Acylation of Iminochitosan: Its Effect on Blending with Cellulose Acetate

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ABSTRACT: Chitosan has many similarities to cellulose, but there are few good direct solvents for codissolving them. Cellulose is cheaper than chitosan, and a blend film of the two would be expected to exhibit the desirable properties of both these polysaccharides. The formation of chitosanic and cellulosic acyl derivatives is an attractive route for the preparation of these fibers and films. They are soluble in common organic solvents, and in many cases it is not difficult to regenerate cellulose or chitosan from these derivatives. However, it may be desirable to protect the amino group from these reactions. Iminochitosan was used in the present study for that purpose. Chitosan at four molecular weights was pre-

pared using an HCl/isopropanol binary system. The hydrolyzed chitosan was allowed to react with salicylaldehyde via Schiff base formation. Acylation of iminochitosan was carried out by its reaction with different acid halides containing a varied number of carbon atoms in the presence of a catalyst. The main factors that affected the dissolution of acylated iminochitosan in different organic solvents are described. Films from blends of *n*-butyryl iminochitosan with cellulose acetate were prepared and are described. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 727–734, 2007

Key words: chitosan; blends; films; biomaterials

INTRODUCTION

Chitin and chitosan are well known but are still not utilized in proportion to how often they occur in nature as cellulose.[1] These materials are biodegradable, relatively nontoxic, nonimmunogenic, and biocompatible; much research has been directed toward their use in medical applications such as drug delivery and hemostasis.[2–4] In many of these uses, chitin and chitosan must be reshaped from a powder in order to be useful. However, they are not thermoplastics, and their solubility is limited.

The intractability of chitin comes mainly from its rigid micelle structure, as a result of close packing of the molecular chains and four types of hydrogen bonding.[5] Chitosan does not have the same degree of hydrogen bonding, but although it has an abundance of hydroxyl groups, it remains generally insoluble in water and organic solvent.

Chitosan fibers and films are some of the physical forms of interest for many applications.[6–8] The formation of chitosanic and cellulosic acyl derivatives is an attractive route for the preparation of fibers and films. Cellulose and chitosan have similar chemical struc-

tures, but there are few direct common solvents for the two. Blending these polymers together in film form could provide many advantages. Cellulose would be expected to contribute strength and is a cheaper material. Chitosan has well-known bioactivity associated with its free amino group. Both are soluble in common organic solvents, and in many cases it is not difficult to regenerate cellulose or chitosan from these derivatives. For example, the spinning of dibutyryl chitin has been reported in the literature. Szosland and East[9,10] have described the preparation of chitin fiber using acetone as a solvent with this derivative.

In our previous work[11] we prepared bromoacylated chitosan by reacting iminochitosan with an equimolar ratio of 2-bromo-isobutyryl bromide and pyridine using dimethylformamide as a solvent. This bromoacylated iminochitosan was found to dissolve in many different organic solvents, such as dimethyl formamide, dimethyl acetamide, and dimethyl sulfoxide. This derivative was used as a trunk polymer for subsequent graft copolymerization.

In the present study we extended the preparation of acyl derivatives of iminochitosan. The main factors affecting the dissolution of acylated iminochitosan in different organic solvents were studied. This was achieved by preparing chitosan at four molecular weights using an HCl/isopropanol binary system. The hydrolyzed chitosan was allowed to react with salicylaldehyde via Schiff base formation to

protect the amino group.[11,12] The iminochitosan was allowed to react with different acid halides containing a varied number of carbon atoms in the presence of pyridine as a catalyst. All factors affecting the acylation process and the dissolution of chitosan, such as molecular weight and kind and concentration of acyl chloride, and of catalyst were studied. A solvent that would dissolve cellulose acetate and the chitosan butyrate derivative was found. Solutions of varying proportions of the cellulose and chitosan esters were prepared. These were cast as films and transesterified. These films were examined by scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Commercial crab shell chitosan was kindly supplied by VANSON, medium viscosity (30 cps) Lot 01-ASCC-0279, (Redmond, WA). The degree of deacetylation of the chitosan was found to be 85% by elemental analysis. Pyridine, triethanolamine, triethylamine, and all other chemicals were purchased from Aldrich (St. Louis, MO).

The acid chlorides used were acetyl chloride, n-butyryl chloride, octanoyl chloride, and benzoyl chloride. The solvents used were dimethylformamide (DMF), dimethylacetamide (DMAc), N-methylpyrrolidone (NMP), trifluoroacetic acid (TFAA), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), methanol, and acetone.

Characterization of chitosan

Hydrolysis of chitosan

Chitosan of four molecular weights was prepared by acid hydrolysis of commercial crab-based chitosan with a 1N HCl/isopropanol (1N HCl/IPL) binary mixture. Then 100 g of chitosan was added gradually to a 4-L reaction kettle containing 2 L of 1N HCl/isopropanol of different ratios. The volume ratios of 1N HCl/isopropanol for the four hydrolysis samples are shown in Table I. The suspension was heated under continuous stirring to 85°C and kept at the same temperature for 2 h. The hydrolyzed chitosan was cooled, filtered, and neutralized

with NaOH. Finally, the chitosan was washed several times un neutral and dried at 50°C for 24 h.

Degree of deacetylation

The degree of deacetylation of chitosan can be calculated by elemental analysis. The hydrolyzed chitosan samples were submitted for C, N, and H elemental analysis to Atlantic Microlab, Inc. (USA). The degree of deacetylation was calculated according to the following equation

$$\%DD = [100\,\% - n_{acetyl}/n_{nitrogen}] \times 100\,\%$$
$$= 100\,\% \frac{-[(C_g/C_{(a.m.)}) - (6 \times N_g/N_{(a.m.)})] \times 0.5}{(N_g/N_{(a.m.)})}$$
$$\times 100\,\% \quad (1)$$

where n_{acetyl} and $n_{nitrogen}$ are the number of moles of the acetyl group and nitrogen, respectively; C_g and N_g are the mass of carbon and nitrogen in grams, respectively; and $C_{(a.m.)}$ and $N_{(a.m.)}$ are the atomic mass of carbon and nitrogen, respectively. The results are explained in Table I.

Determination of molecular weight

Average molecular weight of the hydrolyzed chitosan, M_v, was determined on the basis of intrinsic viscosity, as measured in an Ubbelohde viscometer with a solvent of 0.1M acetic acid/0.2M sodium chloride, and was calculated as 127 kDa for sample 1, 75 kDa for sample 2, 50 kDa for sample 3, and 26 kDa for sample 4. The Mark–Houwink equation was used to calculate the average molecular weight:

$$[\eta] = KM^\alpha \quad (2)$$

Wang et al.[13] established the functional relationship for K and α as a function of percentage of degree of deacetylation (%DD) of chitosan.

$$K = 1.64 \times 10^{-30} \times (\%DD)^{14} \quad (3)$$

$$\alpha = [-1.02 \times 10^{-2} \times (\%DD)] + 1.82 \quad (4)$$

Synthesis of iminochitosan

Iminochitosan was prepared[11] to protect the free amino groups from acylation and also to modify the solubility of chitosan with a hydrophobic group. This was prepared by the reaction of chitosan (100 g) with salicyaldehyde (130 mL) at room temperature for 6 h. The modified chitosan was filtered, washed with distilled water several times, and finally purified by methanol extraction in soxhlet for 6 h and dried at room temperature for 24 h.

TABLE I
Elemental Analysis of Hydrolyzed Chitosan

Sample no.	1N HCl/IPL (mL/L)	Elemental analysis data			Degree of deacetylation
		C	N	H	
1	120	39.52	7.26	7.15	82.42
2	160	39.31	7.20	6.94	81.41
3	200	38.90	7.11	6.90	80.74
4	240	38.54	7.00	6.82	78.73

TABLE II
Elemental Analysis of Chitosan and Iminochitosan

Material	Elemental analysis					
	Theoretical data (%)			Practical data (%)		
	C	H	N	C	H	N
Chitosan	44.44	6.79	8.6	43.9	6.64	7.32
Iminochitosan	58.6	5.64	5.26	55.06	7.15	5.41

O-acylation of iminochitosan

O-acylation of chitosan[11] was accomplished by reacting iminochitosan with different acid chlorides in the presence of a known concentration of catalyst (pyridine or triethylamine or triethanolamine). In a dry ice bath, 3 g of iminochitosan was slurried in 50 mL of DMF. A known concentration of pyridine was added in one portion and stirred for 15 min. A definite amount of acid chloride (in an amount equivalent to the catalyst) in 25 mL of DMF was then added dropwise over 1 h, and stirring was continued for 3 h at 0°C. The reaction solution was poured onto crushed ice and then washed with cold/hot distilled water several times to remove the pyridine salts. The product was finally purified by soxhlet extraction with methanol for 24 h. The O-chitosan derivative was dried at 60°C for 24 h.

Testing and analysis

Elemental analysis

Elemental analysis (C, N, and H) of chitosan and chitosan derivatives was performed at Atlanic Microlab (Atlanta, GA). Theoretical values were calculated for the samples to determine the degree of acylation. The degree of substitution (DS) was calculated on the basis of the percentage of nitrogen according to the following equation:

$$DS = (1400 - MWt_{IC} \times N \%)/MWt_{Fg} \times N \%$$

where MWt_{IC} and MWt_{Fg} are the molecular weights of iminochitosan and the introduced functional groups, respectively, and N is the percentage of nitrogen.

FTIR spectra

FTIR spectra were obtained using a Nicolet 510P FTIR spectrometer (Waltham, MD). Sixty-four scans from 4000–400 cm^{-1} were averaged.

Scanning electron microscopy

A Hitachi S-3200N scanning electron microscope (San Francisco, CA) was used for surface analysis of untreated sodium methylate and hydrochloride-treated blend films of chitosan butyrate/cellulose acetate.

RESULTS AND DISCUSSION

Synthesis of iminochitosan

The intractability of chitosan has been attributed to chain rigidity, bulk crystallinity, and strong intra- and interchain hydrogen bonds. An important component of hydrogen-bonding interactions for this system involves primary amino functionality along the polymer chain. Carrying out appropriate chemical modifications of the chitosan functional groups
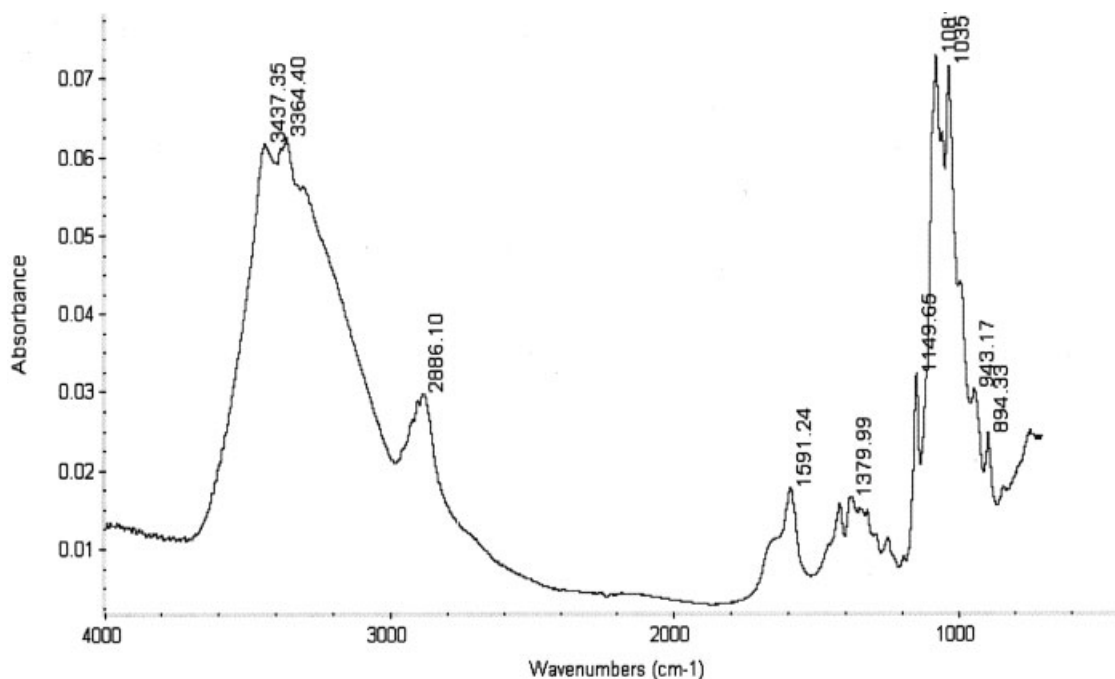

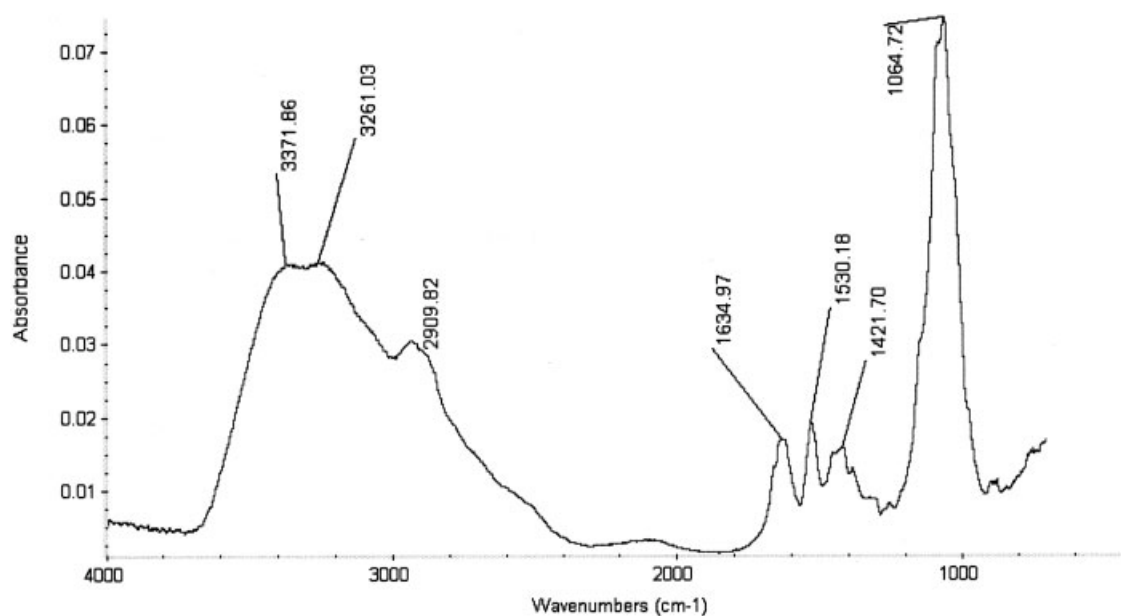**Figure 1** FTIR spectra of deacetylated chitosan.

Figure 2 FTIR spectra of iminochitosan.

can effectively disrupt these hydrogen bond interactions. Chitosan reacts with salicyaldehyde to form the imine, which has been successfully employed to disrupt the amino functionality of the hydrogen-bonding interaction.[12] The elemental analysis of iminochitosan shows an increase in the percentage of C and H, whereas the percentage of N decreased (see Table II). These values were close to the theoretical data, indicating that all the amino groups were masked. The FTIR data for the chitosan and iminochitosan agree with the elemental analysis results. In Figure 1, the absorption peak of the chitosan NH_2 (at 3339 and 3420 cm^{-1}) disappeared after formation of iminochitosan (—N=CH—), as seen in Figure 2.

O-acylation of iminochitosan

Acyl halides

Table III shows the effect of using different acyl halides, as well as chitosan molecular weights, on the percentages of C, N, and H. The acylation reactions were conducted using an equimolar ratio of acyl chlorides to pyridine, 4.14 mol/mol iminochitosan, material to a 1 : 25 liquor ratio and temperature at 0°C for 4 h. The elemental analysis data indicated the efficiency of the acylation reaction because the data showed a decreased percentage of C and H and a decreased percentage of N. For all the acyl halides, as the acylation proceeded, the suspended solids dissolved gradually and gave a very clear and homogenous solution. The DS was calculated from the elemental analysis data. The maximum value was 3, which included the hydroxyl group on the salicyaldehyde group.

The intermediary preparation of chitosan derivatives with superior solubility in organic solvents is an attractive approach to the synthesis of chitosan derivatives that have escaped traditional preparation methods. Our purpose was to investigate the solubility of acylated iminochitosan derivatives in different organic solvents in order to choose the one most compatible with cellulose acetate. The data showed

TABLE III
Effect of Type of Acyl Halide on Degree of Substitution of Iminochitosan at Different Molecular Weights

| Acyl halide | Molecular weight of Chitosan | | | | | | | | | | | | | | | |
| | 26 kDa | | | | 49 kDa | | | | 75 kDa | | | | 127 kDa | | | |
	% C	% N	% H	DS	% C	% N	% H	DS	% C	% N	% H	DS	% C	% N	% H	DS
Acetyl	49.5	4.2	6.14	1.59	49.7	4.24	6.2	1.51	50.01	4.29	6.3	1.43	50.13	4.32	6.3	1.37
Butyryl	60	2.95	6.7	2.95	59.3	3	6.7	2.84	56.6	3.04	6.3	2.76	59.3	3.05	6.6	2.73
Octanoyl	66.1	2.2	9.2	2.93	66.2	2.23	9.2	2.86	65.5	2.29	9.3	2.73	65.6	2.3	9.3	2.7
Benzoyl	65.1	2.4	4.48	3.03	65	2.44	4.6	2.94	63.6	2.49	5.1	2.83	64	2.51	4.8	2.79

[acyl halide, pyridine]; 3.4 mol/mol iminochitosan; M/L ratio 1 : 25; temperature, 0°C.

TABLE IV
Effect of Butyryl Chloride Concentration on DS of Iminochitosan

| Butyryl chloride (mol) | Molecular weight of Chitosan | | | | | | | | | | | | | | | |
| | 26 kDa | | | | 49 kDa | | | | 75 kDa | | | | 127 kDa | | | |
	% C	% N	% H	DS	% C	% N	% H	DS	% C	% N	% H	DS	% C	% N	% H	DS
0	56.3	4.53	6.3	0	55.5	4.6	6	0	54.9	4.64	6.1	0	54.5	4.7	5.5	0
1.38	47.7	4.24	6.83	0.95	44.2	4.25	7.2	0.91	46.6	4.29	6.8	0.86	44.4	4.29	7.22	0.86
2.76	56.7	3.48	6.81	1.94	57.5	3.5	6.65	1.9	57.9	3.5	6.81	1.9	58.1	3.53	6.81	1.85
4.14	60	2.95	6.7	2.95	59.3	3	6.7	2.84	56.6	3.04	6.3	2.76	59.3	3.05	6.6	2.73

[butyryl chloride, pyridine]; 1.38–4.14 mol/mol iminochitosan, M/L ratio 1 : 25; temperature, 0°C.

two types of behavior: higher-molecular-weight chitosan (75 and 127 kDa) showed complete insolubility for all the acyl halides used except in TFAA, whereas lower-molecular-weight chitosan (26 and 49 kDa) showed considerable swelling for the butyrate, octanoylate, and benzoylate derivatives.

Effect of butyryl chloride concentration

Table IV shows the DS values of the acylated derivative of iminochitosan as a function of butyryl chloride concentration, when pyridine was used as a catalyst and in an amount equivalent to that of butyryl chloride. The concentration of butyryl chloride varied from 1.38 to 4.14 mol/mol of iminochitosan. It is clear from the data that the DS values increased with increasing butyryl chloride concentration from 1.38 to 4.14 mol/mol iminochitosan.

Figure 3 shows the FTIR spectra for acylated derivatives of iminochitosan as a function of butyryl chloride. The butyrylation reaction was accompanied by the appearance of a carbonyl peak at 1736 cm^{-1}, indicating the formation of a carbonyl ester derivative of chitosan at the expense of the OH stretching of chitosan at 3420–3250 cm^{-1}.

The solubility of chitosan butyrate derivatives of four molecular weights (26, 49, 75, and 127 kDa) was investigated. It is clear from Table V that the molecular weight of chitosan and the butyryl chloride concentration were the key factors for the dissolution of chitosan derivatives in organic solvents. It is obvious that the optimum concentration of the butyryl chloride was 2.76 mol/mol iminochitosan; below and above this concentration the chitosan derivatives did not show any solubility except in TFAA. The lower-molecular weight chitosan had higher solubility.

Cheevasrirungrueng et al.[14] reported the synthesis of hexanoylated chitosan soluble in chloroform, toluene, and xylene. The N,O-hexanoylated chitosan were synthesized directly by reacting chitosan with hexanoyl chloride to obtain products with various degrees of hexanoylation. However, the O-hexanoy-
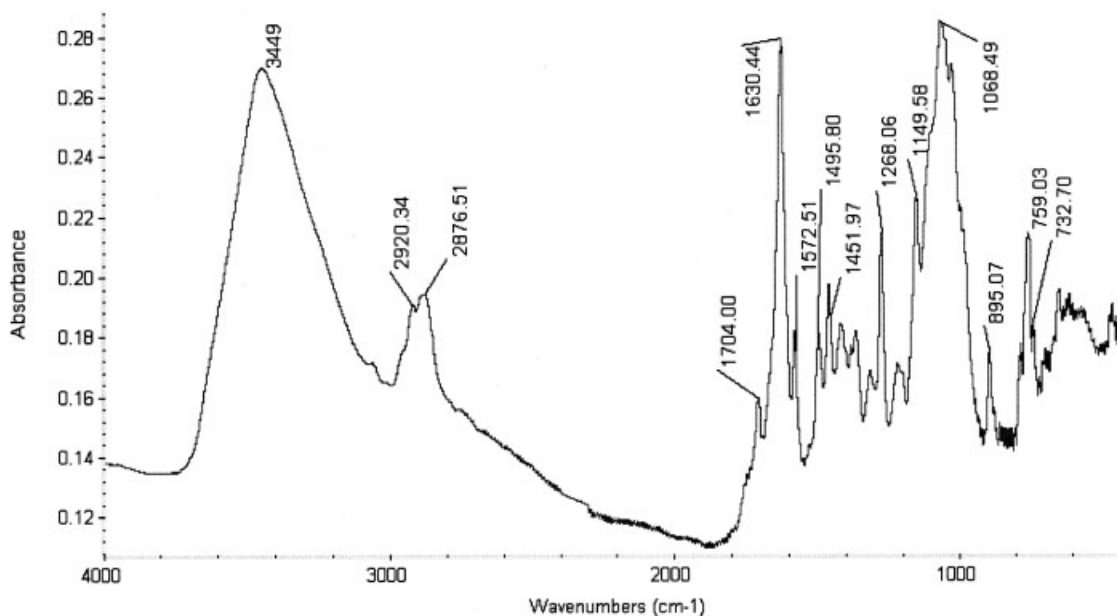

Figure 3 FTIR spectra of chitosan butyrate derivative.

TABLE V
Solubility of Chitosan Butyrate Derivative

Kind of Solvent	26 kDa [Butyryl chloride]			49 kDa [Butyryl chloride]			75 kDa [Butyryl chloride]			127 kDa [Butyryl chloride]		
	1.4	2.4	3.4	1.4	2.4	3.4	1.4	2.4	3.4	1.4	2.4	3.4
DMF	−	+	○	−	+	○	−	○	−	−	−	−
DMS	−	+	○	−	+	○	−	+	−	−	○	−
DMAc	−	+	○	−	+	○	−	○	−	−	○	−
THF	−	○	○	−	○	○	−	−	−	−	−	−
TFAA	+	+	+	+	+	+	+	+	+	+	+	+
NMP	−	+	○	−	+	○	−	○	−	−	○	−
Methanol	−	−	−	−	−	−	−	−	−	−	−	−
Acetone	−	−	−	−	−	−	−	−	−	−	−	−
CHCl$_3$	−	−	−	−	−	−	−	−	−	−	−	−

(+) soluble, (−) insoluble, (○) swellable.

lated chitosan prepared by selective modification of chitosan, and with free amino groups was soluble only in DMSO. Butyrate has the advantage of a wider range of available solvents.

Acid halides

Tables VI and VII illustrate the kind of acid chlorides used at the optimum conditions. Acid chlorides were used in amounts equivalent to pyridine, 2.76 mol/ mol IC, material to liquor ratio of 1 : 25, at 0°C for 4 h. The DS data show that the best acyl chloride was butyryl chloride. The iminochitosan butyrate derivative showed excellent solubility in different organic solvents, such as DMF, DMSO, DMAc, TFAA, and NMP, whereas the other derivatives showed fair solubility in organic solvents, except TFAA.

Catalysts

The efficiency of the acylation process of iminochitosan was highly dependent on the kind of catalyst used. The effect of catalyst type on the extent of the acylation was studied. Pyridine, triethylamine, and triethanolamine were used separately in equivalent

TABLE VI
Effect of Type of Acid Halide on DS of Iminochitosan

Acyl chloride	Effect of acid chloride on elemental analysis of chitosan derivatives			
	% C	% N	% H	DS
Acetyl chloride	47.9	4.44	6.41	1.17
Butyryl chloride	57.45	3.5	6.65	1.9
Octanoyl chloride	64.75	2.4	8.88	2.5
Benzoyl chloride	63.05	2.95	5.33	2.0

[Acid chloride, pyridine]; 2.76 mol/mol iminochitosan, chitosan M_r 49 kDa; M/L ratio 1 : 25; temperature 0°C.

amounts with butyryl chloride (2.76 mol/mol IC). The data (Table VIII) show that the optimum catalyst was pyridine because it acted as a catalyst as well as a solvent for butyryl chloride.

The solubility of iminochitosan derivatives was highly affected by the kind of catalyst used because the solubility of chitosan butyrate derivatives in organic solvents was much better with pyridine than the others. It appears that the solubility depended on the DS of the acylation, which highly relied on the catalyst used. Pyridine had the added advantage of being a solvent for the reaction product. These results are shown in Table IX. NMP (N-methyl-pyrolidinone) was judged the best solvent for both cellulose acetate and the chitosan butyrate.

Seo et al.[15] reported that by using phthalic anhydride as a protecting agent for the amino group of chitosan, followed by acylation in the presence of pyridine, the chitosan derivatives were soluble in different organic solvents, such as CHCl$_3$ and THF. These results indicate that the type of protecting group could affect the selection of organic solvent that would solubilize the chitosan derivative. Also, the

TABLE VII
Effect of Type of Acid Halide on Solubility of Iminochitosan Derivatives

Solvent	Solubility of chitosan derivative in organic solvent			
	Acetate	Butyrate	Octanoylate	Benzoate
DMF	−	+	−	−
DMS	−	+	−	○
DMAc	−	+	−	−
THF	−	○	−	○
TFAA	+	+	+	+
NMP	−	+	−	○
Methanol	−	−	−	−
Acetone	−	−	−	−
CHCl$_3$	−	−	−	−

TABLE VIII
Effect of Type of Catalyst on Acylation Efficiency of Butyryl Chloride

Acyl halide	Effect of acid halide on elemental analysis of iminochitosan derivatives			
	% C	% N	% H	DS
Pyridine	57.45	3.48	6.65	1.9
Triethyamine	55.7	3.91	6.1	1.31
triethanolamine	56.1	3.7	6.6	1.6

deprotection of the phthalate derivative involved the use of hydrazine and more rigorous conditions than that for the Schiff base.

Chitosan butyrate/cellulose acetate blends

Films were prepared by casting on glass plates 4% (w/v) NMP solutions of chitosan butyrate/cellulose acetate derivatives containing different chitosan butyrate contents. The films were treated by first being soaked in 0.05N sodium methylate/methanol solution for 5 h at room temperature and then washed several times with methanol. This transesterified the acyl groups, yielding cellulose, iminochitosan, and the corresponding methyl acyl esters. Then the films were soaked with alcoholic HCl (0.1N HCl/methanol) for 6 h at room temperature to deprotect the amino groups of chitosan, followed by several washings with distilled water. Figures 4–6 show the surface analysis of the treated samples using scanning electron microscopy (SEM). It is obvious that the untreated film showed great smoothness and a homogenous surface, indicating the high compatibility of cellulose acetate and the chitosan butyrate derivatives. The sodium methylate–treated film, however, showed some surface irregularities, which were a result of the hydrolytic action of sodium

TABLE IX
Effect of Type of Catalyst on Solubility of Iminochitosan Derivative

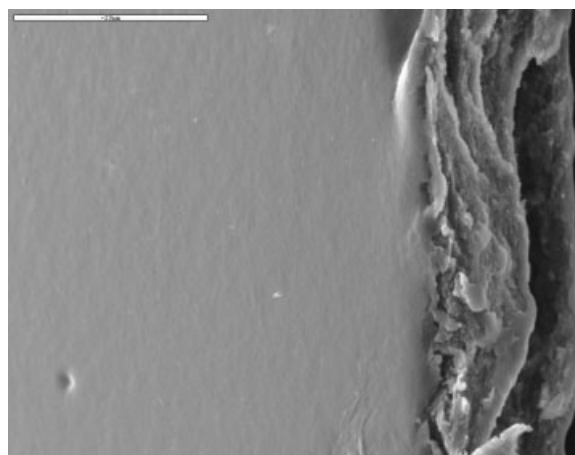| Solvent	Solubility of iminochitosan derivative in organic solvents		
	Pyridine	Triethylamine	Triethanolamine
DMF	+	○	○
DMS	+	+	+
DMAc	+	−	−
THF	○	−	−
TFAA	+	+	+
NMP	+	+	○
Methanol	−	−	−
Acetone	−	−	−
CHCl$_3$	−	−	−

Figure 4 SEM image of the as-cast untreated film. The scale bar is 20 μm.

methylate on the butyrate groups. Figure 6 shows the surface dissolution of chitosan hydrochloride, appearing clearly as irregular cavities on the surface of the film. However, the films represented by elemental analysis in Table X were not washed or treated with water after the HCl treatment. Therefore, we did not expect that any chitosan was extracted. The films prepared for SEM were washed in water and indicated the extraction of some chitosan.

CONCLUSIONS

Acylated chitosan derivatives were synthesized by the reaction of iminochitosan with different acyl chlorides. Chitosan butyrate derivatives of lower molecular weights, 26 and 50 kDa, were dissolved successively in organic solvents, such as NMP, DMF,
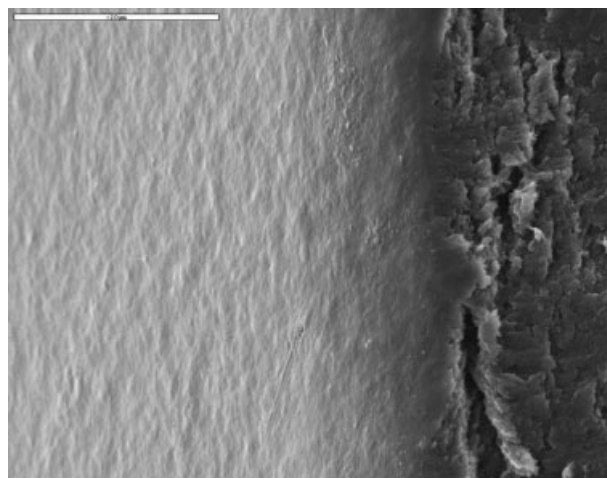

Figure 5 SEM image of the methylate-treated film. The scale bar is 20 μm.

DMAc, TFAA, and DMSO, whereas higher-molecular=weight chitosan derivatives, 75 and 127 kDa, showed fair solubility. The molecular weight of the chitosan and the kind and concentration of acyl chloride were important factors for the dissolution of chitosan derivatives in the organic solvents. The ratio of 2.76 mol butyryl chloride/mol IC was found to be optimum. Films were prepared from a blend of iminochitosan butyrate derivative and cellulose acetate in NMP solution. The cellulose and chitosan were subsequently regenerated. The SEM images indicated that the cellulose and chitosan esters could be blended to yield smooth, homogeneous films.
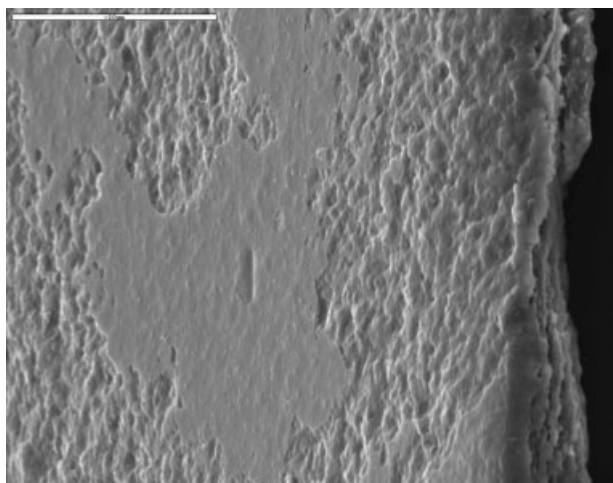

Figure 6 SEM image of the film after the HCl treatment and washing. The scale bar is 20 μm.

TABLE X
Elemental Analysis of Untreated, Sodium Methylate–, and HCl-Treated Films

CB/CA (%)	Untreated blend			Na methylate treated			HCl treated		
	C	H	N	C	H	N	C	H	N
25 : 75	51.7	6.45	1.93	46	7.13	2.36	45.4	7.0	2.41
50 : 50	54.01	6.69	3	49.6	7.0	3.2	48.89	6.95	3.57
75 : 25	57.17	6.8	3.6	51.9	6.9	3.94	50.01	7	4.05

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