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Preparation and physical properties of chitin fatty acids esters

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ABSTRACT

Trifluoroacetic anhydride is an effective promoter for the preparation of chitin single- and mixed-acid esters. Complete dissolution is achieved within 30 min when powdered chitin is heated at 70 °C in a mixed solution of carboxylic acid(s) and trifluoroacetic anhydride. Chitin esters prepared are chitin acetate, chitin butyrate, chitin hexanoate and chitin octanoate, chitin co-acetate/butyrate, chitin co-acetate/hexanoate, chitin co-acetate/octanoate, chitin co-acetate/palmitate, each from a solution of the respective reactants. The products have degrees of *O*-acyl substitution in a range of DS 1–2 depending on the nature of acyl group, as analyzed by gas–liquid and high-pressure liquid chromatography. Acetic acid as a mutual component for the mixed-acid esters increases the total degree of substitution, and the acetyl substitution is close to the relative distribution in the reaction mixture for chitin co-acetate/butyrate. It is favored over hexanoate, octanoate, and palmitate. The parent molecules, as calculated by the composition of the chitin esters and their molecular weights by light-scattering spectroscopy, are 30 kDa for the smallest and 150–151 kDa for the largest. Films of these chitin derivatives when cast from solution are strong and flexible with limited extensibility. By dynamic mechanical analysis of the ester film, it was found that both the glass transition temperature (T_g) and the tensile modulus (E' at 25 °C) are highest for chitin acetate (218 °C and 5.8 GPa), and lowest for chitin octanoate (182 °C and 1.5 GPa). For the other esters, these values lie between the above-cited values, where the T_g and the E' decrease with an increase in the chain length of the acyl constituent.

1. Introduction

Chitin is a linear polysaccharide of β-(1→4)-linked *N*-acetylglucosaminyl repeating units. It is an abundant biopolymer, second only to cellulose in nature, and it is found principally in the structural components of organisms, such as the exoskeleton of crustaceans and insects and the cell walls of fungi.[1] Commercial exploitation of chitin as such has been limited by its poor solubility in common solvents. To improve the solubility, N-deacetylation of the repeating residue is practiced, commonly with aqueous alkali and more cautiously in the presence of reductants[2] (e.g., sodium borohydride) to avoid a potential alkaline degradation[3] of the generated reducing termini. This hydrolysis results in various degrees of primary amine residues being introduced, and the number of these groups determines the solubility of the product in acidic media. The amino groups in the repeating units, unless complete N-deacetylation occurs, are present in a block-type distribution from heterogeneous solution[4] and more randomly from homogeneous solution.[4–6] For practical use of the materials in solution when the N-deacetylation exceeds 50% and the resulting N-deacetylated chitin is called chitosan. Chitosan provides an N-regioselective residue for additional modifications to improve physicochemical properties, such as N-acylation with a longer chain carboxylic anhydride, N-arylidenation or alkylidenation with aldehydes,[7,8] or depolymerization with nitrous acid.[9,10]

O-Acylation with fatty acids is another procedure to improve the solubility of chitin for subsequent applications. The esterification process often makes use of an inorganic acid such as perchloric acid[11–13] as a catalyst for the preparation of chitin acetate and acetate/formate, and methanesulfonic acid[11,14] for chitin formate, acetate, propionate, and butyrate. The resulting derivative, which is depolymerized to some extent, is soluble in organic solvents for highly substituted chitin butyrate,[13] and provides films from solution or fibers by wet/dry spinning.[15]

Trifluoroacetic anhydride (TFAA) proved again to be an effective esterification promoter as seen in our studies of the acylation of granular starch with aliphatic fatty acids,[16,17] with excess reagents acting as the sole solvent. This process was extended for the preparation of chitin single- and mixed-acid esters from partially purified (commercial) chitin, and their films, which were cast from solution, were analyzed for glass transition temperatures and for tensile moduli by a dynamic mechanical analyzer (DMA).

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2. Experimental

2.1. Materials and reagents

Crude powdered chitin (practical grade from crab shell, C7170), chitosan (C3646), formic acid (98–100%), *N,N*-dimethylformamide (DMF), and D_2O (99.9%) were purchased from Sigma–Aldrich (Milwaukee, WI, USA), *N*-acetylglucosamine (GlcNAc) from Pfanstiehl (Waukegon, Il, USA), *N,N*-dimethylformamide-d_7 (99.5 atom% D) from Acros Organics (New Jersey, USA), and palmitic acid from J.T. Baker (Phillipsburg, N.J. USA). All other chemicals used were of analytical grade and had been previously reported.[16]

2.2. O-Acylation of chitin

Powdered chitin (dried in vacuo at 60 °C, 16 h) in a premixed solution of TFAA and carboxylic acid(s) was heated at 70 °C with mechanical stirring (see Table 1). The clear, dark solution, achieved within 30 min, was allowed to cool to ambient temperature and mixed with cold abs EtOH (<−20 °C, 300 mL). The resulting solution was concentrated under reduced pressure (rotatory evaporator, 20 °C) to a syrup, which was hardened by the addition of abs EtOH, crushed with a spatula, and followed by multiple evaporations with abs EtOH. The resulting solid was dispersed in abs EtOH and heated to 80 °C. The colored supernatant was decanted, and the process was repeated multiple times until no color was extracted into the washings. The product was air dried for all derivatives except chitin butyrate.

The wet material of chitin butyrate after washings with hot EtOH was treated with acetone (2 × 300 mL) in order to extract soluble material, and the pooled extract was precipitated by dropwise addition of hexane with 1× volume (for fraction C-B1F2) and >2× volume (for fraction C-B1F3). The precipitates were air dried. The acetone-insoluble material (fraction C-B1F1) was dried. The precipitates from the C-B1F2 fraction, when air dried, were not soluble in acetone, but those from the C-B1F3 fraction were partially soluble in acetone.

In a separate experiment (C-B2), the chitin butyrate was recovered as a slurry from the reaction solution by cold EtOH (1.5 L) precipitation; it was mostly soluble in acetone (325 mL). A portion of the acetone solution was air dried to show an overall yield of acetone-soluble product of 79% (5.98 g), based on the DS 1.5 for butyrate and 67% chitin content of the starting material (see Section 3). The resulting solution after centrifugation was precipitated fractionally with hexane 0.5× volume (for fraction C-B2F1) and 1× volume (for fraction C-B2F1a), and the precipitates were air dried. An acetone-soluble fraction (C-B2F2) was extracted from the dried material of C-B2F1a.

For the reaction to produce the co-acetate/palmitate derivative, a combined mixture of acetic acid, palmitic acid and TFAA was gently heated to dissolve the palmitic acid before adding the powdered chitin.

Each reaction product (1.0 g) was dissolved in 30 mL of formic acid for chitin acetate and in DMF for the other esters, and the solutions were centrifuged (3600*g*) to remove some (<5%) of the insoluble material. The supernatants were used as stock solutions for further studies.

2.3. Analysis of the acid substitution

A portion of stock solution (Section 2.2) was dried in vacuo or by nitrogen gas flow at 40 °C for composition analysis: 5 mg for 4 M HCl hydrolysis (1 mL, 120 °C, 5 h) and 400 μg for butanolysis (400 μL of 2 M BuOH–HCl prepared by acetyl chloride and *n*-butanol, 100 μL of butyl acetate, and 40 μg of palmitate as an internal standard, which was omitted for chitin co-acetate/palmitate, 80 °C, 5 h). High-pressure liquid chromatographic (HPLC) analyses with UV detection (210 nm) for acetate and butyrate released by hydrolysis, and high-performance anion-exchange chromatographic (HPAEC–PAD) analyses with pulsed-amperometric detection for glucosamine were the same as previously reported.[18] Gas-liquid chromatographic analyses with flame ionization detection (GLC–FID) for butanoate, hexanoate, octanoate and palmitate, as their butyl esters released by BuOH–HCl, were as reported previously. *N*-Acetylglucosamine, treated under similar reaction conditions (80 °C, 2 h) as for the chitin esters was determined by GLC–FID for its molar response factor relative to palmitate (RRF

Table 1
Acyl derivatives of powered chitin (Sigma, practical grade from crab shell, C7170) by trifluoroacetic anhydride (TFAA) in single carboxylic acid and in a mixture of acetic acid (A) combined with each of butanoic (B), hexanoic (H), octanoic (O), or palmitic (P) acids

Sample code	Reactants				Yield (g)	DS[c]		M_W[d] × 10^{-3}
	Chitin[a] (g)	TFAA (mL)	Acid[b] (mL)					
			Ac	But		*O*-Ac	But	
C-A	10.0	100	100 (1.0)		10.12	2.3		
C-B1	7.5	75		48 (1.0)				
C-B1F1					3.55		1.3	199 (139)
C-B1F2					3.68		1.9	135 (82)
C-B1F3					1.1		1.7	91 (58)
C-B2	7.5	100		79.5 (1.0)	5.98			
C-B2F1					4.74		1.5	203 (137)
C-B2F2					0.9		1.5	44 (30)
C-AB1	7.5	75	10.1 (0.4)	24.2 (0.6)	8.61	0.8	1.0	
C-AB2	7.5	75	15.2 (0.5)	24.2 (0.5)	9.25	0.7	0.7	
			Ac	Hex		Ac	Hex	
C-H	7.5	75		67	7.54		1.2	233 (150)
C-AH	7.5	75	15.2 (0.5)	33.6 (0.5)	9.89	0.6	0.5	
			Ac	Oct		Ac	Oct	
C-O	7.5	75		84.5	9.5		0.9	229 (151)
C-AO	7.5	75	15.2 (0.5)	42.4 (0.5)	11.25	0.7	0.5	
			Ac	Pal		Ac	Pal	
C-AP	7.5	75	22.4 (0.75)	33.93 (0.25)	10.2	1.3	0.2	

[a] In the case of C-H and C-O, washed chitin was used as noted in the text.
[b] In parentheses, mole fraction of the corresponding fatty acid in the reagent.
[c] Degree of substitution by *O*-acetate corrected from the analyses of chitin butyrates.
[d] Molecular weight by light-scattering spectroscopy of the chitin ester (in DMF; d*n*/d*c* 0.049); in parentheses, the molecular weight of the parent chitin calculated from the composition of acid constituents.

1.000), and the resulting response factor of RRF 0.551 was applied to the quantitative analysis, together with those of other fatty acid esters reported in the previous work: RRF 0.356, butyrate; RRF 0.459, hexanoate; RRF 0.538, octanoate. The extent of *N*-acetyl group relative to the *N*-acetylglucosaminyl unit (DS, degree of substitution), determined by HPLC for acetate and HPAEC–PAD for glucosamine, was DS 0.9 (±0.1) for all the chitin butyrates. The summary correlated to the GLC–FID analysis of the butanolyzate is presented in the Table 1.

2.4. 1D ^{1}H Nuclear magnetic resonance spectroscopy (1D ^{1}H NMR) of selected chitin butyrates

Chitin ester (dried in vacuo at 60 °C) was dissolved in DMF (10 mg mL^{-1}) and centrifuged (27,000*g*, 24 h) to remove a small amount of solid (<1%). A portion of the resulting supernatant (3 mg) was evaporated to dryness by co-evaporation with toluene. The solid sample was dissolved in *N,N*-dimethylformamide-d_7 (0.75 mL) and evaporated to dryness by co-evaporation with toluene, followed by warming in vacuo at 45 °C before re-dissolution for its analysis. For some experiments, a small amount (10 μL) of D_2O was added to a solution of chitin butyrate in *N,N*-dimethylformamide-d_7 before evaporating to dryness. ^{1}H NMR spectra were acquired at 75 °C on a Bruker Avance 600 MHz spectrometer equipped with a 5-mm indirect detection probe. The chemical shifts were referenced to the internal signal (δ 2.74 DMF). The experiments were a single pulse, and the 90° pulse widths were determined for each sample and used accordingly. The delay before the application of the pulse was 8 s, and the acquisition time was 5 s for a total relaxation delay of 13 s between each transient. Longitudinal relaxation times (T_1s) of protons were estimated by using the inversion recovery pulse sequence (180–τ–90°). The T_1s of protons were all found to be lower than 2.6 s ($5 \times T_1 < 13$ s), so that all the protons were completely relaxed. The spectral width was 13,250 Hz, the number of data points was 128 k, the line broadening parameter was 0.2 Hz, and the number of acquired transients was 512.

2.5. O-Deacetylation of chitin butyrate and chitin co-acetate/palmitate

To a stirred solution of NaOMe (8.4 mL of 0.23 M NaOMe and 50 mL of MeOH) was added slowly a solution of chitin butyrate (DS 1.9, 1.0 g in 30 mL DMF) or chitin co-acetate/palmitate (DS 0.2, 1 g in 30 mL DMF). An aliquot of stirred slurry was withdrawn at intervals and centrifuged. The resulting precipitate was washed multiple times with abs MeOH until the washings were neutral (pH strip pre-wetted with water) and dried before analysis by GLC–FID of its butanolyzate.

2.6. Determination of molecular weight by light-scattering spectroscopy (LS)

A portion of the same chitin ester supernatant (10 mg mL^{-1}) as used for the above-mentioned Section 2.4 was diluted (2 and 5 mg mL^{-1}) and analyzed, as described in the previous report,[19] for a refractive index increment (d*n*/d*c*) of 0.049 mL mg^{-1} (±2%) and molecular weight (M_w) by batch mode using an RI detector (ERC-7517 RI, ERC Inc., Tokyo, Japan) and multi-angle light scattering (LS, DAWN DSP Laser Photometer and its software ASTRA V.473.04, Wyatt Technology Company, Santa Barbara, CA, USA).

2.7. Preparation and dynamic mechanical analysis (DMA) of a film strip

Each stock solution (0.7–1 g solid equivalent, Section 2.2) was poured slowly to avoid air bubbles into plate-glass wells (40 × 65 × 10 mm) that had been sprayed with PTFE (1,1,1,2-tetrafluoroethane) release agent (Dry Lubricant MS-122AD, Miller-Stephenson Chemical, Inc., Connecticut, USA). The wells were covered with a few layers of filter paper before loosely placing a glass lid on top. The samples were allowed to slowly evaporate to dryness (in a hood, 2–3 days) at ambient temperature for the formic acid solution of chitin acetate, and at 38–40 °C (hot plate) for the DMF solution of the other esters. The resulting film recovered from the wells was sliced into rectangular strips using a razor blade. The resulting strips were further dried in vacuo at <65 °C to remove bound solvent, and stored at ambient conditions (55–65% relative humidity) until they were taken for analysis.

The glass transition temperature was measured with a TA dynamic mechanical analyzer (Model DMA Q800, Newcastle, DE, USA) using a tension film clamp (Table 2). The conditions for acquisition of data were similar to those used for the starch ester films reported in the previous work and otherwise noted:[17] a preloaded force of 0.04% strain; 1 Hz, amplitude of 15 μm, and a ramp rate of 3.0 °C min^{-1}, from 20 °C with soaking time for 2 min to 250 °C or until the force track of 110% was reached. Necking (i.e., a reduction in width) was rarely observed for each of the samples that were analyzed under these conditions.

3. Results and discussion

3.1. Preparation of chitin esters

The crude chitin used for most of the esterifications was a light-yellow powdered material with chitin content of 67 ± 2% by weight and DS 1.0 ± 0.1 of acetate, as determined by HPLC of acid hydrolyzate (4 M HCl, 5 h, 120 °C) for acetic acid and HPAEC–PAD for glucosamine. The chitin content slightly increased to 73 ± 3% with a DS 1.0 ± 0.1 of acetate when the material was washed sequentially with 50 mM HCl, deionized water, 2% Na_2CO_3, deionized water, and finally with acetone. This process removed some extraneous materials including paramagnetic metals, as trapped by the stirring bar during those washings. The washed chitin, used in some instances, such as chitin hexanoate and chitin octanoate (C-H and C-O in Table 1), did not remove the color of the ester products. Further improvement in chitin content of 79 ± 1% and DS 0.8 of acetate was seen when the crude powdered chitin was briefly heated in 20% NaOH (400 mL, 105 °C, 10 min), and the dispersed product

Table 2
Thermal and elastic properties of chitin single- and mixed-acid ester films

Sample code[a]	Glass transition temperature[b] (°C)	E' (MPa)[c]
	Tan δ	
C-Ac	218	5850
C-B1		
C-B1F1	214	3245
C-B1F2	203	3234
C-B2		
C-B2F1	203	3460
C-B2F2	182 (200)	2099
C-AB2	211(177)	3802
C-H	194	1884
C-AH	196	3380
C-O	183	1511
C-AO	203	2619
C-AP	203	2719

[a] Sample code as noted in Table 1.
[b] Average of three measurements (±2 °C) as detected by the major distinct peak, and the value in parentheses noted for the second T_g as detected as a distinct minor peak.
[c] Average of three measurements (<6%) for storage modulus (E') taken at 25 °C.

was recovered by filtration. The filtrates when neutralized with glacial acetic acid resulted in a varied amount of precipitates, with the yield being 26% in one instance. The precipitate neither contained sugar nor was charred by a flame.

The presence of such non-carbohydrate material necessitated, in part, excess reagents (528 mmol of TFAA) relative to the available hydroxyl groups (7.5 g: 50 mmol based on 67.4% chitin content) of the *N*-acetylglucosaminyl residue for effective dissolution of the crude chitin, which generally occurred prior to reaching the reaction temperature of 70 °C. When TFAA was reduced to half of the above amount, the reaction was not as efficient, resulting in a hazy and viscous solution.

The dried reaction products were light yellow to brown in either of the preparations derived from crude or washed chitin. It was difficult to remove the color by washing the ester product with hot ethanol, and the color persisted even after fractional precipitation with hexane from the product dissolved in acetone (C-B2F2). It appears that the colored materials are tightly bound to the chitin molecule, and are also stable enough under acidic conditions, such as those employed for the reaction.

Cold ethanol precipitation, suitable for recovery of chitin acetate, resulted in some loss (20%) for chitin butyrate, and the loss was greater (>50%) with increased chain length of acid for the reaction solutions from hexanoate and octanoate. All dried chitin esters were insoluble in water, but readily soluble in DMF except for chitin acetate, which dissolved only in formic acid among the common solvents. Chitin butyrate, recovered as a slurry by cold ethanol precipitation, was soluble in acetone, but the air-dried product was only partially soluble in acetone, dimethyl sulfoxide and pyridine. The soluble chitin butyrates were principally molecules of a smaller molecular weight with a relatively high substitution of butyrate (see below). Insolubility is frequently seen for carbohydrate polymers or their derivatives due to inter- and intramolecular hydrogen bonding that results from drying.

3.2. Composition and molecular weight of chitin esters

The carboxylic acids released from chitin esters by BuOH–HCl varied with the nature of the *O*-acyl substituent over the period of heating at 80 °C, as determined by GLC–FID analysis of the released butyl ester and the butyl glucosaminide as the per-*O*-acetyl derivative (Ac_2O–pyridine system). Liberation of butyrate from chitin butyrate was complete within 2 h, and the palmitate from chitin co-acetate/palmitate continued to increase up to 5 h (Fig. 1), reflecting the apparent inhibition by a longer chain fatty acid. Such inhibition of palmitate had also been observed with starch palmitate and was more pronounced when the starch palmitate was heated in MeOH–HCl, where the material did not go into solution even after extended heating (80 °C, 16 h). The release of the acyl groups appears to occur concomitantly with that of the monosaccharide, as seen for an asymptotic value each reached simultaneously for either chitin butyrate or chitin co-acetate/palmitate.

The extent of *O*-acyl substitution was DS 1.3–1.9 for chitin butyrate, where the higher substitution was seen for the molecules with smaller masses. The substitution has a tendency to decrease with an increase in the *O*-acyl chain length (C-H and C-O in Table 1). The insertion of acetate as a mutual component for the mixed esters increased the total degree of substitution, where the relative distribution of the *O*-acyl substituent, determined by its mole fraction, was in a similar proportion to the acid added to the reaction mixture. This is noted particularly for chitin co-acetate/butyrate. The apparent steric hindrance increased with an increase in chain length, whereby acetate was favored over hexanoate (C-AH), octanoate (C-AO) and palmitate (C-AP), the hindrance being most substantial for palmitate.

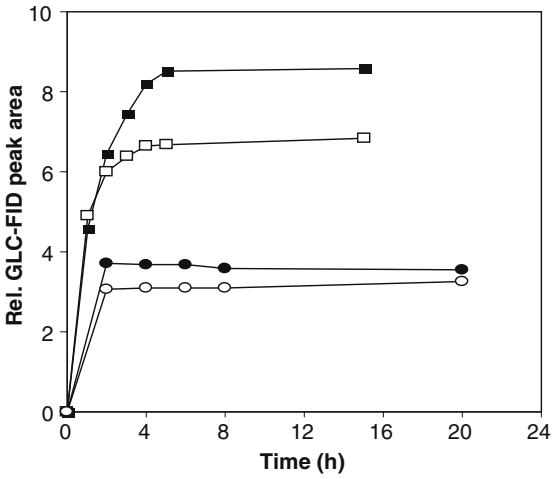

Figure 1. Analysis of butyric acid released from chitin butyrate (circle, C-B1F1) and palmitic acid from chitin co-acetate/palmitate (square, C-AP), together with the released monosaccharide, by GLC–FID of butanolysis (2 M BuOH–HCl, 80 °C). Filled: butyl per-*O*-acetylglucosaminide; open: butyl esters of fatty acid. Peak area of butyl palmitate in GLC was doubled for clarity.

Chitin esters of chitin butyrate (C-B1F2) and chitin co-acetate/butyrate (C-AB2) were analyzed again for carbohydrate content using the same hydrolysis conditions (4 M HCl) as that used above for crude chitin. The analyses accounted for 92 ± 1% of the weight with DS 1.9 ± 0.1 of butyrate for the former and for the latter 84 ± 1% with DS 2.1 ± 0.1 of the combined value for *N*- and *O*-acetyl and DS 0.9 ± <0.1 of butyrate. The extent of acid substitution was comparable to the analysis of the butanolyzate by GLC–FID. The apparent yield of the chitin esters from the starting materials of either 67% or 73% chitin content was higher than the calculated value (data not shown), due to the incomplete dryness of the ethanol-washed products. This esterification removed the non-carbohydrate material from the crude chitin and resulted in an increased carbohydrate content, the increase being more by partial fractionation with hexane from its solution in acetone than by hot ethanol washings alone.

The analysis of chitin acetate resulted in a DS of 2.3, which is more than the theoretical maximum substitution of *O*-acetyl. This excess might have been due to the additional presence of partial N-acetylation of acetamido residues. The occurrence of such *N,N*-diacyl substitution on the glucosamine residue was reported previously when chitosan and acyl chloride were refluxed in pyridine and chloroform, resulting in a 4-mole acyl substitution per monosaccharide.[20] In contrast, acylation of *N*-acetylglucosaminitol, when reacted in the premixed solution of TFAA and butanoic acid at a much elevated temperature (80 °C, 30 min), did not support the incidence of *N,N*-dibutanoyl substitution by GLC analyses of the resulting product, which co-eluted with the acyl preparation of the same molecule in butyric anhydride and pyridine (rt, 16 h). Furthermore, there is no implication from 1D ^{1}H NMR analysis for such disubstitution, as detected by the signal close to quantitative amido *N*–H proton (see discussion later) noted especially for chitin butyrates. At this stage, the nature of the overestimation of acetate in the chitin acetate remains to be determined.

Light-scattering data of chitin butyrates dissolved in DMF reflected the parent polymers as polydispersed in size. Distinct differences were noted in molecular weights among the chitin butyrates as fractionated. The lowest mass of 44 kDa was identified in an acetone-soluble fraction of the butyl ester that was extracted from an air-dried product (C-B2F2), and the highest mass of 199–203 kDa was found in acetone-insoluble products (C-B1F1 and CB2F1). These molecular weights, when corrected for the composi-

tions of acid constituents, permitted the molecular weight of the parent chitin to be estimated. The smallest mass of the parent molecules from these fractions was 30 kDa compared to the largest species at 137–139 kDa (Table 1). The presence of the smaller masses as seen in chitin butyrates was not apparently perceived for the analysis of chitin hexanoate (233 kDa) or chitin octanoate (229 kDa) when the whole preparations of ester were determined by light scattering. This is due to the nature of molecular weights characterized by light scattering in a batch mode, which is more sensitive to larger molecules when mixed randomly with smaller ones, and also to the weight average of the molecules distributed. Thus molecular weights determined from the whole preparation, either of chitin hexanoate or of chitin octanoate, lean mostly to the higher masses, particularly when smaller ones exist relatively sparsely, as in chitin butyrate (<15% by w/w). The parent molecules of 150 kDa calculated for chitin hexanoate and 151 kDa for chitin octanoate are close to each other, and are comparable to the high-molecular weight fraction (137–139 kDa) from the chitin butyrate.

The crude powdered chitin was used as purchased, and the process for its preparation is not known. The occurrence of smaller masses as detected in these preparations might be naturally inherent, but more likely results from depolymerization of the parent molecules to some extent during the preliminary washings with acid to remove minerals or with alkali to remove lipids and proteins. A sample of chitin isolated from the shell of the crab *Scylla serrata*, when in lithium thiocyanate solution, is reported to have molecular weight (M_w) of more than a million daltons by light-scattering studies.[21]

O-Deacylation of chitin ester should have provided another process for purification of chitin from crude materials. Sodium methoxide in absolute methanol, when mixed with starch triacetate dissolved in chloroform, resulted in starch that was regenerated to near-complete deacetylation within one hour. The same approach made for chitin esters was different, and the O-deacylation for chitin esters was somewhat incomplete in the first 4 h of the reaction for either chitin butyrate or chitin co-acetate/palmitate, and then leveled off without complete removal of the remaining acyl groups (Fig. 2). The nature of such partial deacylation is not known, but it has been suggested that the resistant acyl groups are attached to the nitrogen. By comparison, the butyrate release from *N*-acetyl-per-*O*-butanoylglucosamintol (prepared by a butyric anhydride–pyridine system) in NaOMe solution was completed within 1 h, as determined by GLC–FID of the product as its per-*O*-propanoyl derivative.

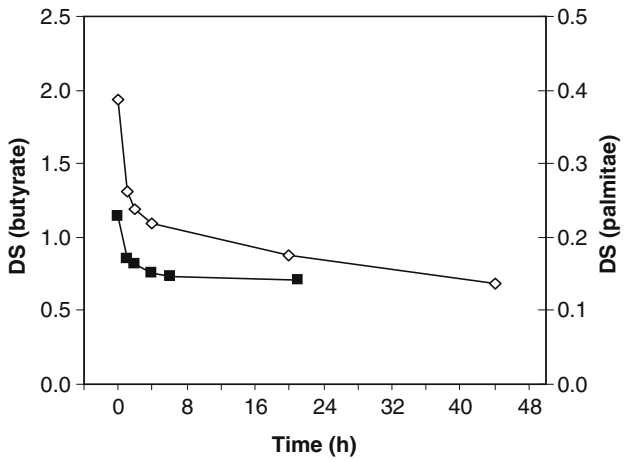

Figure 2. The extent of O-deacylation by NaOMe for chitin butyrate (DS 1.9; open square) and chitin co-acetate/palmitate (DS 0.2 of palmitate; filled square) by GLC–FID of butanolyzate. Repeated treatment was conducted after the 18 h for the resulting chitin butyrate with freshly prepared 0.3 M NaOMe in methanol.

3.3. Analysis of chitin butyrate by 1D ^{1}H NMR

Regenerated chitin was prepared by selective N-acetylation of chitosan in aqueous methanolic acetic acid with acetic anhydride.[8] The resulting chitin, when acylated in a mixed solution of butanoic acid and TFAA at ambient temperature, had the composition of DS 1.90 ± 0.14 for *O*-butyrate and 1.05 ± 0.07 for *N*-acetate, as determined by liquid chromatography of the 4 M HCl hydrolyzate. This butyl ester, dissolved in DMF-d_7, was also analyzed by 1D ^{1}H NMR spectroscopy. The spectrum revealed signals of sugar backbone (Fig. 3) for the H1 to H6 protons: δ 5.14–5.08 (set to 1.00H, broad), 4.70 (0.97H, broad), 4.47 (0.81H, 9.8Hz), 4.18 (1.05 H, broad), 3,80–3.64 (3.44H, broad), and 3.34 (0.17, broad); amide *N*–H proton at 7.42 (0.92H, broad); acetamido methyl at 1.81 (3.07H, singlet). Additional signals derived from butanoyl substituents were detected at δ 2.36 and 2.24 (total 3.95H, broad), each for the proximal methylenes to carbonyl group (–OCO–CH_2–); at δ 1.67 and 1.58 (total 4.12H, broad), each for the distal methylenes; at δ 0.98 and δ 0.92 (total 6.35H, broad), each for methyl groups.

These signals in the spectra were broad and overlapped, which is in contrast to well-resolved resonances for the per-*O*-acetyl derivative of pre-reduced chitobiose.[22] The peak areas of proton signals, as used for quantitative determination of *N*-acetyl content in partially N-deacetylated chitin,[5] were useful for estimation of relative distribution of butyrate per repeating sugar unit. The average peak area of each proton (1.06) for H1 to H6, as calculated from the total integrated area (7.44 for 7H's), is close to that (1.02) for the acetamido methyl (3.07 for 3H's) and also to that (1.03) for butyrate ester (14.42 for 14H's). The ratios among those values reflecting the repeating sugar residues are 96% (1.02/1.06) for N-acetylation and 97% (1.03/1.06) for O-butyration, compared to 95% butyrate substitution by chromatographic analysis of acid hydrolyzate. The signal ratio of the amide *N*–H proton at δ 7.42 is 87% (0.94/1.06). The lower detection, compared to 96% for the acetamido methyl protons, would be due to a small amount of water present in the sample.[22] Its signal disappeared almost completely (<3%) when D_2O was added to the sample.

The same analyses made for chitin butyrate of DS 1.9 (C-B1F2 in Table 1) resulted in 1.10 for each proton from H1 to H6 (total 7.69), 1.07 from the acetamido methyl group (total 3.21) and in 1.08 from the butanoate esters (total 15.86), revealing 97% acylation for *N*-acetyl and 98% for *O*-butyryl, compared again to 95% butyrate substitution. The evaluations of butyrate substitution, even with the poorly resolved ^{1}H NMR spectra, were close to those by HPLC analyses of acid hydrolyzate, which had also been previously applied to acetate determination from various biopolymers including chitosan.[18] The unidentified signal at δ 1.30, detected for the same sample with different degrees of intensities, appeared to be a contaminant.

3.4. Dynamic mechanical analyses (DMAs) of chitin ester films

The films that were cast from DMF solution at 40 °C were light-brown, clear, and flexible. These films were susceptible to deformation when exposed to acetone or absolute ethanol, necessitating removal of the remaining DMF by drying in vacuo at elevated temperature (<65 °C) before analyses. Such dry film was used for DMA with a ramp gradient from 20 °C to 250 °C. The measurement was directed principally to the assessment of the primary glass transition (T_g), defined as the temperature at which the mechanical damping peak (tanδ) occurs.[23] Thermal expansion of the resulting film specimen was minimal (1–3%), as determined by the increase in length after the analysis, reflecting that the films are behaving

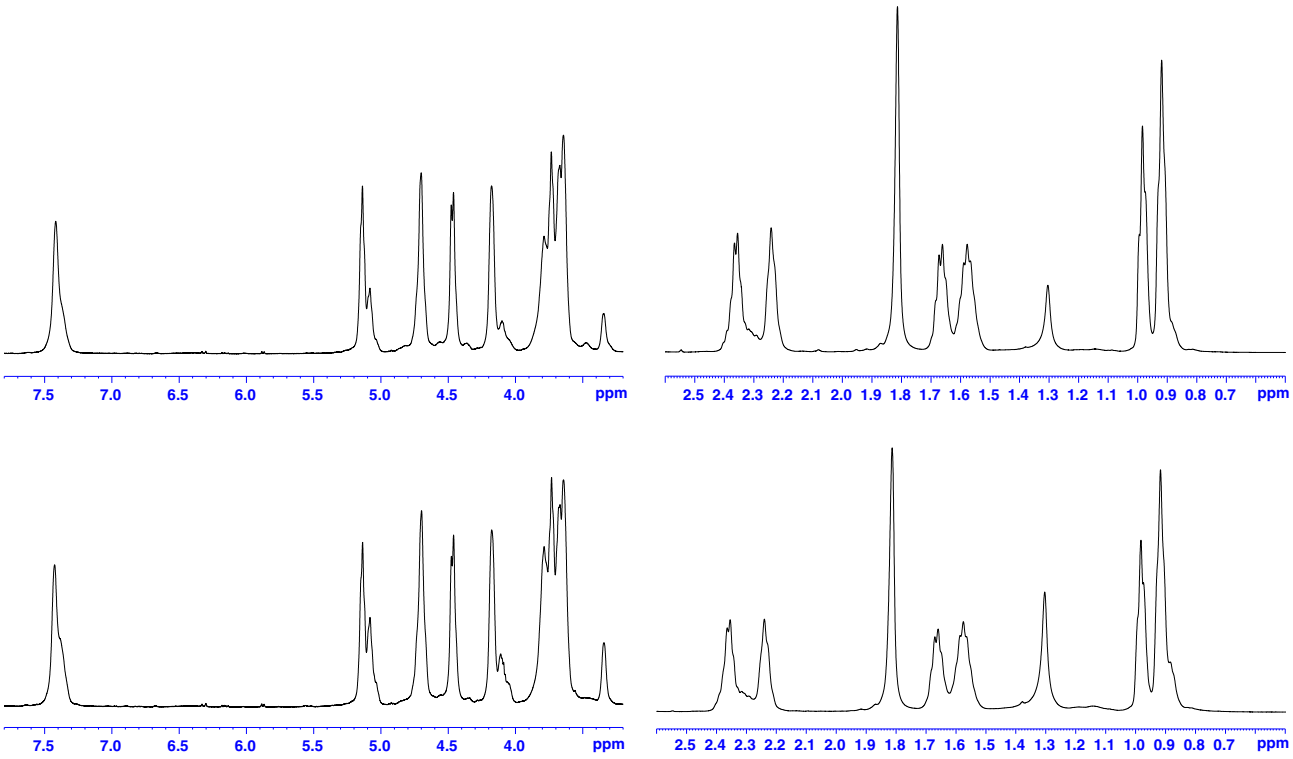

Figure 3. 600 MHz ^{1}H NMR spectra of chitin butyrates (4 mg/mL DMF-d_7). The regions of frequencies, referenced to the internal signal of DMF (δ 2.74), comprising the sugar backbone proton (left) and methyl and methylene proton signals (right), are shown. Top, DS 1.9 regenerated chitin butyrate; bottom, DS 1.9 chitin butyrate (C-B1F2 in Table 1).

like a fiber and are thermally stable and rigid over the range of temperatures. The measurements, when conducted to a ramp temperature of 300 °C, ended with all the ester films becoming charred. Abrupt changes in their storage moduli were noted around 250 °C, indicating thermal degradation of the ester films.

The changes in the moduli with temperature are presented in Figure 4 for various sizes of chitin butyrates, and the T_gs are summarized in Table 2, together with those of other chitin esters. The T_g was highest for chitin acetate (218 °C), lowest for chitin octanoate (183 °C), and between these values for other esters. The T_g was depressed with an increase in the chain length of the acyl group for the single-acid esters, and increased with insertion of acetic acid for the mixed-acid esters.

The T_gs for various chitin butyrates, which were fractionated on the basis of their solubility in acetone, increased with increase in molecular weight (C-B2F2, C-B1F2, and C-B1F1), and decreased with the increased substitution of butyrate within molecules of the similar size (B1F1 and B2F1). The detection of two different T_gs for the C-B2F2 chitin butyrate reflected two thermally differentiated domains of two distinctly different molecular sizes, one lower T_g for the molecules of smaller mass and the other for the larger molecules. The detection of two different T_gs was also noted for other chitin ester preparations, but rather as a hump or smeared peak for the smaller molecules on the shoulder before the principal peak emerged for larger molecules (Fig. 5).

The glass transition temperature of viscoelastic materials is considered to depend on the chain stiffness of the polymers and the effectiveness of intermolecular forces. The stiffness of the ester films, determined by the tensile moduli (E') at 25 °C (Table 2), was truly reflective of the glass transition behavior as seen above: the highest modulus for the chitin acetate (5850 MPa) and the lowest for chitin octanoate (1511 MPa), and values between these for the other esters. The tensile modulus decreased with an increase in the chain length of the acyl group for the single-acid esters, and increased with insertion of acetate for the mixed-acid esters. The tensile modulus increased with an increase in the molecular weight among the chitin butyrates, and had a tendency to level off for esters of molecular weight $\geqslant$135 kDa.

The decreases in E' were inversely related to the increase in the acyl chain lengths, particularly noted for the single-acid esters (Table 2): the stiffness of chitin acetate was 1.7–1.8 times for that of chitin butyrate (3245–3460 MPa), 3.1 times for chitin hexanoate (1884 MPa), and 3.9 times for chitin octanoate (1511 MPa), which is close to the inverse of the 2-, 3-, and 4-fold increments of the acyl chain length of the corresponding acid to acetic acid. Such correlation reflects somewhat the variation in the tensile modulus resulting from the nature of the homogeneous distribution of these

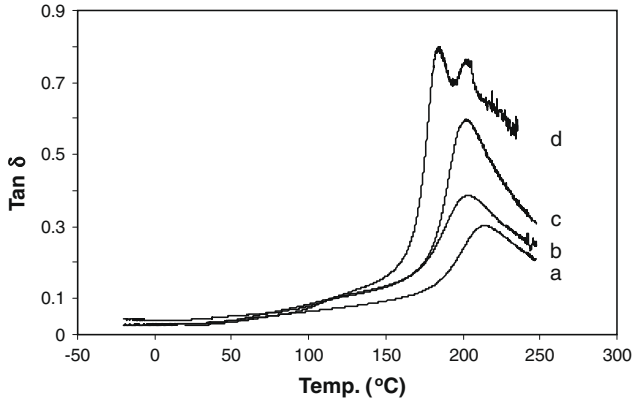

Figure 4. Glass transition temperature (T_g) for different molecular weights of chitin butyrates, determined as the maximum loss tangent (tanδ) by dynamic mechanical analyses of ester films over the temperature of −20 to 250 °C at 3 °C min^{-1}. (a) C-B1F1 (199 kDa); (b) C-B2F1 (203 kDa); (c) C-B1F2 (135 kDa); (d) C-B2F2 (44 kDa).

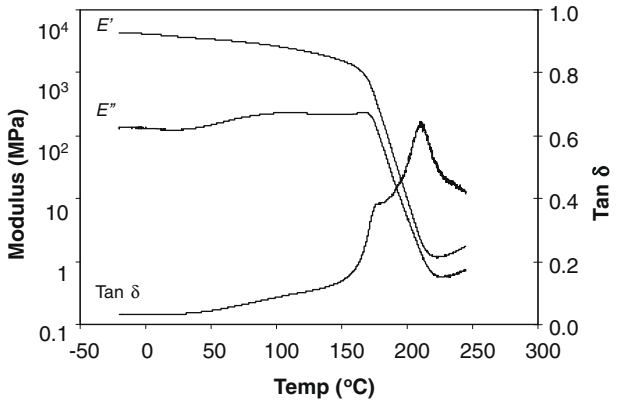

Figure 5. DMA analysis of chitin co-acetate/butyrate (C-AB2) film as a representative for chitin esters, reflecting the two principal domains of different molecular weights thermally differentiated. The shoulder peak for smaller molecules and the principal peak for the larger molecules. E', storage modulus; E'', loss modulus; tan δ, loss tangent (E''/E').

acyl groups along the chain and a cumulative effect of acyl chain length of the acid. These properties were different for starch esters, and the variation in the tensile modulus was multiple orders of magnitude, depending on the nature of the acyl chain length: the tensile modulus of the starch acetate (2158 MPa) was about seven times higher than that of starch hexanoate, 20 times higher than that of starch octanoate, and 75 times higher than that of starch palmitate. Thus, the insertion of longer chain fatty acids alone, such as starch octanoate and palmitate esters, resulted in much softer film with poor mechanical properties. For chitin esters, such a relatively smaller variation of tensile modulus would be attributed to the linear molecules of the backbone sugar residues for the rigidity and flexibility of the ester films.

4. Conclusion

The abundant biorenewable nature of chitin and its proven biocompatibility have drawn tremendous interest for industrial and medical applications.[24,25] The existence of chitin in a complex with minerals, protein, or lipids necessitates harsh conditions for obtaining the purified products, resulting in some degradation of the polymers. Limited solubility and increased viscosity are other constraints for the effective usages of chitin.

The facile and gentle preparation of chitin esters with the use of TFAA as a promoter provided materials that readily dissolved in DMF. The films cast from these esters were hard and flexible, and had tensile moduli of 1.5–5.8 GPa, close to those of commercial polymers (Matweb.com/tensilestrength), such as polystyrene (3 GPa), high-density polyethylene (0.8 GPa), or nylon 6 (1.8 GPa). The insertion of acetic acid as a mutual component further improved the tensile modulus. The T_gs of the films revealed the potential of chitin esters being moldable at temperatures of 180–220 °C, with thermal stabilities of up to about 250 °C. It is also conceivable that the efficient esterification of chitin, followed by O-deacylation, would serve as an alternative to the harsh and lengthy process for chitin purification.

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