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The use of intense femtosecond laser pulses for the fragmentation of chitosan

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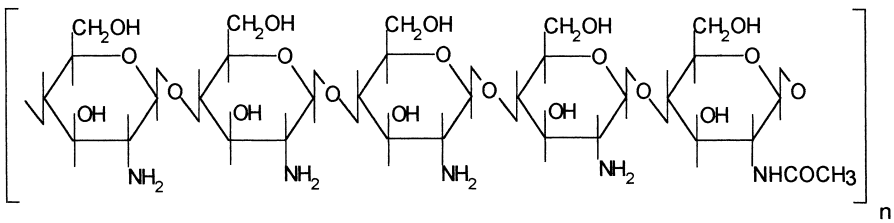
Abstract

The use of ultrashort laser pulses for the fragmentation of chitosan was investigated. Femtosecond Ti-saphire laser pulses were focused into a flask containing 1.0% chitosan in 0.1 M acetic acid. The effects of the pulse energy (between 0.1 and 0.82 mJ) and the focal length on the laser-induced fragmentation were followed by viscometry and size exclusion chromatography. The chemical structure and degree of acetylation of chitosan and its fragments were studied using elemental analysis, IR and ^{1}H NMR spectroscopy. The experimental results showed that (i) Ti-saphire laser irradiation induced chain scission in the chitosan macromolecules, (ii) the chemical structure, including the degree of acetylation, did not change significantly upon laser irradiation, (iii) the number of chain scission dependence on laser energy suggests that fragmentation was a two-photon process, and (iv) at constant pulse energy, the molecular weight dropped to a minimum as a function of the focal length (between 45 and 330 mm), indicating that the efficiency of fragmentation was very sensitive to the geometry of the laser beam.

Keywords: Chitosan; Fragmentation; Femtosecond; Ti-saphire laser; Molecular weight

1. Introduction

Chitosan is a linear copolymer of glucosamine and *N*-acetylated glucosamine. The chemical structure of chitosan with a degree of acetylation of 20% is shown below:



This cationic biopolymer exhibits a variety of biological activities, especially antifungal activity [1]. A method to fragment chitosan without losing its biological activity would facilitate its applications in biodegradable films for food coating and as an antimicrobial agent for the preservation of fresh fruits and vegetables [2].

Chitosan with different degrees of polymerization can be obtained by various methods, including acid hydrolysis, oxidation with sodium nitrite and nitrous acid [3–5]. Use of energy from an electromagnetic radiation can be an alternative, and does not require chemical agents. The absorption of light by a polymer molecule can result in scission of chemical bonds. If the broken bond belongs to the backbone of the polymer, a decrease in the polymer molecular weight would result [6].

Photodegradation, photodecomposition, photodegradation and ablation of polymers has been mainly studied in the solid state [7–12]. However, there are some reports on the degradation of synthetic polymers [13,14] and biopolymer [15] in solution by UV or visible light. UV laser ablation

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of organic polymers has been reviewed by Srinivasan and Braren [16]. Ablation with shortened pulse duration, i.e. femtosecond, yields results that are different from those obtained with longer (nanosecond) pulses [17]. On the other hand, flash photolysis of polystyrene solutions using a Neodymium glass laser ($\lambda = 265$ nm) was shown to induce main-chain cleavage but only in the presence of oxygen [18].

The objective of this study was to investigate the possibility of chitosan fragmentation in solution by femtosecond laser light, and to evaluate the molecular weight and the structure of the resultant fragments.

2. Experimental details

2.1. Materials

Shrimp-shell chitosan, with a nominal degree of acetylation (DA) of 20%, was purchased from Nova-Chem. Ltd. (Halifax, Nova Scotia, Canada) and purified. Pullulan standards ($5.88 \leq M_w \leq 1660$ kDa; $1.06 \leq M_w/M_n \leq 1.19$) were purchased from the American Polymer Standards Corporation (Mentor, OH). Acetic acid (HAc) and sodium acetate (NaAc) were of HPLC grade. KBr, CD_3COOD and D_2O were of analytical grade. All other chemicals were reagent grade.

2.2. Fragmentation

Forty grams of a 1.0% (w/v) chitosan solution in 0.1 M HAc were placed in a 50 ml spherical flask. A femtosecond Ti-saphire laser beam (pulse duration: 160 ± 20 fs; repetition rate: 1 kHz; central wavelength: 800 nm) was focused into the flask for 90 min at room temperature while the solution was constantly stirred. Measurements were performed as a function of the pulse energy in the range 0.1–0.82 mJ, with a 38 mm focal length lens. The fluctuation of the pulse energy was $\pm 10\%$. Another set of experiment was carried out at a constant energy of 0.57 mJ with different focal lengths in the range of 45–330 mm. In all cases, the position of the lens respective to the flask was adjusted in order to focus the beam at the center of the solution. After exposure to the laser radiation, the solution was neutralized with 1.0 N NaOH to precipitate the chitosan, and the suspension was centrifuged, washed with deionized water, and lyophilized.

2.3. Viscometry

The intrinsic viscosities of the original chitosan and its fragments were measured in a capillary viscometer (Model AMV-200, Paar Physica USA, Edison, NJ) in 0.1 M HAc/0.02 M NaCl at 25°C. The viscosity-average molecular weight was calculated according to [19]:

$$[\eta] = 3.04 \times 10^{-5} M_v^{1.26} \quad (1)$$

2.4. Size exclusion chromatography

Size exclusion chromatography (SEC) was used for qualitative determination of the reduction in molecular weight and its distribution. A HPLC/SEC instrument (Hewlett-Packard, Series 1050) was used with a refractive index detector, whose response is directly proportional to the polymer concentration in the eluting solution. Separation was achieved at 35°C using a TosoHaas-TSK gel column ($GMPW_{XL}$, 30 cm × 7.8 mm) with 0.25 M HAc/0.25 M NaAc as the eluent at a flow rate of 0.4 ml min^{-1}. A calibration curve was constructed from the peak molecular weights of pullulan standards as a function of elution volume, in order to ascertain the performance of the SEC columns and assembly. However, conversion of elution volume to molecular weight was not performed in the present work, since chromatograms were used only to evaluate the relative reduction in molecular weight and the changes in distribution of the fragments compared to the original chitosan.

2.5. Structural analysis

The structure of the original chitosan and a low molecular weight fragment were compared by elemental analysis, ^{1}H NMR and IR spectroscopy, in order to assess any structural modification during fragmentation. The elemental composition was determined using a Carlo-Erba 1108 Elemental Analyzer (Model EA 1109, CHN, FISONS Instruments). The degree of acetylation (DA), for the original chitosan and chitosan fragments was calculated from C/N, the carbon/nitrogen ratio. This ratio varies from 5.145 in fully *N*-deacetylated chitosan (repeat unit $C_6H_{11}NO_4$) to 6.861 in chitin, the fully *N*-acetylated polymer (repeat unit $C_8H_{13}ON_5$). The degree of acetylation was calculated according to:

$$DA = \frac{C/N - 5.145}{6.861 - 5.145} \times 100 \quad (2)$$

^{1}H NMR spectra of chitosan solutions in 2%(w/w) CD_3COOD/D_2O were obtained at 70°C using (Model AC-300 MHz spectrometer, Bruker, Billerica, MA). The experimental conditions were essentially the same as Hirai et al. (1991) [20], except for the polymer concentration (2.5 mg ml^{-1} for the original chitosan), the pulse repetition delay (6 s), the use of a single pulse sequence (40°) and the number of scans (16 and 64 for the fragment and the original chitosan, respectively). The degree of acetylation, DA, expressed in mole %, was determined using the following relation:

$$DA = \frac{(1/3 \text{ surface area at } 2.1 \text{ ppm})}{(\text{surface area at } 3.2 \text{ ppm}) \times 100} \quad (3)$$

where the resonances at 2.1 and 3.2 ppm correspond to methyl protons and the main-chain proton closer to the *N*-acetyl group respectively. IR spectra were obtained from

KBr pellets using a Sirius 100 FT-IR (Mattson Instruments, Madison, WI, USA).

3. Results and discussion

Fig. 1 shows the SEC chromatograms of the original chitosan and the typical samples resulting from fragmentation at different pulse energies. The viscosity-average molecular weight (M_v) of original chitosan and its fragments are presented in Table 1. In size exclusion chromatography, the elution volume decreased linearly with the logarithm of molecular weight. Therefore, the shift of the chromatograms towards higher elution volume with increase in pulse energy indicates that laser irradiation induces chitosan fragmentation, and that with increase in pulse energy, the molecular weight of the fragment decreases. The chromatograms did not broaden significantly when the molecular weight decreased, suggesting that the larger macromolecules were preferentially fragmented.

Fig. 2A shows the effect of the laser pulse energy on $[(M_{v,original}/M_{v,fragment})-1]$, the average number of chain scission [21]. A curvilinear relationship was obtained, indicative of a multi-photon process [22,23]. The plot was linear as a function of the square of the pulse energy (Fig. 2B), suggesting that the fragmentation of chitosan is a two-photon process in the energy range applied (0.1–0.82 mJ). Such an hypothesis is consistent with the excitation wavelength used in this study (800 nm or 150 kJ per mole of photon) and the dissociation energy of a typical C–O glucosidic linkage, the C–O bond between two repeat units (300–400 kJ mol^{-1}). Although the photochemical fragmentation of chitosan appears to proceed mainly through multi-photon absorption, secondary processes involving free

Table 1
Viscosity-average molecular weight (M_v) of the original chitosan and the fragmented chitosan samples irradiated with different laser energies (E) (at constant focal length of the lens, F) and at different focal lengths (at constant energy, E)

Chitosan	E (mJ)	F (mm)	$M_v \times 10^5$ (Da)
Original			21.0
Fragmented			
1	0.10	38	11.4
2	0.26	38	9.84
3	0.40	38	7.12
4	0.45	38	5.60
5	0.55	38	3.23
6	0.60	38	2.73
7	0.82	38	2.29
5	0.57	330	9.96
6	0.57	175	2.64
7	0.57	125	2.07
8	0.57	75	2.31
9	0.57	45	4.05

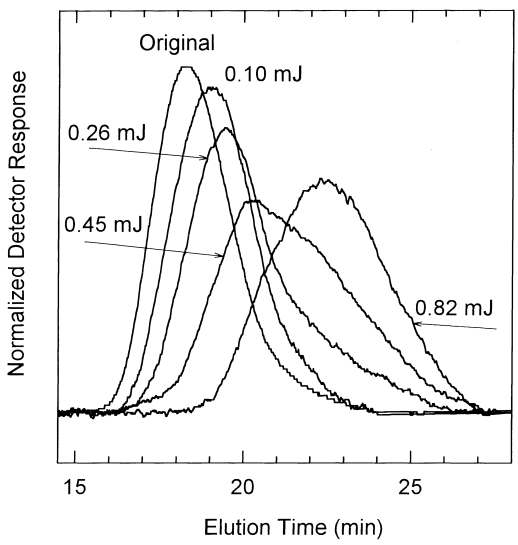

Fig. 1. Size-exclusion chromatograms of the original chitosan and typical fragments resulting from irradiation at different pulse energies (E). Focal length of the lens was 38 mm.

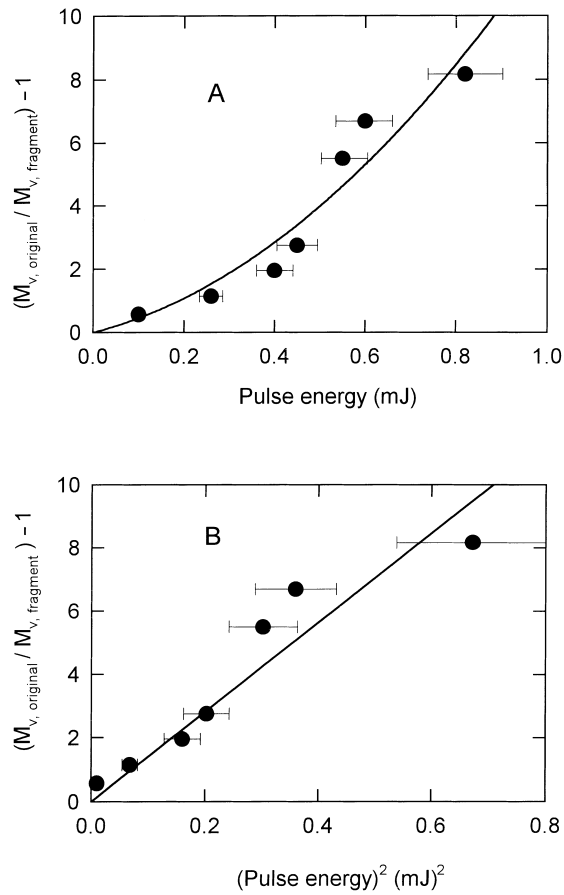

Fig. 2. Average number of chain scission, $[(M_{v,\ original}/M_{v,\ fragment})-1]$, as a function of (A) the laser pulse energy; and (B) square of the laser pulse energy. Horizontal bars represent the fluctuation of the laser pulse energy (10%). Focal length of the lens was 38 mm.

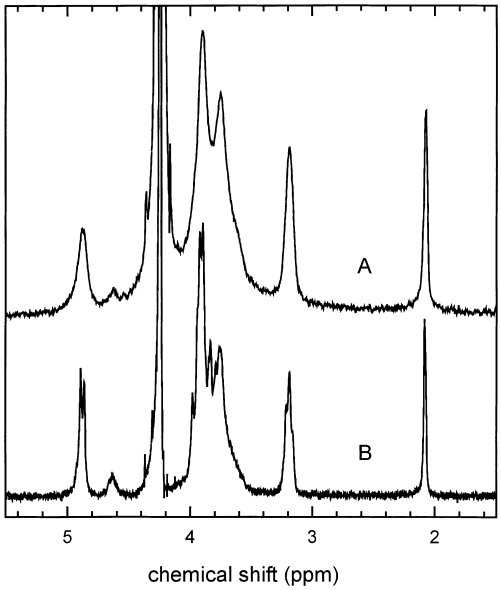

Fig. 3. ^{1}H NMR spectra of (A) original chitosan and (B) the fragment obtained with pulse energy of 0.82 mJ in 2% (w/w) CD_3COOD/D_2O at 70°C.

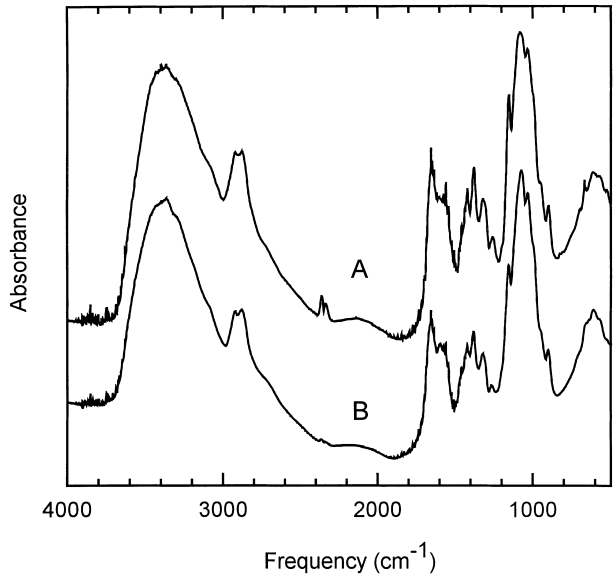

Fig. 4. IR spectra of (A) original chitosan and (B) the fragment obtained with pulse energy of 0.82 mJ.

radicals (oxidation–reduction reactions) may also play a minor role. Femtosecond laser irradiation has been shown to be sufficiently intense to photolyse water molecules, forming free radicals which can lead to the formation of hydrogen peroxide [24]. However, analysis of laser-irradiated chitosan solutions by both iodometric and permanganate methods revealed the presence of only traces of hydrogen peroxide, suggesting its participation in the fragmentation of chitosan can only be minor at best.

In order to assess the effect of laser irradiation on the chemical structure of the polymer, the fragment obtained from pulse energy of 0.82 mJ was analyzed by ^{1}H NMR, FTIR and elemental analysis. Fig. 3 shows the NMR spectra of the original material (spectrum A) and the fragment obtained with pulse energy of 0.82 mJ (spectrum B) in CD_3COOD/D_2O. The two spectra were nearly identical, except for the lower resolution for original chitosan, which is commonly observed for high molecular weight samples. They were similar to the spectra obtained by Hirai, Odani, and Nakajima [20]. In addition, the original polymer and the fragment exhibited essentially the same FTIR spectrum (Fig. 4), which is quite comparable to the one reported by Domszy and Roberts [25]. The degree of acetylation was calculated by ^{1}H NMR spectroscopy and elemental analysis. ^{1}H NMR spectroscopy yielded DA values of 21.9% and 20.2% for the original polymer and the fragment respectively, while the corresponding results from elemental analysis were 22.6 and 24%. These data suggest that no significant change in DA occurred during fragmentation.

A change in size and/or shape of the laser beam at the focal point in the solution was found to affect significantly the fragmentation of chitosan. Fig. 5 shows the SEC chromatograms of the original chitosan and the fragments obtained with lenses of different focal lengths. Again, no significant broadening of the molecular weight distribution occurred upon chain scission. The viscosity-average molecular weight of the fragments are listed in Table 1. Fig. 6 shows the viscosity-average molecular weight of the fragments as a function of the focal length. At constant pulse energy, the curve exhibits a minimum in M_v indicating that there exists an optimum focal length in the focal length range applied (45–330 mm) at which the efficiency of fragmentation is optimum. Presumably, a change in focal length affects the beam geometry, the fragmentation being

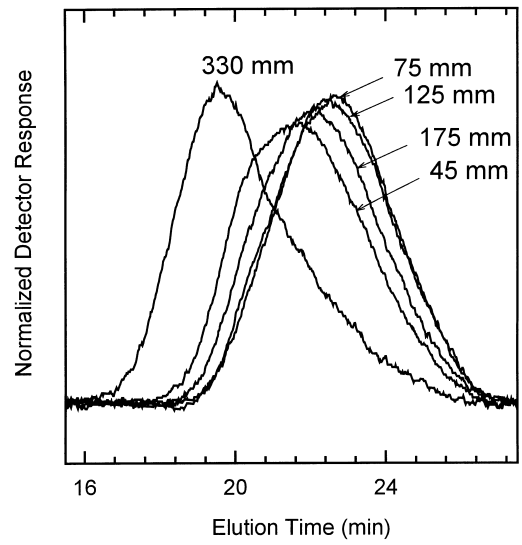

Fig. 5. Size-exclusion chromatograms of the original chitosan and the fragments resulting from irradiation at constant pulse energy (0.57 mJ) and with lenses of different focal length (*F*).

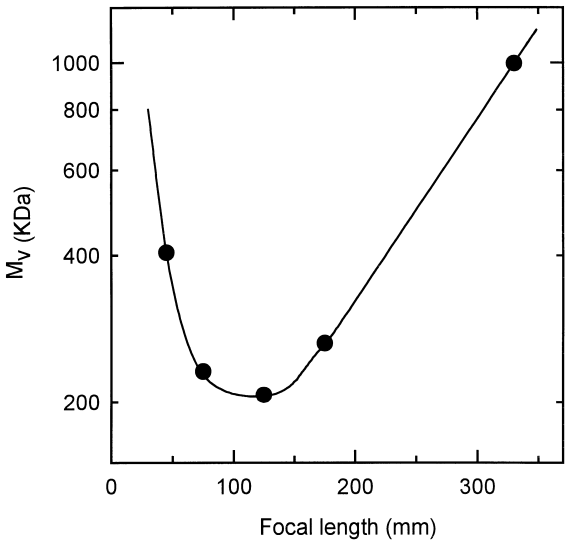

Fig. 6. Viscosity-average molecular weight of chitosan fragments irradiated at constant pulse energy (0.57 mJ) and with different lenses as a function of the focal length of the lens (F).

less efficient when the volume of the irradiated solution decreases.

4. Conclusions

Pulses from a Ti-saphire laser induce fragmentation in chitosan macromolecules in solution. The fragmentation appears to be a two-photon absorption process. The molecular weight of the fragment decreases as the pulse energy increases. At constant pulse energy, the efficiency of fragmentation is very sensitive to the geometry of the laser beam. Elemental analysis, FTIR and ^{1}H NMR spectroscopy shows that the chemical structure of chitosan, including the degree of acetylation, does not change significantly during laser irradiation.

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