

Article

Depolymerization of Chitosan Using a High Pressure Homogenizer

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Abstract: Large molecular weight chitosan was depolymerized by a high pressure homogenizer with a micro orifice module. 1 % (w/v) chitosan solution was prepared with 1 % (v/v) acetic acid solution. The chitosan solution was passed through a high pressure homogenizer at 500, 1000 and 1500 bar for 1 to 5 cycles. An increase in the pressure and the number of passes causes the viscosity and molecular weight to decrease without the use of any additives, such as acid/alkali solutions. The FT-IR spectra indicated no obvious modifications of the chemical structure of chitosan before and after the high pressure homogenizer treatment. The UV spectra showed a new absorption band of carbonyl groups at 255 nm. The carbonyl groups might be formed during depolymerization. The XRD results showed that the crystallinity of the chitosan decreased with increases in the pressure and the number of passes.

Keywords: Chitosan; Depolymerization; High pressure homogenizer; Micro orifice module

1. Introduction

Chitosan is a natural polysaccharide and can be extracted by a deacetylation process from chitin, an abundant matter from crustacean shells. It is a biocompatible, biodegradable and non-toxic polymer, which makes it attractive for applications in the medical, pharmaceutical, cosmetic and food industries [1-4]. In addition, chitosan is used in many other industries, such as sewage treatments, in particular to

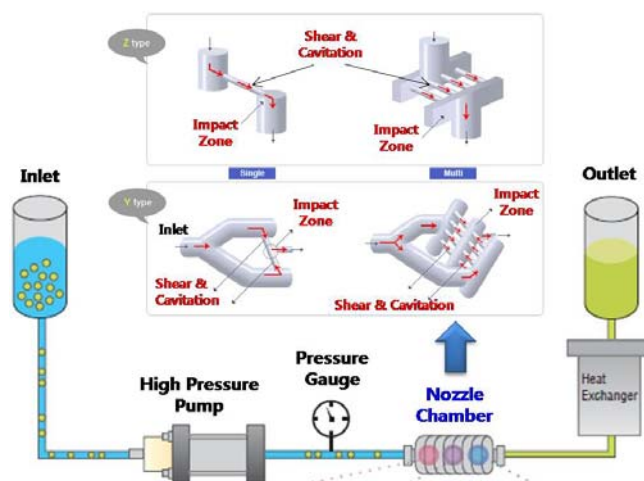
minimize heavy metals [5,6]. However, its large molecular weight and viscosity limit its applications. Low molecular weight chitosan of high solubility in water would have great potential in applications, especially in improving medicines, pharmaceuticals and cosmetics such as drug loaded nanocomposites [7,8].

The molecular weight of chitosan can be controlled by acid or enzymatic hydrolysis. Acid hydrolysis has several disadvantages, such as not being environmentally friendly, not being easy to control, and the non-uniformity of the final product. In contrast, enzymatic hydrolysis produces specific products as the reaction can be precisely controlled. However, it is very expensive and unavailable in bulk for commercial exploitation [9-11]. In addition, scientists are focusing on reducing the molecular weight of chitosan without changing its chemical structure by using different hydrolysis methods, such as hydrogen peroxide, plasma, ultrasound, radiation, and so on [12-15].

Using a high pressure homogenizer is a novel approach for the depolymerization of large molecular weight chitosan. Fig. 1 shows a schematic of the high pressure homogenizer with a reaction nozzle chamber. The fluid passes an orifice module under ultra high pressure, and supersonic speeds result due to a rapid decrease in pressure. During the high pressure homogenization process, the fluid is affected by high energy processes such as impacts, cavitation and shear forces due to turbulence. The implosive collapse of bubbles generates localized hot spots with a transient temperature of 5000 K, pressure of 500 atm and cooling rates in excess of 10^9 K^{-1} by cavitation phenomena [16-18]. In addition, this can produce oxidizing species radicals ($\text{H}\cdot$, $\text{O}\cdot$, $\text{OH}\cdot$, *etc.*) and molecules (H_2O_2 , O_3 , *etc.*). The high pressure homogenizer produces high energy greater than other technologies, such as using a homomixer, ball mill and ultrasonic [19-21]. Therefore, the high energy of the high pressure homogenizer can lead to depolymerization because of the intense mechanical and chemical effects associated with shear, cavitation, and impact effects.

This work reports the effect of depolymerization using a high pressure homogenizer through the control of pressure and the number of passes. In addition, the depolymerized chitosan was characterized by viscometry, gel permeation chromatography (GPC), Fourier-transform infrared spectra (FT-IR), and UV-Vis, X-ray diffraction (XRD).

Fig. 1. Schematic of the high pressure homogenizer with a reaction nozzle chamber.

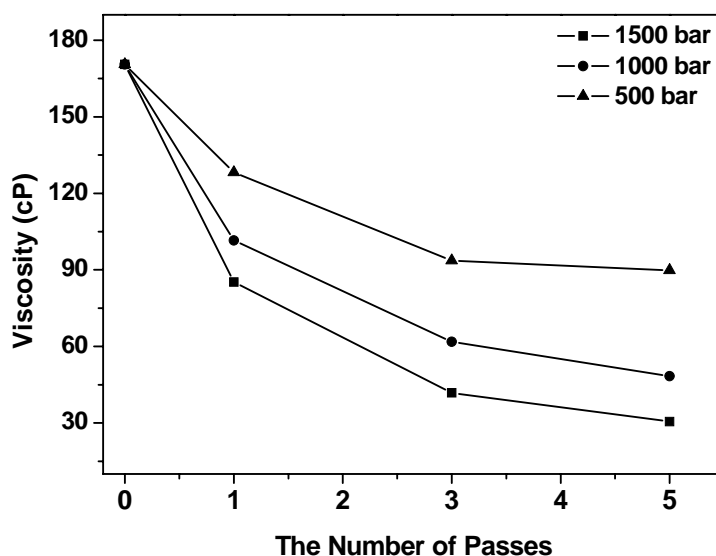


2. Results and Discussion

2.1. Viscosity of the Chitosan solution

Fig. 2 shows the effect of high pressure homogenization on the viscosity of 1 % (w/v) chitosan solution. High pressure homogenization produces significant changes in the viscosity of the chitosan solution. As the pressure and the number of passes increase, the chitosan solution shows a significant decrease in viscosity. The viscosity of the original chitosan solution was 170.5 cp, whereas the viscosity of the 5 passes sample at 1500 bar was 30.5 cp, with a decrease of about 82.1 % compared with the original chitosan solution. The decrease in the viscosity was hypothesized to occur due to depolymerization through the breakage of the glycosidic bond in the chitosan. In addition, this result is consistent with other studies on polymers with a polysaccharide structure [9,22,23]. The viscosity of the chitosan solution can be effectively controlled by the high pressure homogenizer without any chemical additives.

Fig. 2. Effect of the homogenization pressure and number of passes on the viscosity of chitosan solution.

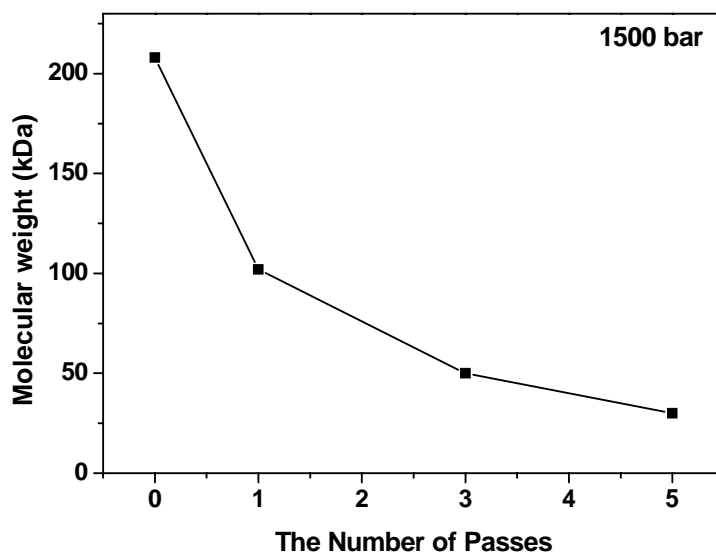


2.2. Molecular weight of Chitosan

The average molecular weight was analyzed by GPC to determine the molecular weight of chitosan after the high pressure homogenization process. The molecular weight of the original chitosan was about 208 kDa. The change in the average molecular weight of the chitosan by high-pressure homogenization is shown in Fig. 3. When the original chitosan solution was high pressure homogenized at 1500 bar, the corresponding molecular weight of chitosan decreased from 208 to 30 kDa. The decrease in the molecular weight was 85.6 %. As the number of passes increase, the chitosan shows a significant decrease in its molecular weight. This result appears to be in good agreement with the data on the viscosity of the chitosan solution. We speculate that the decrease in the molecular weight occurred through the high energy of the high pressure homogenizer through shearing,

cavitation, and impact effects. The entanglement and stretching of the chitosan polymer chain was disentangled through shear forces. In addition, the large molecular weight of the chitosan polymer was depolymerized through cavitation effects. The high pressure homogenizer process could be used in commercial processes as an effective method to resolve the physical problems involved in the use of chitosan with high viscosity and a large molecular weight.

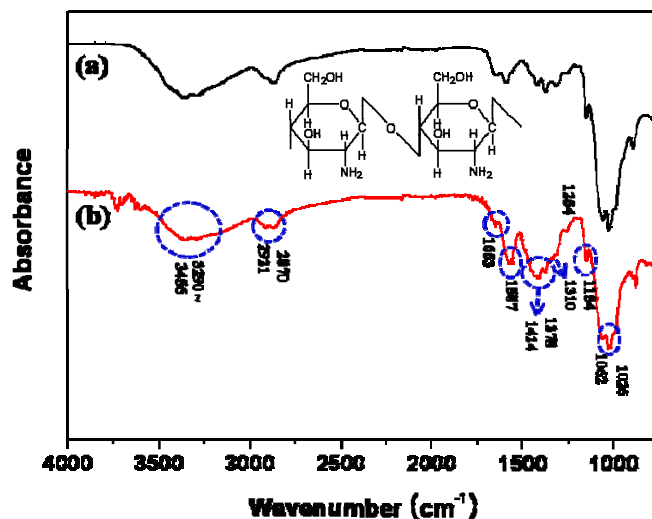
Fig. 3. Effect of the homogenization pressure and number of passes on the molecular weight of chitosan.



2.3. FT-IR Spectrum of Chitosan

Fig. 4 shows the FT-IR spectrum of the original chitosan and the depolymerized chitosan by high pressure homogenization with 5 passes at 1500 bar. The broad absorbent band centered at $3456\text{--}3290\text{ cm}^{-1}$ is characteristic of the stretching vibration of --OH and --NH_2 . The band near $2921\text{--}2870\text{ cm}^{-1}$ corresponds to C--H stretching of the alkyl substituent. The bands at 1653 and 1587 cm^{-1} are attributed to the binding vibrations of the amide groups. The bands in the range $1158\text{--}1026\text{ cm}^{-1}$ are assigned to the characteristics of the glycosidic linkage in chitosan [24,25]. In addition, the band at 1735 cm^{-1} , which is assigned to the carboxyl group, was not obvious, indicating that the carboxyl group of chitosan was not formed during depolymerization [12]. The spectrum of the depolymerized chitosan was similar to that of the essential one. This indicates that the essential chitosan and high pressure homogenized chitosan both have similar FT-IR spectrum patterns, without any notable changes in the functional group status.

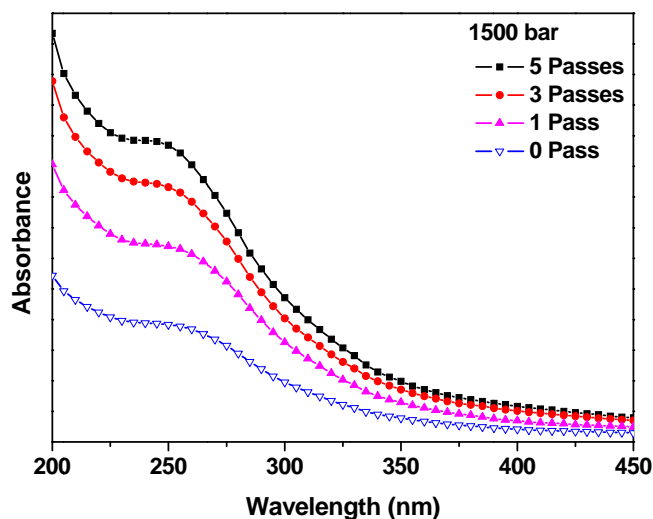
Fig. 4. FT-IR spectra of (a) essential chitosan and (b) depolymerized chitosan with 5 passes at 1500 bar.



2.4. UV-Vis spectrum of Chitosan solution

Fig. 5 shows the UV-Vis spectrum of the original chitosan and the depolymerized chitosan by high pressure homogenization with 5 passes at 1500 bar. The absorption band of the chitosan solution was observed at 255 nm, which was ascribed to carbonyl groups [26]. The relative absorption intensity of the peaks increased with increasing numbers of passes at 1500 bar. This result indicates that the carbonyl groups might be formed during high pressure homogenization by the breakage of the glycosidic bond. This result is consistent with other studies on polysaccharides [27].

Fig. 5. UV spectrum of essential chitosan and depolymerized chitosan.

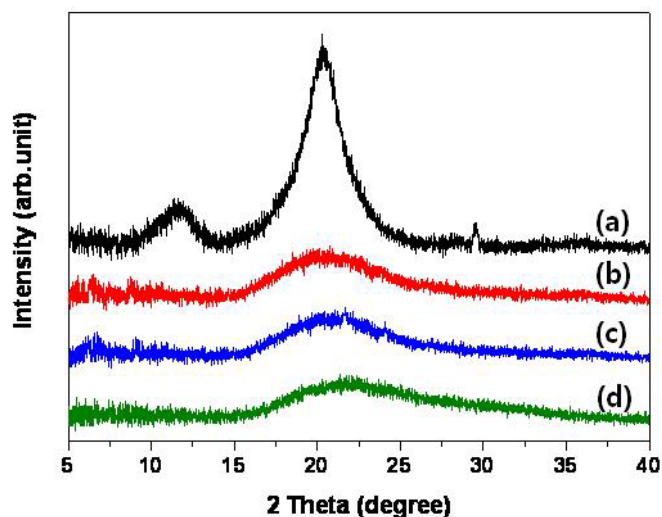


2.5. XRD Patterns of Chitosan solution

The XRD patterns of the original chitosan and depolymerized chitosan are shown in Fig. 6. The peaks appeared at 2θ values of 11.5° and 20.3° which match well with the literature values [28]. The intensity characteristics of the peaks tend to decrease with increasing numbers of passes under high pressure homogenization. A decrease in crystallinity occurs due to the destruction of the crystal

structure of chitosan as a result of the cleavage of the glycosyl bonds [22]. This result appears to be in good agreement with the data on the viscosity and molecular weight of chitosan.

Fig. 6. XRD patterns of the essential chitosan and depolymerized chitosan. (a) essential chitosan; (b) 1 pass; (c) 3 passes; (d) 5 passes.



3. Experimental Section

3.1. Materials

Chitosan was supplied by Acros Chemical Reagent Co., Ltd. The degree of deacetylation (DD) of chitosan was not less than 90 %. The average molecular weight was about 100,000 – 300,000 kDa. Acetic acid (CH_3COOH) was purchased from Samchun. All chemicals used were reagent grade.

3.2. Preparation of Depolymerized Chitosan

1 % (w/v) chitosan solution was prepared with 1 % (v/v) acetic acid solution. The chitosan solutions were then passed through a high pressure homogenizer (Nano Disperser NLM-100, ILSHIN AUTOCLAVE) with a micro orifice module operated at 500, 1000 and 1500 bar for 1 to 5 homogenization cycles. The micro orifice module of 75 μm inside diameter (Z type) was used in the high pressure homogenizer. The chitosan solution after high pressure homogenization was precipitated with 1N NaOH to pH 8.0. The precipitated chitosan was recovered by centrifugation, washed several times with deionized water, and freeze dried.

3.3. Characterization of the Depolymerized Chitosan

The viscosity of the chitosan solution was measured at room temperature using an A&D company SV-10 viscometer. GPC was used for the qualitative evaluation of the reduction in molecular weight of chitosan on an Agilent 1100 GPC instrument equipped with a refractive index detector. The GPC measurement was carried out at 40 °C with 0.2 M CH_3COOH /0.1 M CH_3COONa solution as an eluent at a flow rate of 1 mL/min. The UV-Vis spectrum was taken on a Shimadzu UV-1601 PC in the range of 200 to 500 nm. The XRD patterns of the depolymerized chitosan were obtained on a Rigaku

diffractometer using Cu K α radiation operating at 40 kV and 50 mA with a scanning rate of 2 ° min⁻¹ from 5 to 40 °. The FT-IR spectra were measured on a Thermo Nicolet 6700 spectrophotometer by the potassium bromide pellet method in the range of 400 - 4000 cm⁻¹.

4. Conclusions

The chitosan was efficiently depolymerized by high pressure homogenizer. In the high pressure homogenization, an increase in the pressure and the number of passes causes a viscosity and molecular weight decrease without the use of any acid/alkali solution and without causing any notable changes in the functional-group status. The results of XRD and UV-Vis confirmed this claim that the reduction in the molecular weight of the resulting chitosan led to the transformation of the crystal structure. The depolymerization of chitosan using a high pressure homogenizer is a green chemical process for potential medicine, pharmacy and food industries applications.

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