

Kinetic aspects of chitosan fragmentation by femtosecond laser

Mohammad R. Kasaai^a, See L. Chin^b, Joseph Arul^{a,*}

^a Department of Food Science and Nutrition, Université Laval, Sainte-Foy, Que., Canada G1K 7P4

^b Department of Physics and COPL, Université Laval, Sainte-Foy, Que., Canada G1K 7P4

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Abstract

Kinetics of chitosan fragmentation by ultrafast laser pulses, and the effects of reactor shape, saturation of solution with oxygen and chitosan molecular weight on fragmentation were investigated. The degree of fragmentation and molecular weight distribution of the fragments were followed by viscometry and size exclusion chromatography. Chain scission increased with light pulse energy, irradiation time in sigmoidal manner, and dose in biphasic manner. It also increased with molecular weight of chitosan, indicating that larger molecules were preferentially fragmented. The polydispersity of fragments increased with irradiation time. There was only a minor change in chain scission when chitosan solution was saturated with oxygen suggesting that secondary processes involving oxidation–reduction reactions may play only a minor role, and fragmentation proceeds mainly through multi-photon absorption.

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1. Introduction

Chitosan is a cationic polysaccharide. The cationic nature of this polymer confers to its diverse biological properties, in addition to its film and fiber forming ability. Thus, it has potential for application in many areas such as food, agriculture, medicine, cosmetics, paper, textile and wastewater treatment [1]. The effectiveness and properties of chitosan appear to be dependent on its degree of acetylation (DA) and molecular weight in various applications [2–5], where high molecular weight of chitosan is either less effective or its application can generate practical difficulty owing to its high solution viscosity. Thus, the development of an efficient process for fragmentation and reduction in molecular weight without altering its chemical structure and DA of chitosan is required.

Chemical (HCl, NaNO₂ and H₂O₂) [6–9], enzymatic [10–12] and ultrasonic irradiation methods [13] have been previously employed for chitosan fragmentation. Physical methods such as laser irradiation can be an alternative, since they do not need chemical agents. We have earlier reported on the feasibility of chitosan fragmentation by femtosecond laser light and that chain scission proceeds mainly through multi-photon process [14]. We report here the kinetics of

chitosan fragmentation in solution including the effect of reaction conditions such as reactor shape and dissolved oxygen in solution as well as initial molecular weight of chitosan (a hydrodynamic parameter), which may affect chain scission.

2. Experimental details

2.1. Materials

High viscosity shrimp-shell chitosan, with a nominal DA of 25%, was obtained from Nova-Chem Ltd. (Halifax, NS, Canada) and purified. Acetic acid (HAc) and sodium acetate (NaAc) were of HPLC grade. CD₃COOD and D₂O were of analytical grade. All other chemicals were of 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 flask with curved section on one side replaced by a flat fused silica window (25 mm in diameter and 3.0 mm thick) as light port. A femtosecond Ti-sapphire laser beam (pulse duration: 160 ± 20 fs; repetition rate: 1 kHz; central wavelength: 800 nm) was focused into the flask through the flat light port using a lens with a focal length of 70 mm at room temperature while the

* Corresponding author. Tel.: +1-418-656-2839; fax: +1-418-656-3353.
E-mail address: joseph.arul@aln.ulaval.ca (J. Arul).

solution was constantly stirred. Rate studies were carried out as a function of irradiation time (0–90 min) at three different pulse energies of 0.36, 0.55 and 0.80 mJ in flasks. The effects of dissolved oxygen in solution and initial molecular weight of chitosan ($M_v = 1965, 1375$ and 749 kDa) on fragmentation were examined at constant pulse energy of 0.80 mJ and irradiation time of 45 and 90 min. The position of the lens respective to the flask was adjusted in order to focus the beam at the center of the solution in all experiments. After laser irradiation, chitosan was recovered as described elsewhere [14]; and characterized by viscometry and size exclusion chromatography (SEC).

2.3. Characterization

Intrinsic viscosities of chitosan and its fragments in 0.1 M HAc/0.02 M NaCl were measured on a capillary viscometer (model AMV-200, Paar Physica USA, Edison, NJ) at 25 °C. The viscosity-average molecular weight was calculated according to Roberts and Domszy [15].

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

The average number of chain scission (α) was calculated from the viscosity-average molecular weight of original chitosan ($M_{v,o}$) and that of fragments ($M_{v,f}$) according to Nemzek and Guillet [16].

$$\alpha = \frac{M_{v,o}}{M_{v,f}} - 1 \quad (2)$$

SEC was used for qualitative evaluation of the reduction in molecular weight and its distribution on a SEC/HPLC instrument (model 1050, Hewlett-Packard) equipped with a refractive index detector, using the protocol described earlier [14].

3. Results and discussion

Fig. 1 shows the SEC chromatograms of the original chitosan and the typical samples resulting from fragmentation at different irradiation times with a pulse energy of 0.8 mJ. The shift of the chromatograms towards higher elution volume with irradiation time indicates that the molecular weight of the fragment decreases with irradiation time. The chromatograms broaden gradually with irradiation time, indicating that the polydispersity increases with longer exposure.

The rate of chain scission at pulse energies of 0.36, 0.55 and 0.80 mJ is shown in Fig. 2. The increase in chain scission (α) with irradiation time was sigmoidal. The increase in α was initially slow for all the three pulse energies, followed by period of high scission rate and a final period of slow rate which was nearly the same as the initial period. The initial slow rate periods were 35, 30 and 12 min for the pulse energies of 0.36, 55 and 0.80, respectively. The intermediate high rate periods were between 35 and 70; 30 and 60; and

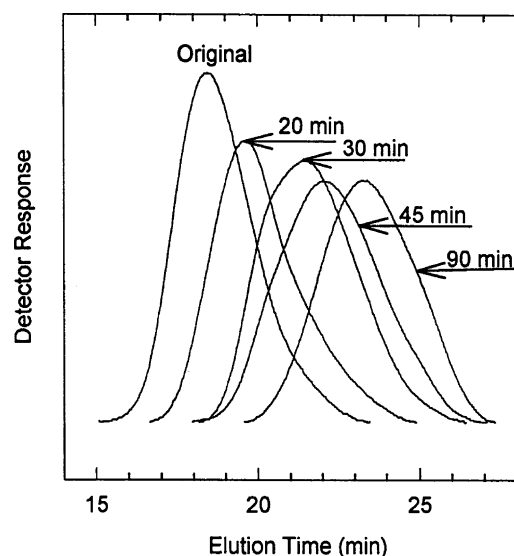


Fig. 1. Size-exclusion chromatograms of the original chitosan and typical fragments obtained by laser irradiation of 1.0% (w/v) chitosan solution with pulse energy of 0.80 mJ for 20, 30, 45 and 90 min. The focal length of the lens was 70 mm.

12 and 35 min for the pulse energies of 0.36, 55 and 80, respectively. With higher pulse energy, the initial slow rate and the intermediate high rate periods were shorter.

The initial lag in fragmentation presumably arises due to the presence of larger chitosan hydrodynamic units or aggregates. Reduced concentration or Berry number, $c[\eta]$, of the original chitosan at 1.0% concentration being 25.86 dl g^{-1} (Table 1), which is greater than 15.5 at its critical concentration of 0.6% (w/v) [17]. This hydrodynamic status would result in significant entanglements, and the hydrodynamic units are aggregated molecules. Thus, much of the light energy is expended initially in the disaggregation of the

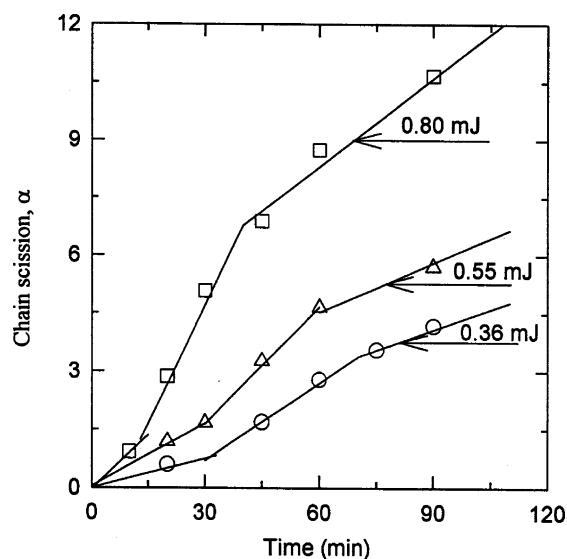


Fig. 2. Average number of chain scission, α , as a function of irradiation time with pulse energies of (○) 0.36 mJ; (△) 0.55 mJ; and (□) 0.80 mJ.

Table 1

Intrinsic viscosity, $[\eta]$, in 0.1M HAc/ 0.02M NaCl, viscosity-average molecular weight, M_v , and average number of chain scission, α , for fragments prepared by laser irradiation of 1.0% (w/v) chitosan solution under various conditions of pulse energy (E) and irradiation time

| Chitosan sample code | Irradiation conditions | | $[\eta]$ (dl g ⁻¹) | $M_v \times 10^5$ (Da) | α |
|----------------------|------------------------|------------|--------------------------------|------------------------|----------|
| | E (mJ) | Time (min) | | | |
| Original | 0 | 0 | 25.86 | 19.65 | 0 |
| F1 | 0.36 | 20 | 14.40 | 12.35 | 0.59 |
| F2 | 0.36 | 45 | 7.45 | 7.32 | 1.69 |
| F3 | 0.36 | 60 | 4.83 | 5.19 | 2.79 |
| F4 | 0.36 | 75 | 3.82 | 4.31 | 3.56 |
| F5 | 0.36 | 90 | 3.26 | 3.80 | 4.17 |
| F6 | 0.55 | 20 | 9.69 | 9.01 | 1.18 |
| F7 | 0.55 | 30 | 7.53 | 7.38 | 1.66 |
| F8 | 0.55 | 45 | 4.16 | 4.61 | 3.26 |
| F9 | 0.55 | 60 | 2.91 | 3.47 | 4.66 |
| F10 | 0.55 | 90 | 2.35 | 3.03 | 5.49 |
| F11 | 0.80 | 10 | 11.40 | 10.26 | 0.92 |
| F12 | 0.80 | 20 | 4.71 | 5.09 | 2.86 |
| F13 | 0.80 | 30 | 2.66 | 3.23 | 5.08 |
| F14 | 0.80 | 45 | 1.92 | 2.50 | 6.88 |
| F15 | 0.80 | 60 | 1.47 | 2.02 | 8.74 |
| F16 | 0.80 | 90 | 1.17 | 1.68 | 10.67 |

Focal length of the lens was 70 mm.

hydrodynamic unit, and thereafter the rate of fragmentation increases as long as high concentration of large molecules is present. With increase in the concentration of smaller fragments, the rate of fragmentation decreases to rates comparable to the initial rate. Although the rate of scission falls, chain scission does not reach a saturation value, suggesting that even smaller molecules continued to be subject to scission, albeit at a slower rate. Consequently, the molecular weight distribution widens as seen in Fig. 1.

Furthermore, the rate of fragmentation was linear as a function of square of pulse energy during both the high rate-intermediate phase and slow rate-terminal phase (Fig. 3), indicating that the reaction takes place through a two-photon process all along. The dissociation energy of a typical glycosidic linkage, the C–O bond between two repeat units is 300–400 kJ. Chain scission thus can be accomplished either by the energy of two photons provided by high intense femtosecond laser at 800 nm (150 kJ mol⁻¹ photon) or by a single photon at 400 nm (300 kJ mol⁻¹). But chitosan solution does not absorb light at 400 nm (spectrum not shown). In addition, during irradiation of the solution by femtosecond laser (800 nm), emission of white light (which includes wavelength of 400 nm) was observed, indicating that the solution did not absorb light at 400 nm. This confirms our earlier report that photochemical fragmentation of chitosan is a two-photon process [14].

Fig. 4 shows a plot of chain scission as a function of light dose. The dose, D , was calculated using the relation:

$$D = (ER)^2 t \quad (3)$$

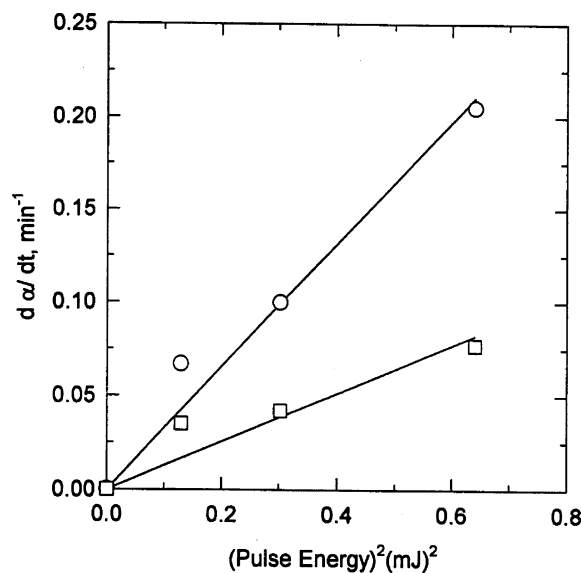


Fig. 3. Chain scission rate, $d\alpha/dt$, as a function of the square of the laser pulse energy (E^2): (○) intermediate phase; and (□) terminal phase.

where E is pulse energy, R is the repetition rate (1 kHz), and t is irradiation time. The product of E and R is squared to account for two-photon process in chitosan depolymerization reaction [14]. The relationship between chain scission and light dose was biphasic, with a greater chain scission rate at low doses of up to 1400 J² s⁻¹ which corresponds to chain scission of about 6.0 (Fig. 4). Beyond the dose of 1400 J² s⁻¹, the scission rate falls. This indicates that the same photochemistry occurs at all pulse energies, but the scission rate slows down at higher doses as the susceptible molecular targets are depleted. The sagging rate also indicates that larger macromolecules are preferably fragmented, and the resulting smaller macromolecules undergo scission only at higher doses but at a slower rate.

As the macromolecules get smaller, the viscosity of the solution decreases. Viscosity of the solution can also decrease through heating of solution by weak one-photon water absorption at 800 nm (or 150 kJ mol⁻¹). However, thermal effect on viscosity may not be significant, since the temperature of irradiated solution for 90 min with pulse energy of 0.8 mJ increased from 22 to only 25 °C. Reduction in molecular size and solution viscosity should lead to increased diffusivity of the molecules. Electronic energy is transformed into vibrational energy in the excited molecules by the mechanism of internal conversion. When several vibrational quanta accumulate in a certain glycosidic bond, chain scission can occur. As the photochemical reaction proceeds and the macromolecules get smaller, their diffusivity or mobility increases. Consequently, the probability of energy deposition in a certain bond becomes smaller, leading to a slower rate of chain scission, although scission of smaller macromolecules continue to occur at higher doses.

Larger macromolecules were more susceptible to fragmentation by laser irradiation than smaller molecules. For

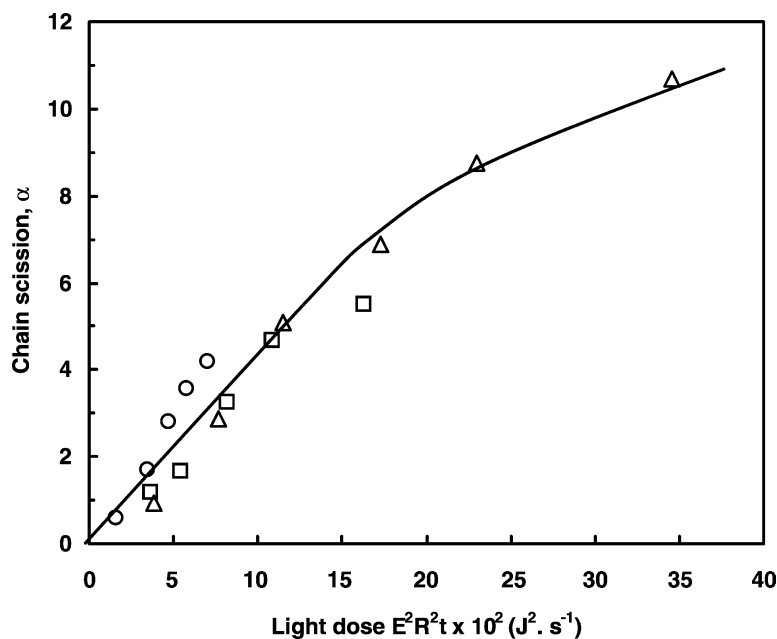


Fig. 4. Chain scission rate (α) as a function of light dose (E^2R^2t). Pulse energy of (○) 0.36 mJ; (□) 0.55 mJ; and (△) 0.80 mJ.

Table 2

Intrinsic viscosity, $[\eta]$, in 0.1 M HAc/ 0.02 M NaCl, viscosity-average molecular weight, M_v , and average number of chain scission, α , for fragments prepared by laser irradiation of either air or oxygen saturated chitosan solution (1.0%, w/v) with pulse energy of 0.80 mJ for 45 or 90 min in flat light port reactor

| Chitosan sample code | Irradiation conditions | | $[\eta]$ (dl g ⁻¹) | | $M_v \times 10^5$ (Da) | | σ |
|----------------------|------------------------|---------------------------|--------------------------------|-------------------|------------------------|-------------------|----------|
| | Exposure time (min) | Dissolved gas in solution | Initial | After irradiation | Initial | After irradiation | |
| Original | 0 | – | 25.86 | 25.86 | 19.65 | 19.65 | 0 |
| F15 | 45 | Air | 25.86 | 1.92 | 19.65 | 2.50 | 6.88 |
| F16 | 90 | Air | 25.86 | 1.17 | 19.65 | 1.68 | 10.67 |
| F17 | 45 | Oxygen | 25.86 | 1.90 | 19.65 | 2.47 | 6.94 |
| F18 | 90 | Air | 16.49 | 1.07 | 13.75 | 1.57 | 7.58 |
| F19 | 90 | Air | 7.47 | 0.76 | 7.49 | 1.20 | 5.24 |

Focal length of the lens was 70 mm.

instance, the value of α was found to be 10.67 when chitosan with molecular weight of 1965 kDa was exposed to light with pulse energy of 0.80 mJ for 90 min, whereas the values of α were 7.58 and 5.24 with initial chitosan molecular weights of 1375 and 749 kDa, respectively (samples F16, F18 and F19, Table 2). This is also evident from time course and dose–effect curves that the rate of chain scission falls as fragmentation is continued after 60 min (Fig. 2), or at higher doses (Fig. 4).

There was only a minor change in the fragmentation of chitosan when the solution was saturated with oxygen at room temperature compared to saturation with air (samples F15 and F17, Table 2). Femtosecond laser irradiation was shown to photolyze water molecules, forming free radicals which can lead to the formation of hydrogen peroxide [18]. However, only traces of H₂O₂ were found in laser-irradiated chitosan solution [14], suggesting that chitosan plays a free-radical scavenging role. Although, it is

not known whether chitosan possesses antioxidant property, other amino-groups bearing substances such as creatinine [19], polyamines [20], amino acids, peptides and proteins [21] have been demonstrated to possess antioxidant properties. Since there was no significant change in chain scission in chitosan solution saturated with oxygen, secondary processes involving oxidation–reduction reactions may play only a minor role, and fragmentation occurs mainly through multi-photon absorption confirming our earlier report [14].

4. Conclusions

Fragmentation of chitosan increased with pulse energy, irradiation time in sigmoidal manner, and with dose in biphasic manner. Larger molecules were more susceptible to fragmentation than smaller molecules, and polydispersity of the fragments increased with irradiation time. The resulting

smaller molecules undergo scission only at higher doses but at a slower rate. Fragmentation of chitosan appears to be a two-photon process, and secondary oxidation–reduction reactions may play only a minor role.

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