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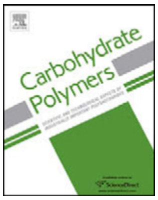

Short communication

Preparation of low molecular weight chitosan using solution plasma system

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ABSTRACT

The solution plasma system was introduced to treat chitosan solution in order to prepare low molecular weight chitosan. The plasma treatment time was varied from 0 min to 300 min. The plasma-treated chitosan was characterized including viscosity, molecular weight by GPC, and chemical characteristics by FT-IR. The results showed that after treated with plasma for 15–60 min, the viscosity of chitosan solution and apparent molecular weight of chitosans were remarkably decreased, compared to those of untreated sample. Longer treatment time had less effect on both viscosity and molecular weight of samples. Eventually, long treatment time (≥180 min) showed no influence on both viscosity and apparent molecular weight. This suggested that the degradation process of chitosan occurred during plasma treatment. FT-IR analysis revealed that chemical structure of chitosan was not affected by solution plasma treatment. TOF-MS results showed that chitooligosaccharides with the degree of polymerization of 2–8 were also generated by solution plasma treatment. The results suggested that solution plasma system could be a potential method for the preparation of low molecular weight chitosan and chitooligosaccharides.

1. Introduction

Chitosan, linear heterocopolymers of β-1,4-linked 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acet-amido-2-deoxy-D-glucopyranose (GlcNAc) units, is derived from chitin by deactylation process. Due to many unique properties such as biocompatibility, biodegradability, and bioactivity, chitosan is widely used in many applications such as food processing, pharmaceutics, and cosmetics (Dodane & Villivalam, 1998; Jayakumar, Nwe, Tokura, & Tamura, 2007; Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010; Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011; Kumar, 2000; Li et al., 2005; Shahidi, Arachchi, & Jeon, 1999). Many studies have reported that the molecular weight of chitosan could greatly affect its biological properties. Kittur, Vishu Kumar, and Tharanathan (2003) reported that low molecular weight chitosan (LMWC) with weight average molecular weight (M_w) in the range of 5000–10000 Da showed superior biological properties, compared to that high molecular weight chitosan (HMWC). Tangsadthakun et al. (2007) showed that LMWC (Viscosity-averaged molecular weight (M_v) ~ 74000 Da) was more effective to promote the proliferation of mouse fibroblast than HMWC (M_v ~ 880000 Da). In addition, Ratanavaraporn, Kanokpanont, Tabata, and Damrongsakkul (2009) found that very low molecular weight chitooligosaccharide (COS, M_w < 10 kDa) was shown to be a more favorable material for the growth and osteogenic differentiation of both adipose-derived stem cell and bone marrow-derived stem cell, compared to high molecular weight chitosan. Therefore, low molecular weight chitosan and chitooligosaccharide were served as potential materials for biomedical applications.

Several methods, including chemical treatment, enzymatic treatment, and radical treatment, have been suggested to prepare LMWC and COS (Chang, Tai, & Cheng, 2001; Xie, Hu, Wei, & Hong, 2009). Chemical treatment is an easy and low cost process. However, chemical waste and contamination are the main problems. Enzymatic treatment is an effective way to achieve the specific cleavage to COS. This process is very complicate and inconvenient for large-scale production, since it requires multi-steps, particularly enzyme preparation and product purification. For radical treatment, the degradation process of chitosan was performed by free radical such as hydroxyl radical, generated by hydrogen peroxide (Chang et al., 2001). However, the removal of hydrogen peroxide must be required.

Solution plasma is a new plasma system which has been proposed by Takai (2008). It is liquid-phase plasma which has been widely utilized in nanomaterial synthesis, surface modification, water treatment, sterilization, and decomposition of organic compound (Takai, 2008). This system is able to produce highly

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active species such as hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$), free electron (e^-), superoxide anion (O_2^-), and atomic oxygen anion (O^-) (Potocký, Saito, & Takai, 2009). Since solution plasma does not involve any chemical reagents, the removal of chemical residue is not required. Therefore, in this study, the solution plasma was first introduced to treat chitosan solution in order to investigate the effects of solution plasma on the preparation of low molecular weight chitosan. Characteristics of chitosan, including viscosity, molecular weight, and chemical components were investigated.

2. Materials and methods

2.1. Materials

Shrimp shell chitosan with the degree of deacetylation and average molecular weight of 95% and 2.1×10^5 Da, respectively, was obtained from Seafresh Chitosan Co. Ltd., Thailand.

2.2. Solution plasma setup

The solution plasma system reported by Takai (2008) was set up as shown in Fig. 1. The pulsed electric discharge was generated between two needle electrodes, made of tungsten, using a high frequency bipolar pulsed DC power supply. The two electrodes, of which the distance is 0.2 mm, are set inside a glass reactor where polymer solution is filled. Once the power is applied, the plasma was generated.

2.3. Solution plasma treatment of chitosan solution

Chitosan powder was dissolved in 1 M acetic acid to obtain 1% w/v chitosan solution and filled in the glass reactor. The solution plasma was produced at the fixed frequency, voltage and pulse width of 15 kHz, 1.6 kV and 2 μs, respectively. During plasma treatment, the temperature of chitosan solution was controlled at 25–30 °C. The treatment time of solution plasma was varied in the range of 0–300 min. The obtained chitosan after treatment were characterized as described in Section 2.4. In addition, the precipitate of chitosan solution treated with solution plasma for 300 min was collected by adjusting the pH of plasma-treated solution to 7 using NaOH. The first precipitate was then removed by centrifugation at 5000 rpm for 30 min. The supernatant was mixed with equal volume of acetone to give a second precipitate. The second precipitate was dried prior to characterization using a matrix-assisted laser desorption time-off-flight mass spectrometer (MALDI-TOF-MS, Bruker, USA).

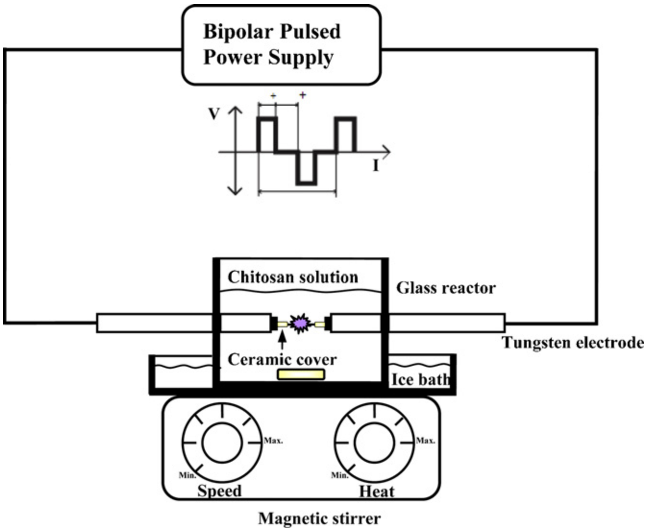


Fig. 1. Schematic diagram of solution plasma system.

2.4. Characterization of plasma-treated chitosan

2.4.1. Viscosity measurement

The viscosities of plasma-treated and untreated chitosan solutions were determined at 25 °C using a viscometer (Vibro SV-100, Japan).

2.4.2. Determination of apparent molecular weight (M_{app})

The apparent molecular weight of plasma-treated and untreated chitosan solution were characterized by Gel Permeation Chromatography (GPC, Water 600E, Waters, USA). The concentration of chitosan solution was 0.4 mg/ml. Eluent and chitosan sample solutions were filtered through 0.45 μm Millipore filters. The flow rate was maintained at 0.6 ml/min. The pullulans (M_w 5900–708,000 Da) were used as standard samples.

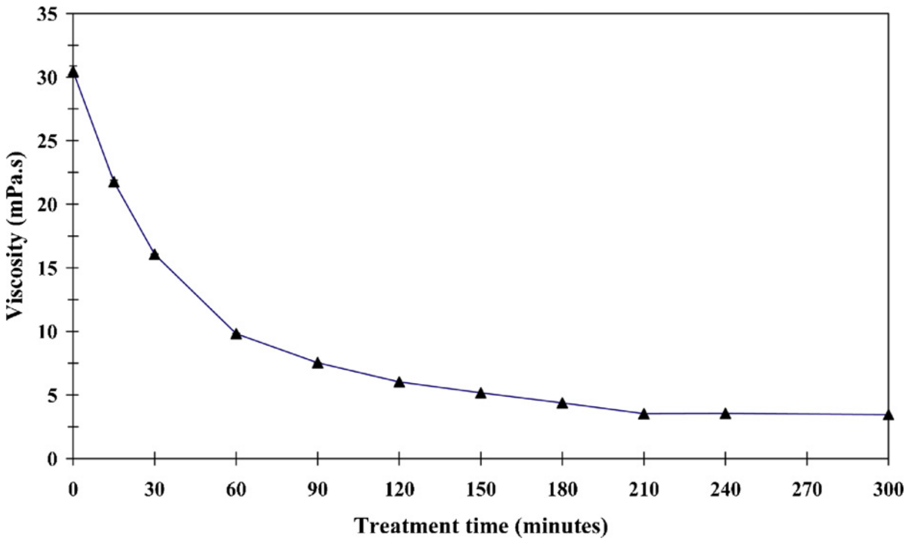


Fig. 2. The viscosity of untreated and plasma-treated chitosan solution (1% chitosan solution and pH 3.7) as a function of treatment time.

Table 1
The kinetic parameter of degradation process of chitosan by solution plasma.

Treatment time (min)	Viscosity (ν) (mPa s)	α	$-\ln(1-\alpha)$
0	30.47	0.0000	0.0000
15	21.77	0.3222	0.3889
30	16.07	0.5333	0.7620
60	9.81	0.7650	1.4480
90	7.53	0.8494	1.8931
120	6.03	0.9048	2.3521
150	5.17	0.9367	2.7595
180	4.38	0.9661	3.3830
210	3.73	0.9902	4.6303
240	3.55	0.9968	5.7417
300	3.46	1.0000	6.9204

2.4.3. Chemical analysis

The plasma-treated and untreated chitosan solutions were casted and air-dried to form thin films with the thickness of 110 ± 20 μm. The chemical analysis of the obtained films was then characterized using FT-IR spectroscopy (Digilab, FTS 7000 Series, USA).

3. Results and discussion

In this study, the solution plasma system was introduced to treat chitosan solution. Fig. 2 shows the viscosity of plasma-treated and untreated chitosan solution. It was observed that the viscosity of chitosan solution after plasma treatment was continuously decreased as increasing the treatment time. During the first 60 min of treatment, the viscosity was sharply decreased by 67%. After that a slow decrease in viscosity was noticed until the treatment time reached 180 min. Further solution plasma treatment longer than 180 min seemed to have no effect of the viscosity of chitosan solution. According to the result of viscosity reduction, the rate constant of the reaction (k) could be determined. The studied reaction was pseudo-monomolecular and seemed to be first order. The kinetic equation was evaluated as follows:

$$\frac{d\alpha}{dt} = k(1-\alpha_{(t)}) \tag{1}$$

where k is rate constant and α is the degree of conversion which was presented as follows:

$$a = \frac{(\nu_i - \nu_t)}{(\nu_i - \nu_f)} \tag{2}$$

where ν_i is the initial viscosity, ν_t is the actual viscosity at the corresponding time, and ν_f is the finally viscosity. The values of kinetic parameters were summarized in Table 1. In order to determine the rate constant, the time dependence of $-\ln(1-\alpha)$ was plotted. The results showed that the viscosity decreasing of chitosan solution by solution plasma at 25 °C was a first order reaction with the rate constant of 0.0225 min^{-1}.

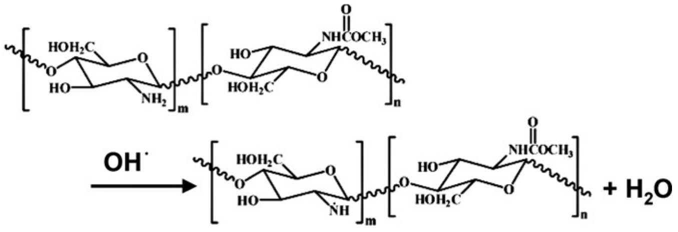
Considering on the apparent molecular weight of chitosan obtained from GPC, shown in Table 2. The apparent molecular weight of untreated chitosan (2.1×10^5 Da) was markedly decreased to 6.2×10^4 Da after treated with plasma for 30 min. After treated with plasma for 180 min, the apparent molecular weight of treated chitosan was at 2.9×10^4 Da. The apparent molecular weight remained consistent, when the solution plasma was treated longer than 180 min. This corresponded to the result on the viscosity of chitosan. This suggested that chitosan was degraded during plasma treatment. This could be attributed to the free radical such as hydroxyl generated by plasma treatment caused the degradation process of chitosan. The hydroxyl radical could be performed during solution plasma treatment by the reaction of oxygen radical and water (Baroch, Anita, Saito, & Takai, 2008).

Table 2
Molecular weight of plasma-treated and untreated chitosan solution determined by GPC.

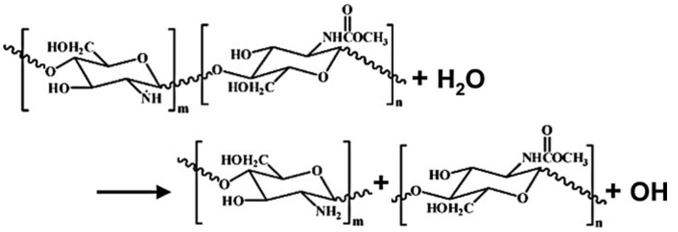
Samples	Treatment time (min)	M_{app} (Da)	Polydispersity (M_{app}/M_n)
t-0	0	2.1×10^5	4.1
t-30	30	6.2×10^4	2.7
t-60	60	5.6×10^4	2.7
t-180	180	2.9×10^4	2.7
t-300	300	3.2×10^4	2.7

$$\mathrm{O}^{\bullet} + \mathrm{H_2O} \rightarrow 2\mathrm{OH}^{\bullet} \tag{3}$$

As previously reported by Chang et al. (2001) the hydroxyl radicals subsequently broke the β-1-4 glycosidic linkage and decreased the molecular weight of chitosan. They proposed the degradation mechanism of chitosan as follows:



(4)



(5)

where m and n represented the number of glucosamine residue.

The degradation process of chitosan occurred during solution plasma treatment, resulting in low molecular weight chitosan. However, after treated with plasma longer than 180 min, the viscosity and apparent molecular weight of chitosan seem to be consistent. This corresponded to the results of the kinetic analysis of the data, i.e. $1/M_w$ versus treatment time plot, as shown in Fig. 3. The kinetic analysis implied that after treated with solution plasma longer than 180 min, the degradation rate constant seems to be consistent. This might be the result from the α-chitosan structure of the sample. The α-chitosan is known to have poor reactivity due to its rigid crystalline structure (Takai et al., 1992). This could inhibit the degradation process of chitosan caused by plasma treatment, resulting in constant viscosity and apparent molecular weight of plasma-treated chitosan.

The FT-IR spectra of plasma-treated and untreated gelatin samples were shown in Fig. 4, demonstrated the characteristic peaks of chitosan films at 1650, 1575 and 1152 cm^{-1}, which corresponded to C=O stretching, $-\mathrm{NH_2}$ bending, and –C–O–C– glycosidic linkage between chitosan monomer, respectively (Tangsadthakun et al., 2007). After treated with solution plasma, the characteristic peaks of chitosan remained unchange. This indicated that the degradation process of chitosan caused by plasma treatment only had an effect on the molecular weight not on the chemistry component of chitosan.

Not only the preparation of LMWC was observed by solution plasma treatment, COS was also obtained. After the plasma-treated

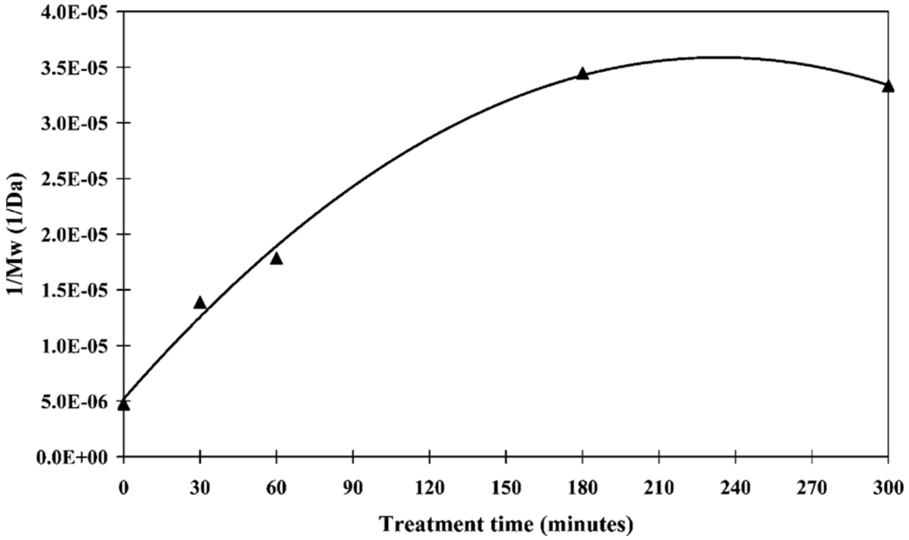


Fig. 3. The change of apparent molecular weight of chitosan as a function of plasma treatment time.

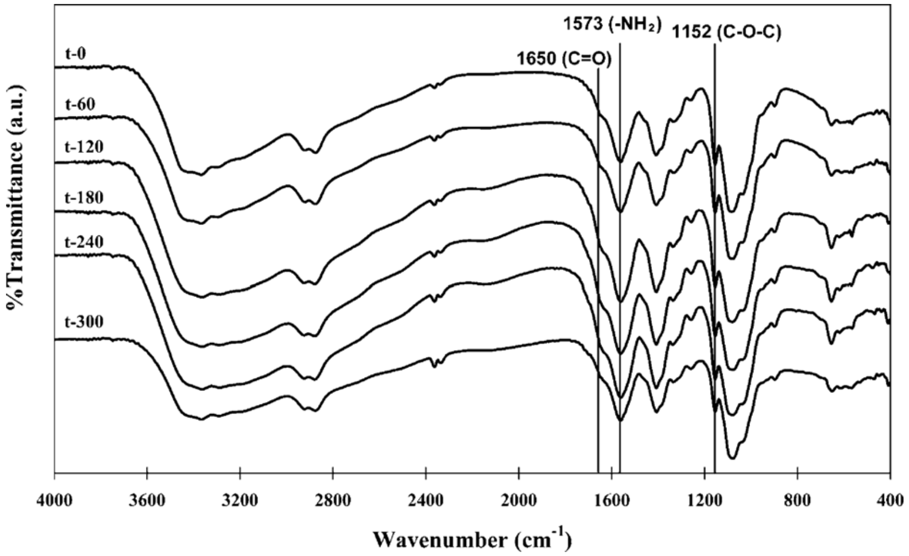


Fig. 4. FT-IR spectra of untreated and plasma-treated chitosan films for various treatment time.

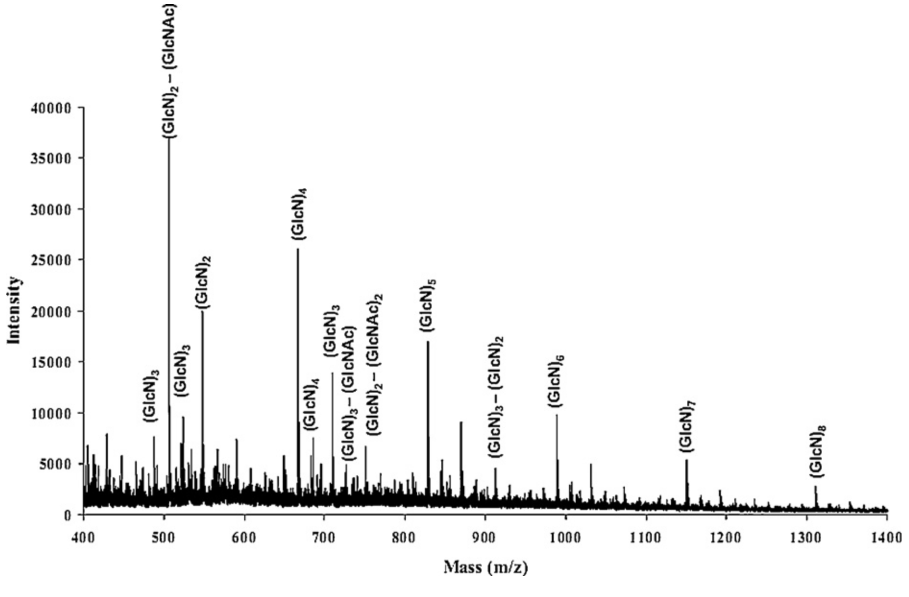


Fig. 5. TOF-MS of the second precipitate of plasma-treated chitosan.

chitosan solution was second precipitated by acetone, the obtained sample characterized by MALDI-TOF-MS (Fig. 5), showed that the product was mainly composed of chitooligosaccharides with the degree of polymerization (DP) 2–8 (Xie et al., 2009). This revealed that the solution plasma system could be used to prepare both LMWC and COS.

4. Conclusion

In this study, the solution plasma system was first introduced to treat chitosan solution. The viscosity and apparent molecular weight of plasma-treated chitosan solution was decreased with increasing plasma treatment time, compared to that of untreated sample. The degradation process of chitosan caused by plasma treatment only had an effect on the molecular weight but not on the structure of chitosan. We have shown that solution plasma system could be employed to produce both LMWC and COS. The plasma conditions, such as plasma power and frequency, for high efficiency of production would be further investigated.

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