Carbohydrate Polymers 87 (2012) 2745–2749

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Preparation of low molecular weight chitosan using solution plasma system

I. Prasertsung[a], S. Damrongsakkul[b,*], C. Terashima[d], N. Saito[c], O. Takai[d]

[a] Chemical Engineering Program, Department of Industrial Engineering, Faculty of Engineering, Naresuan University, Phitsanulok 65000, Thailand
[b] Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
[c] Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
[d] EcoTopia Science Institute, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 16 October 2011
Accepted 16 November 2011
Available online 25 November 2011

Keywords:
Solution plasma
Chitosan
Chitooligosaccharide

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

* Corresponding author. Tel.: +66 2 2186862; fax: +66 2 2186877.
E-mail address: siriporn.d@chula.ac.th (S. Damrongsakkul).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.11.055

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.

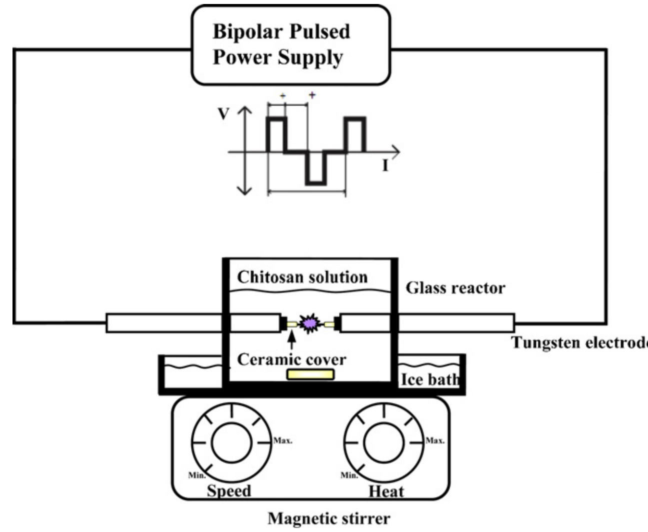

Fig. 1. Schematic diagram of solution plasma system.

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).

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.

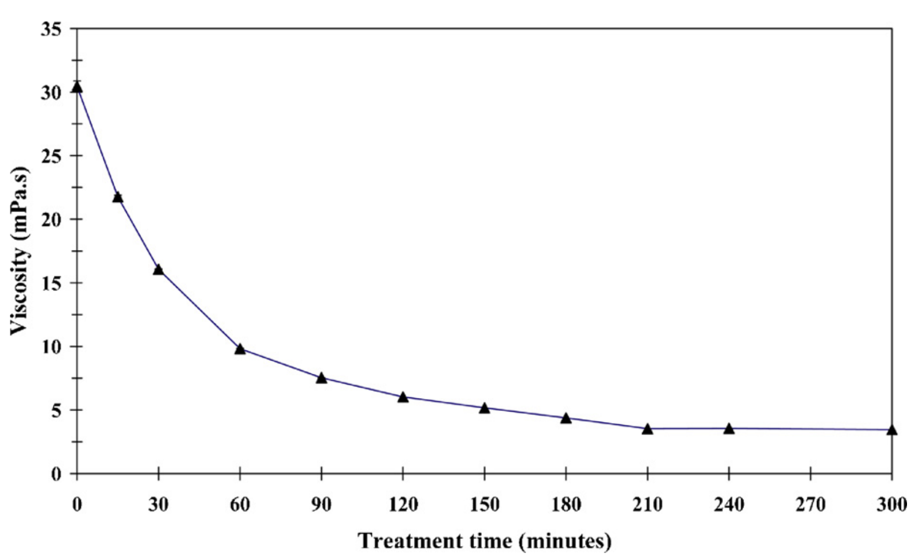

Fig. 2. The viscosity of untreated and plasma-treated chitosan solution (1% chitosan solution and pH 3.7) as a function of treatment time.