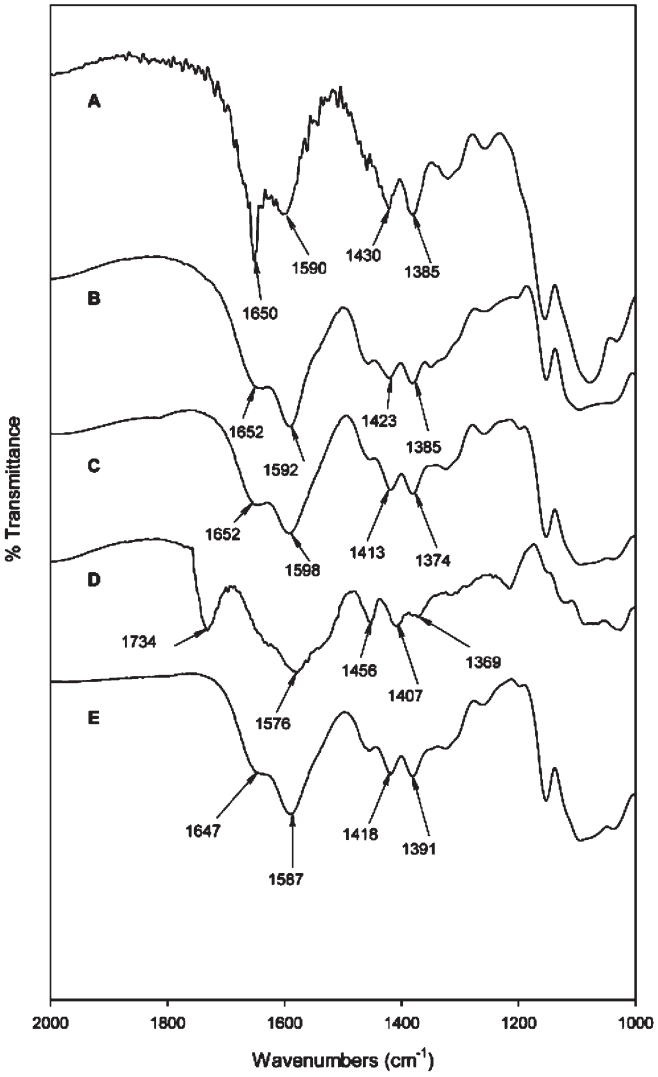

Figure 1. FTIR spectra of high-deacetylated chitosan films cast from different acid solutions at pH 3.0: (a) high deacetylated (HDA) chitosan powder; (b) formic acid; (c) acetic acid; (d) lactic acid; and (e) propionic acid

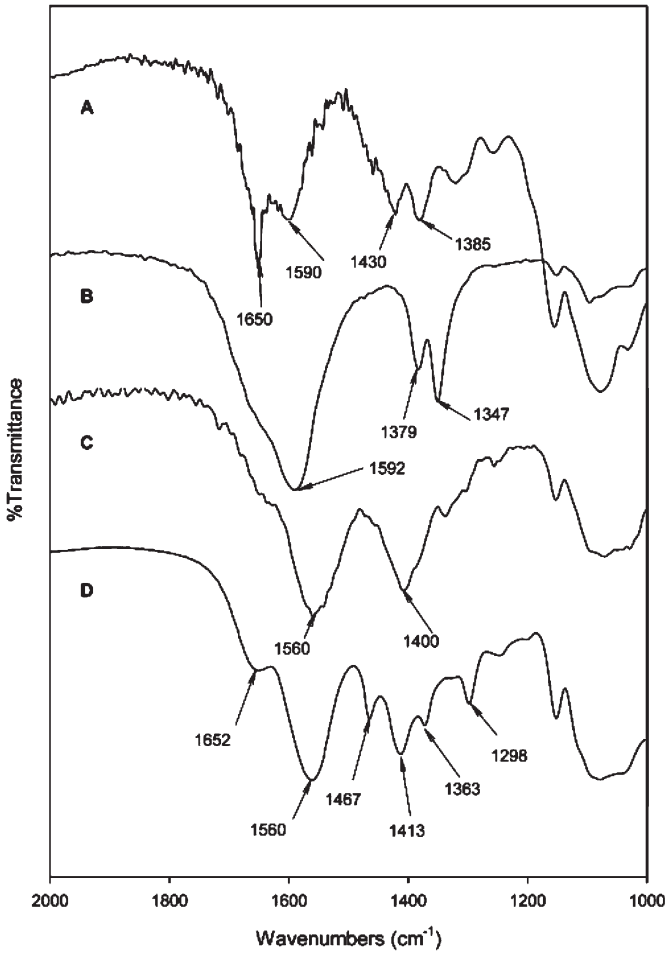

Figure 2. FTIR spectra of high-deacetylated chitosan films cast from different acid solutions after pH adjusted to 5.0: (a) high deacetylated (HDA) chitosan powder; (b) formic acid; (c) acetic acid; and (d) propionic acid

FTIR absorbance

FTIR spectroscopy is expected to be especially valuable in analyzing the phase structure and the interaction between chitosan and organic acids. Figure 1 through 4 show the FTIR spectra of pure HDA and LDA chitosan powder and films in the range of 2000/cm to 1000/ cm. FTIR spectroscopy was used to give information about the molecular state of chitosan in the cast films. Chitosan-lactic acid films at pH 5.0 could not be tested because of extreme flexibility. Stretching vibration of hydroxyl groups (-OH) appeared around 3450/cm in chitosan films (not shown in figures) and indicated intermolecular hydrogen bonding of chitosan molecules. They also overlapped in the same region of an NH stretching (Nunthanid and others 2001). The carbonyl, C = O-NHR, amine, NH_2 and ammonium, NH_3^+ bands were situated in the region between 1400/cm and 1700/cm. The carbonyl, C = O-NHR band was observed at 1650/cm, and the amine NH_2 band at 1590/cm appeared as a small shoulder in pure chitosan powders. It has been reported that the cation of the organic acid interacted with the nitrogen atom of amine group (Rinaudo and others 1999). The change in the characteristic shape of the chitosan spectrum, as well as the peak shifts to a lower frequency range in chitosan organic acid films, showed the same patterns as reported by Park and others (2001). They suggested that increased hydrogen bonding between –OH of additive (polyvinyl alcohol) and –OH or $-NH_2$ of chitosan in the blended films affected the change of peak pattern in their experiment. The absence of the NH_3^+ band in the pH-adjusted films was probably due to the interaction between NH_3^+ of the chitosan and the additive for pH adjustment of film solution. The characteristic amine peak at 1590/cm and amide I peak of the acetyl group at 1650/cm were shifted or disappeared. The change of patterns indicates that films prepared by adjusting pH levels formed chitosonium organic acid, which caused electrolyte instability in the film-forming solution by addition of sodium hydroxide. Especially, chitosan lactic acid films showed distinguishable changes in peak patterns at pH 3.0 compared with other chitosan films. This fact can explain the relationship between physical properties and changes of peak pattern of chitosan films. Nunthanid and others (2001) reported a strong peak at 1550/cm to 1600/cm and the weak peak near 1400/cm in spectra and concluded it was attributable to an asymmetric and a symmetric carboxylate anion stretching, respectively. Spectra showed a weak peak near 1400/cm, which was the same pattern as reported by Nunthanid and others (2001).

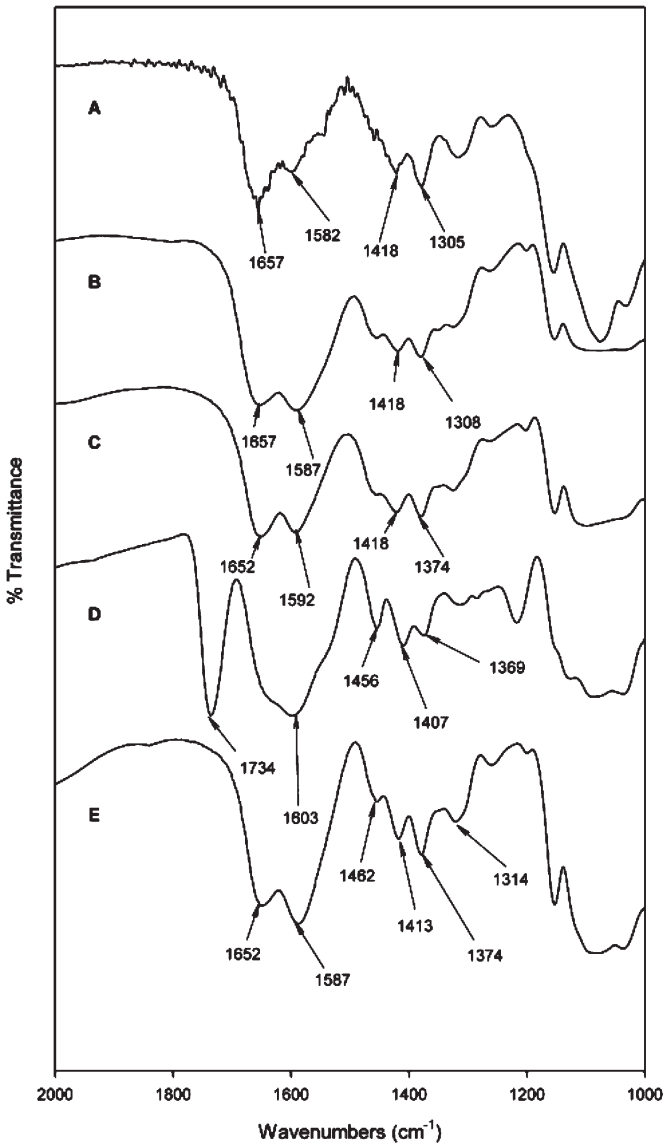

Figure 3. FTIR spectra of low-deacetylated chitosan films cast from different acid solutions at pH 3.0: (a) low deacetylated (LDA) chitosan powder; (b) formic acid; (c) acetic acid; (d) lactic acid; and (e) propionic acid

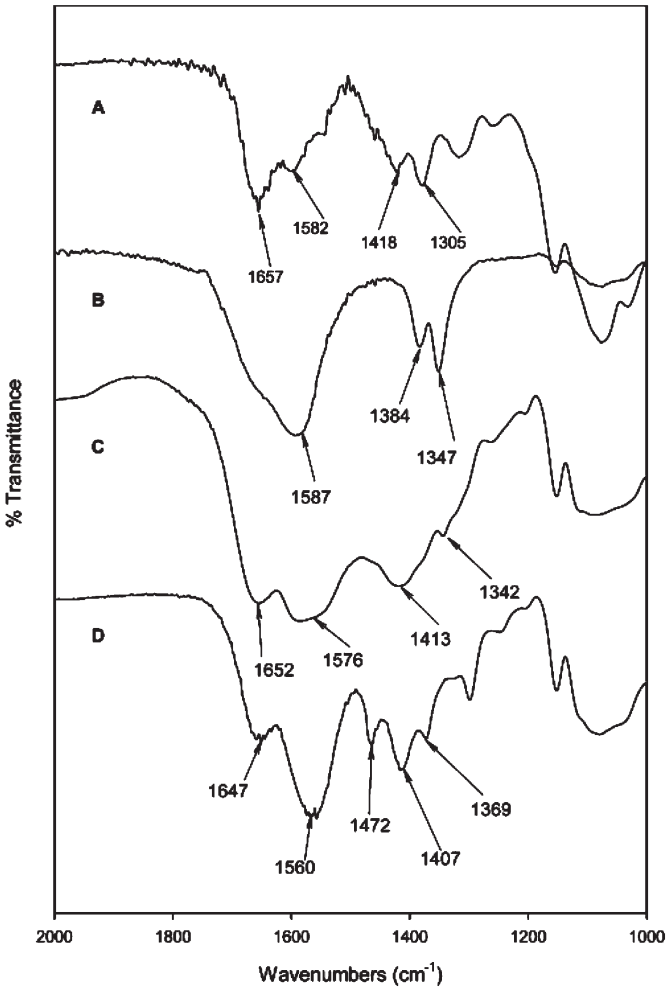

Figure 4. FTIR spectra of low-deacetylated chitosan films cast from different acid solutions after pH adjusted to 5.0: (a) low deacetylated (LDA) chitosan powder; (b) formic acid; (c) acetic acid; and (d) propionic acid

Conclusions

Chitosan showed different film properties when different solvents and degrees of deacetylation were used to prepare the film-forming solutions. The structure or size of acids, as counter ions, may have influenced the intramolecular and intermolecular interactions. HDA chitosan solutions showed greater changes in WVP, TS, E, and TSM due to pH and acid type than did LDA. Chitosan lactic acid films showed distinguishable shift of peak patterns in FTIR. That may be attributed to instability of the electrolyte complex of film forming solutions, which may affect inferior characteristics in film properties. This indicated there was a proper balance of electrolyte complex between the amino group of chitosan and solvents. Chitosan-acetic acid solvent systems showed lower WVP and higher integrity, higher values of TS, and lower values of E and TSM compared with the other organic acids in this experiment.

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