ELSEVIER

Carbohydrate Polymers 48 (2002) 203–207

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Novel *N*-saturated-fatty-acyl derivatives of chitosan soluble in water and in aqueous acid and alkaline solutions

Shigehiro Hirano*, Yasuhiro Yamaguchi, Mitsutomo Kamiya

Chitin/Chitosan R and D Center, 445-Sakuradani, Tottori 680-0853, Japan

Received 14 September 2000; revised 14 March 2001; accepted 12 April 2001

Abstract

Novel *N*-saturated fatty acyl derivatives of chitosan soluble in water and in aqueous alkaline and acid solutions were prepared in 45–72% yields by *N*-deacylation of sodium *N*-acylchitosan salts, and in 75–85% yields by *N*-acylation of chitosan. The *N*-acyl derivatives soluble in water and 2% aqueous sodium hydroxide were in the degree of substitution (d.s.) ranges of 0.42–0.82 (d.s. 0.40 in width) for *N*-acetyl, 0.37–0.76 (d.s. 0.39 in width) for *N*-propionyl, 0.52–0.71 (d.s. 0.19 in width) for *N*-butyryl and 0.54–0.64 (d.s. 0.10 in width) for *N*-pentanoyl, and 0.58 (narrow d.s. in width) for *N*-hexanoyl. The *N*-acyl derivatives soluble in 2% aqueous acetic acid were in the d.s. ranges of <0.82 for *N*-acetyl, <0.76 for *N*-propionyl, <0.71 for *N*-butyryl, <0.64 for *N*-pentanoyl, <0.58 for *N*-hexanoyl, <0.41 for *N*-octanoyl, <0.24 for *N*-decanoyl, <0.15 for *N*-lauroyl, <0.05 for *N*-myristoyl. However, *N*-palmitoyl and *N*-stearoyl derivatives in all the d.s. ranges were insoluble in water, 2% aqueous sodium hydroxide and 2% aqueous acetic acid solutions.

Keywords: *N*-Acylation; Chitin; Chitosan; *N*-Deacylation; Water-soluble *N*-fatty acyl chitosan

1. Introduction

Chitin is insoluble in water, aqueous alkaline and acid solutions because of its intra- and inter-molecular bonds. Chitosan is soluble in aqueous acid solutions when forming its quaternary ammonium salt with organic acids. The so called 'water-soluble chitin', which has amino and *N*-acetyl groups in about 1:1 ratio, was originally prepared by *N*-deacetylation of sodium chitin salt (alkaline chitin) with 14% aqueous sodium hydroxide (San-nan, Kurita & Iwakura, 1975) and by *N*-acetylation of chitosan with acetic anhydride and pyridine (Kurita, Koyama, Nishimura & Kamiya, 1989; Kurita, Koyama & Nishimura, 1991). The water-soluble derivative has been used as a starting material for the chemical modification of chitosan (Kurita, Yoshida & Koyama, 1988), as a substrate for chitinase and chitosanase (Hutadilok et al., 1995), and as ecological and environmental friendly materials in agricultural, biomedical, cosmetic and food additive fields (Hirano, 1996). On the other hand, *N*-long-chain saturated fatty acyl derivatives insoluble in water were used as a media for collecting and purifying some important oils (Muzzarelli, Frega, Milians, Muzzarelli & Cartolari, 2000) and for affinity chromatography (Hirano, 1996). Little is known about *N*-long-chain saturated fatty acyl derivatives soluble in water and in aqueous alkaline and acid solutions with respect to the structure (chain-length), degree of substitution (d.s.) and distribution of *N*-acyl groups.

Now we report a facile method for the preparation of novel *N*-saturated-fatty-acyl derivatives of chitosan soluble in water and in aqueous alkaline and acid solutions, and the effects of the structure and degree of substitution of *N*-acyl groups on the solubility are discussed.

2. Experimental

2.1. Materials

A sample (Young Deok Chitosan, Young deok, South Korea) of crab shell chitosan was purified by treating it with 40% aqueous sodium hydroxide at 115–117°C for 4 h to give rise to a purified sample. Anal. calc. for $[C_6H_{11}NO_4{\cdot}0.69H_2O]_n$: C, 41.52; H, 7.14; N, 8.07. Found: C, 41.82; H, 7.10; N, 7.93. $[\alpha]_D^{16} = -8°$ (*c* 0.5, 2% aqueous acetic acid). *N*-Formyl (d.s. 1.0), *N*-acetyl (d.s. 1.0), *N*-propionyl (d.s. 1.0), *N*-butyryl (d.s. 1.0) and *N* pentanoyl (d.s. 0.9) derivatives of chitosan were prepared by the conventional method (Hirano & Yagi, 1980). Sodium *N*-propionylchitosan salt, $[\alpha]_D^5 = -16°$ (*c* 0.4, 14% aqueous

* Corresponding author.

0144-8617/02/$ - see front matter
PII: S0144-8617(01)00243-0

Table 1
The d.s. for *N*-acyl group, and the appearance and solubility for the products obtained in the reactions of chitosan with saturated fatty acid anhydrides

N-Acyl group	Acid anhydride added[a] (d.s. for *N*-acyl)	Product appearance[b]	Solubility of the products[c]		
			Water	2% aqueous NaOH	2% aqueous AcOH
Acetyl	0.3	L	i	i	s
	0.5	L	i	i	s
	0.7 (0.42)[d]	L	s	s	s
	0.9	L	s	s	s
	1.1	L	s	s	s
	1.3	L	s	s	s
	1.5 (0.82)[e]	L	s	s	s
	1.7	L	i + s	i	i
	1.9	G + L	i	i	i
	2.1	G	i	i	i
	3.1	G	i	i	i
Propionyl	0.3	L	i	i	s
	0.5	L	i	i	s
	0.7 (0.45)[f]	L	s	s	s
	0.9	L	s	s	s
	1.1	L	s	s	s
	1.3	L	s	s	s
	1.5 (0.76)[g]	L	s	s	s
	1.7	G	s + i	i	i
	1.9	G	i	i	i
	3.1	G	i	i	i
Butyryl	0.1	L	i	i	s
	0.3	L	i	i	s
	0.5	L	i	i	s
	0.7 (0.52)[h]	L	s	s	s
	0.9	L	s	s	s
	1.1	L	s	s	s
	1.3	L	s	s	s
	1.5 (0.71)[i]	L	s	s	s
	1.7	L	s	s	s
	1.9	G	i	i	i
	2.1	G	i	i	i
Pentanoyl	0.1	L	i	i	s
	0.3	L	i	i	s
	0.5	L	i	s	s
	0.7 (0.54)[j]	L	s	s	s
	0.9	L	s	s	s
	1.1	L	s	s	s
	1.3	L	s	s	s
	1.5 (0.64)[k]	L	s	s	s
	1.7	L	i + s	i	i
	1.9	G	i	i	i
Hexanoyl	0.1	L	i	i	s
	0.3	L	i	i	s
	0.5	L	i	i	s
	0.7	L	i	i	s
	0.9	L	i	i	s
	1.1 (0.58)[l]	L	s	s	s
	1.3	G	i	i	i
	1.5	G	i	i	i
	1.9	G	i	i	i
Octanoyl	0.5 (0.42)[m]	L	i	i	s
	0.7	L	i	i	w
	0.9	L + G	i	i	i

Table 1 (*continued*)

N-Acyl group	Acid anhydride added[a] (d.s. for *N*-acyl)	Product appearance[b]	Solubility of the products[c]		
			Water	2% aqueous NaOH	2% aqueous AcOH
	1.1	G	i	i	i
	1.3	G	i	i	i
	1.5	G	i	i	i
Decanoyl	0.1	L	i	i	s
	0.5 (0.24)[n]	L	i	i	s
	0.7	L	i	i	i
Lauroyl	0.1 (0.15)[o]	L	i	i	s
	0.5	L	i	i	i
Myristoyl	0.1 (0.05)[p]	L	i	i	s
	0.5	L	i	i	i
Palmitoyl	0.1	L	i	i	i + s
	0.3	L	i	i	i
	0.5	L	i	i	i
Stearoyl	0.1	L	i	i	i + s
	0.3	L	i	i	i

[a] Mol for the corresponding anhydride per GlcN.
[b] L, liquid; P, precipitate; G, hydrogel.
[c] i, insoluble; s, soluble; w, swelling.
[d] Anal. calc. for $[C_6H_{10}O_4N(C_2H_3O)_{0.42}(H)_{0.58}\cdot 1.14H_2O]_n$: C, 41.04; H, 7.46; N, 7.00. Found: C, 41.08; H, 7.47; N, 7.03. $[\alpha]_D^{17} = -6°$ (*c* 0.2, 2% aqueous AcOH).
[e] Anal. calc. for $[C_6H_{10}O_4N(C_2H_3O)_{0.82}(H)_{0.18}\cdot 1.23H_2O]_n$: C, 42.14; H, 6.94; N, 6.43. Found: C, 42.26; H, 6.57; N, 6.50.
[f] Anal. calc. for $[C_6H_{10}O_4N(C_3H_5O)_{0.45}(H)_{0.55}\cdot 0.98H_2O]_n$: C, 43.27; H, 7.24; N, 6.87. Found: C, 43.23; H, 7.23; N, 6.92. $[\alpha]_D^{17} = -4°$ (*c* 0.2, 2% aqueous AcOH).
[g] Anal..calc. for $[C_6H_{10}O_4N(C_3H_5O)_{0.76}(H)_{0.24}.1.01H_2O]_n$: C, 44.81; H, 7.24; N, 6.31. Found: C, 44.75; H, 7.24; N, 6.35.
[h] Anal. calc. for $[C_6H_{10}O_4N(C_4H_7O)_{0.52}(H)_{0.48}\cdot 1.48H_2O]_n$: C, 43.28; H, 7.62; N, 6.25. Found: C, 43.21; H, 7.63; N, 62.4. $[\alpha]_D^{17} = -7°$ (*c* 0.2, 2% aqueous AcOH).
[i] Anal. calc. for $[C_6H_{10}O_4N(C_4H_7O)_{0.71}(H)_{0.29}\cdot 1.18H_2O]_n$: C, 45.74; H, 7.60; N, 6.04. Found: C, 45.67; H, 7.60; N, 6.03.
[j] Anal. calc. for $[C_6H_{10}O_4N(C_5H_9O)_{0.54}(H)_{0.46}\cdot 0.86H_2O]_n$: C, 47.06; H, 7.68; N, 6.31. Found: C, 47.04; H, 7.71; N, 6.36. $[\alpha]_D^{17} = -3°$ (*c* 0.2, 2% aqueous AcOH).
[k] Anal. calc. for $[C_6H_{10}O_4N(C_5H_9O)_{0.64}(H)_{0.36}\cdot 1.31H_2O]_n$: C, 46.32; H, 7.86; N, 5.87. Found: C, 46.32; H, 7.82; N, 5.94.
[l] Anal. calc. for $[C_6H_{10}O_4N(C_6H_{11}O)_{0.58}(H)_{0.42}\cdot 1.17H_2O]_n$: C, 47.62; H, 8.01; N, 5.86. Found: C, 47.74; H, 7.63; N, 5.89. $[\alpha]_D^{12} = -12°$ (*c* 0.2, 2% aqueous AcOH).
[m] Anal. calc. for $[C_6H_{10}O_4N(C_8H_{15}O)_{0.41}(H)_{0.59}\cdot 0.90H_2O]_n$: C, 44.80; H, 7.46; N, 5.63. Found: C, 44.89; H, 7.56; N, 5.54.
[n] Anal. calc. for $[C_6H_{10}O_4N(C_{10}H_{19}O)_{0.24}(H)_{0.76}\cdot 1.10H_2O]_n$: C, 46.29; H, 8.05; N, 6.43. Found: C, 46.30; H, 7.95; N, 6.44.
[o] Anal. calc. for $[C_6H_{10}O_4N(C_{12}H_{23}O)_{0.15}(H)_{0.85}\cdot 0.83H_2O]_n$: C, 46.05; H, 7.85 N, 6.89. Found: C, 46.5; H, 7.96; N, 6.81.
[p] Anal. calc. for $[C_6H_{10}O_4N(C_{14}H_{31}O)_{0.05}(H)_{0.95}\cdot 1.17H_2O]_n$: C, 42.01; H, 7.81; N, 7.17. Found: C, 41.69; H, 7.85; N, 7.15. $[\alpha]_D^{20} = -1°$ (*c* 0.2, 2% aqueous AcOH).

sodium hydroxide) and sodium *N*-acetylchitosan salt, $[\alpha]_D^5 = -6°$ (*c* 0.4, 14% aqueous sodium hydroxide) were prepared as reported previously (Hirano & Midorikawa, 1998).

2.2. Methods

FTIR spectra (KBr) were recorded on a Jasco FTIR 5300 spectrometer (JASCO, Tokyo), and specific rotations on a Horiba SEPA-200 autopolarimeter (Horiba, Kyoto). Elemental analyses were performed at the Micro-analytical Center at Kyoto University, Kyoto, Japan. The d.s. for *N*-acyl group was calculated on the basis of C/N ratio in the elemental analysis data.

2.3. Partial N-deacylation

A 2% solution of sodium *N*-propionylchitosan salt in 14% aqueous sodium hydroxide was heated at 60, 80 and 100°C, respectively. An aliquot was withdrawn from each of the solutions at an interval of 1 h, and the solution was adjusted at pH 7.5 with 3% aqueous HCl for the solubility test. No precipitate appeared after heating the sodium *N*-propionylchitosan salt at 60°C for 17 h (the reaction condition 1), 80° for 14 h (the reaction condition 2) and 100°C for 12 h. Each of the reaction products was dialyzed against running water and in distilled water for one day at room temperature. The produced solution was concentrated in vacuo at <45°C to a ca. 20 ml-volume, and treated in two ways: (1) the solution

was lyophilized to give rise to an amorphous white product which was soluble in water with slight turbidity, and (2) three volumes of ethanol were added to give rise to a white precipitate which was insoluble in water. However, both the products were soluble in 2% aqueous acetic acid at room temperature. D.s. for *N*-propionyl was in the range 0.37–0.46. A product (d.s. 0.54 for *N*-propionyl) was obtained in 61% yield under the reaction condition 1. ν_{max} (KBr) 1658 and 1567 cm^{-1} (C=O and NH for *N*-propionyl). Anal. calc. for $[C_6H_{10}O_4N(C_3H_5O)_{0.54}(H)_{0.46}{\cdot}0.05H_2O]_n$: C, 47, 68; H, 6.90; N, 7.30. Found: C, 47.60; H, 7.01; N, 7.28. $[\alpha]_D^{25} = -12°$ (*c* 0.2, 2% aqueous acetic acid); ν_{max} (KBr) 1656 and 1555 cm^{-1} (C=O and NH for *N*-propionyl). The other product (d.s. 0.37 for *N*-propionyl) was obtained in 41% yield under reaction condition 2. Anal. calc. for $[C_6H_{10}O_4N(C_3H_5O)_{0.37}(H)_{0.67}{\cdot}0.08H_2O]_n$: C, 38.31; H, 6.37; N, 6.27. Found: C, 38.19; H, 6.42; N, 6.26. $[\alpha]_D^{25} = -15°$ (*c* 0.2, 2% aqueous acetic acid): ν_{max} (KBr) 1658 and 1557 cm^{-1} (C=O and NH for *N*-propionyl). *N*-Deacetyl derivatives (d.s. 0.48 and 0.58 for *N*-acetyl) soluble in water and in 2% aqueous sodium hydroxide were obtained by heating a 2% alkaline chitin solution in 14% aqueous sodium hydroxide at 60°C for 3 h and 80°C for 2 h, respectively, $[\alpha]_D^{15} = -5°$ (*c* 0.2, 2% aqueous acetic acid).

2.4. Partial N-acylation of chitosan

Chitosan (0.16 g) was dissolved in 2% aqueous acetic acid (20 ml)–methanol (40 ml), and a fatty acid anhydride (0.1–3.1 mol/GlcN) was added with mechanical stirring. The mixture solution was retained at room temperature overnight to give rise to a product in liquid (L), hydrogel (G) or swelling (W) state (Table 1). The reaction mixture was adjusted at pH 8–10 with 15% aqueous sodium hydroxide. After retaining at room temperature overnight, the mixture was dialyzed against running water overnight to give rise to a product. The product was adjusted at pH 4–5 with 2% aqueous hydrochloride, and the free long chain fatty acids generated were extracted with a portion of chloroform several times. The resulted aqueous solution was adjusted to pH 9 with 2% aqueous sodium hydroxide, and three volumes of ethanol were added. The produced precipitate was collected by filtration, washed with ethanol, and dried to give rise to the corresponding white product in 75–85% yields. The elemental analysis data are shown in the footnote of Table 1, and the structures were also confirmed by FTIR spectral analysis: ν_{max} (KBr) 2960–2900 (CH), 1654–1648 and 1558–1553 (C=O and NH for *N*-acyl), ~1070 (C–O) cm^{-1}.

3. Results and discussion

3.1. Partial N-deacylation

The *N*-depropionylation reaction of sodium *N*-propionylchitosan salt with 14% aqueous sodium hydroxide began in a slight turbidity state, but a clear solution was obtained soon after heating. The *N*-propionyl derivatives (d.s. 0.37–0.46) soluble in water and in 2% aqueous sodium hydroxide were obtained in 45–61% yields by heating a 2% sodium *N*-propionylchitosan salt solution in 14% aqueous sodium hydroxide at 60°C for 17 h, 80° for 14 h, and 100°C for 12 h. The *N*-deacetyl derivatives (d.s. 0.48–0.58) soluble in water and in 2% aqueous sodium hydroxide were obtained in 56–72% yields by heating a 2% alkaline chitin solution in 14% aqueous sodium hydroxide at 60°C for 3 h and at 80°C for 2 h. These data indicate that a slightly severe condition is required for *N*-depropionylation in reference to that for *N*-deacetylation. A slight turbidity appeared in the solution of partially *N*-propionyl derivatives within a few hours by retaining at room temperature, but no turbidity appeared in the solution of partially *N*-acetyl derivatives even after retaining for 12 h at room temperature. The lyophilized products were slightly soluble in water, but the products that precipitated with ethanol were insoluble in water. Both the products were soluble in 2% aqueous acetic acid. Sodium *N*-acetylchitosan salt and sodium *N*-propionylchitosan salt were soluble in 14% aqueous sodium hydroxide. However, the preparation of sodium salts of *N*-formylchitosan, *N*-butyroylchitosan and *N*-pentanoylchitosan was unsuccessful under the current conditions.

3.2. Partial N-acylation of chitosan

Table 1 shows the d.s. for *N*-acyl groups and the appearance and solubility of the products. The *N*-acylation was performed in 2% aqueous acetic acid–methanol (1:2, v/v) (Hirano, Ohe & Ono, 1976). Neither precipitate nor hydrogel appeared in the reactions in the range 0.7–1.5 mol acetic, propionic, butyric, pentanoic or hexanoic anhydrides per GlcN. Each of the reaction products was dialyzed against running water overnight, and a clear solution was obtained. No precipitate appeared at pH 3 and 10. To each of the dialyzed solutions were added three volumes of ethanol to give rise to a white precipitate, which was filtered, washed with ethanol and air-dried. The dried sample was soluble in 2% aqueous acetic acid but insoluble in water and in aqueous 2% sodium hydroxide. For their solubilization again in water, the sample was dissolved in 2% aqueous acetic acid, and the solution was adjusted to pH ca. 8 with 2% aqueous sodium hydroxide. The solution was dialyzed again against running water to give rise to an aqueous clear solution containing the products.

3.3. Effects of the structure and d.s. of N-acyl group on the solubility

The partial *N*-deacylation was performed on sodium *N*-acylchitosan salts in a 14% aqueous sodium hydroxide solution, and the partial *N*-acylation was performed on chitosan in a 2% aqueous acetic acid–methanol solution.

It is considered that similar stretched chain conformations exit in each of the solutions because –ONa groups are ionized into $-O^{-}Na^{+}$ in the aqueous alkaline solution for alkaline chitin, and $-NH_2$ groups are ionized into $-NH_4^{+}$ groups in the aqueous acetic acid–methanol solution for chitosan. Since both the reactions were performed in clear homogeneous solution conditions, the residual *N*-acyl groups over the chains in the products are distributed randomly but are not localized as blocks (Kurita et al., 1977). A random distribution of *N*-acyl groups was also previously confirmed by the gel permeation chromatography of their oxidized products with nitrous acid, followed by the reduction with sodium borohydride (Hirano, Tsuneyasu & Kondo, 1981).

The *N*-fatty acyl derivatives soluble in water and in aqueous alkaline solutions were in the d.s. ranges 0.42–0.82 for *N*-acetyl (d.s. 0.40 in width), 0.37–0.76 (d.s. 0.39 in width) for *N*-propionyl, 0.52–0.71 (d.s. 0.19 in width) for *N*-butyryl and 0.54–0.64 (d.s. 0.1 in width) for *N*-pentanoyl, and at d.s. 0.58 (narrow d.s. in width). The d.s. width becomes narrow and the d.s. values are astringent at ca. 0.58 with an increase in the chain length up to *N*-hexanoyl.

The derivatives soluble in 2% aqueous acetic acid were in the d.s. ranges of <0.82 for *N*-acetyl, <0.76 for *N*-propionyl, <0.71 for *N*-butyryl, <0.64 for *N*-pentanoyl, <0.58 for *N*-hexanoyl, <0.42 for *N*-octanoyl, <0.24 for *N*-decanoyl, <0.15 for *N*-lauroyl, <0.05 for *N*-myristoyl. *N*-Palmitoyl and *N*-stearoyl derivatives in all the d.s. values were insoluble in water, 2% aqueous sodium hydroxide and 2% aqueous acetic acid solutions.

In the aqueous solutions, the hydrophobic *N*-fatty acyl groups are present in the inner part of chain clusters, and the hydrophilic amino groups are present at the outer part, and their solubility is controlled by the proportion of the hydrophobic and the hydrophilic groups. A hydrogel was produced in the reactions with >1.9 mol per GlcN for acetic, propionic, butyric and pentanoic anhydrides, >1.3 mol per GlcN for hexanoic anhydride, and >1.1 mol per GlcN for octanoic anhydride under the current conditions. A precipitate appeared in the reactions with >ca. 0.5 mol per GlcN for decanoic, lauric, myristic, palmitic and stearic anhydrides.

4. Conclusions

Novel partially *N*-saturated fatty acyl derivatives of chitosan soluble in water and in aqueous alkaline and acid solutions were prepared in 45–72% yields by heating a 2% sodium *N*-propionylchitosan salt solution in 14% aqueous sodium hydroxide and in 75–85% yield by treating chitosan with saturated-fatty acid anhydrides in 2% aqueous acetic acid–methanol (1:2 v/v). The latter method is more convenient than the former method for the preparation of *N*-partial acyl derivatives of chitosan soluble in water and in aqueous alkaline and acid solutions.

References

Hirano, S. (1996). Chitin biotechnology applications. *Biotechnology Annual Review*, *2*, 237–258.

Hirano, S., & Midorikawa, T. (1998). Novel method for the preparation of *N*-acylchitosan fiber and *N*-acylchitosan–cellulose fiber. *Biomaterials*, *19*, 293–297.

Hirano, S., & Yagi, Y. (1980). The effects of *N*-substitution of chitosan and the physical form of the products on the rate of hydrolysis by chitinase from *Streptomyces griseus*. *Carbohydrate Research*, *83*, 103–108.

Hirano, S., Ohe, Y., & Ono, H. (1976). Selective *N*-acylation of chitosan. *Carbohydrate Research*, *47*, 314–320.

Hirano, S., Tsuneyasu, S., & Kondo, Y. (1981). Heterogeneous distribution of amino groups in partially *N*-acetylated derivatives of chitosan. *Agricultural and Biological Chemistry*, *88*, 172–175.

Hutadilok, N., et al. (1995). The effect of *N*-substitution on the hydrolysis of chitosan by an endo-chitinase. *Carbohydrate Research*, *268*, 143–149.

Kurita, K., Yoshida, A., & Koyama, Y. (1988). Studies on chitin 13 new polysaccharide/polypeptide hybrid materials based on chitin and poly(γ-methyl-L-glutamate). *Macromolecules*, *21*, 1579–1583.

Kurita, K., Koyama, Y., Nishimura, S., & Kamiya, M. (1989). Facile preparation of water-soluble chitin from chitosan. *Chemistry Letter*, *1989*, 1597–1598.

Kurita, K., Koyama, Y., & Nishimura, S. (1991). Solubilization of a rigid polysaccharide: Controlled partial *N*-acylation of chitosan to develop solubility. *Carbohydrate Polymers*, *16*, 83–89.

Muzzarelli, R. A. A., Frega, N., Milians, M., Muzzarelli, C., & Cartolari, M. (2000). Interactions of chitin, chitosan, *N*-lauroylchitosan and *N*-dimethylaminopropionyl chitosan with olive oil. *Carbohydrate Polymers*, *43*, 263–268.

San-nan, T., Kurita, K., & Iwakura, Y. (1975). Chitin 1. Solubility change by alkaline treatment and film casting. *Makromolekular Chemie*, *176*, 1191–1195.