

PROCEEDING

19th Regional Symposium on Chemical Engineering (RSCE2012)



Strengthening the Role of ASEAN
Chemical Engineers in the world economy dynamic



November 7 - 8, 2012
Bali, Indonesia

Hosted By



Department of Chemical Engineering
Institut Teknologi Sepuluh Nopember (ITS)
Surabaya, Indonesia



ISBN : 978-602-9494-30-3

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Emma Savitri^{a*}, Azra Yuliana^a, Linggar Septy Pradeckta^a, Anitarakhmi Handaratri^a, Sumarno^a and Achmad Roesyadi

^aDepartment of Chemical Engineering, Sepuluh Nopember Institute of Technology, Surabaya 60111, Indonesia

*Corresponding Author's E-mail: savitri_ma@yahoo.com

Abstract

Degraded chitosan was prepared by sonication of chitosan. The effects of time period and temperature on molecular structure, intrinsic viscosity and conversion of dissolved substances were investigated. Chitosan decomposition was conducted through sonication process with a high intensity ultrasonic processor at temperatures from 20 to 60 °C on sonication period (10 - 120 min). The structure of the product was confirmed by FT-IR spectrum analysis. The intrinsic viscosity of degraded chitosan was determined by the method of viscometry. The decomposition of chitosan which is dissolved in water was followed by total organic carbon analyzer. The experimental results show that sonication caused the decrease of inter and intramolecular hydrogen bonding and intrinsic viscosity, eventhough the degraded chitosan which is dissolve in the water was still below 1 %.

Keywords: Chitosan; Degraded chitosan ; Sonication; Intrinsic Viscosity; TOC; dissolved substances

1. Introduction

Chitosan, (1→4)-2-amino-2-deoxy-β-D-glucan, is a natural polymer generally produced by deacetylation of chitin isolated from crustacean shells. Chitosan and its derivatives have interesting properties such as non toxicity, biocompatibility, controllable biodegradability, and non antigenicity [Cheng *et al.*, 2003; Carmen and Roland, 1997]. These properties cause chitosan as an attractive and potential biopolymer for applications in wide areas such as biotechnology, pharmaceuticals, wastewater treatment, cosmetics, agriculture, food science, and textiles [Q. Li *et al.*, 1997].

Generally, chitosan obtained from the deacetylation of chitin has a high molecular weight and low solubility in water or neutral pH solution, which limits its applications, especially in medicine, pharmacy and in the food industry. For some applications, such as in medicine, there is a necessity to decrease chitosan degree of polymerization, since low molecular weight chitosan and chitoooligomers are responsible for chitosan biological activity [Folkman and Pospieszny, 2001; Suzuki *et al.*, 1986]. However, when chitosan is degraded, its solubility can be enhanced. Moreover, low molecular weight of chitosan and chitosan oligomers obtained from chitosan possess improved biological, chemical, and physical properties compared to chitosan [Jeon *et al.*, 2001]. Muzzarelli *et al* demonstrated that chitosan with an average molecular weight in the range of 1000– 10,000 Da is most interesting for a number of medical and biotechnological applications [Muzzarelli *et al*, 1994]. Thus the development of an efficient process for reducing the molecular size of chitosan, without altering its chemical structure, is of great interest.

Like other polysaccharides, chitosan is susceptible to a variety of degradation techniques, including acid hydrolysis [Varum *et al.*, 2001; Pankaj and Lawrence, 1999], oxidative degradation [Allan and Peyron, 1995; Jian *et al*, 2003] and enzymatic methods [Wenshui *et al*, 2008].

Sonication is a common tool for the preparation and processing of polymer nanoparticles. It is particularly effective in breaking up aggregates, and in reducing the size and polydispersity of nanoparticles. It is generally agreed that sonication causes main chain scissions at the 1,4-glycosidic bond [Chen *et al*, 1997] without affecting the DD of chitosan samples. The process has, therefore, been conveniently applied to produce chitosan samples of lower molecular weights of the same DD [Tsaih and Chen, 1999].

Recently, ultrasonication of chitosan solution has been studied [Mohammad *et al*, 2008; Renata *et al*, 2005]. The technique is based on the formation of acoustic cavitation, which generates hot spots of short lifetimes with intense local heating of ~5000 °C, pressures of ~1000 atm, and heating and cooling rates above 1010 K/s [Kenneth, 1998]. These, together with free-radical formation, may mediate redox reactions and intramolecular regroupings in the samples. Cavitation also generates rapid streaming of solvent molecules around the cavitation bubble, as well as shock waves during bubble collapse, which in turn generate very large shear forces [Kenneth and Price, 1999]. In addition, rarefactions and compressions of the liquid media can cause dispersive (particle separation) and coagulative (collision and adhesion of particles) phenomena, respectively. However, degradation of chitosan solution needs to recover and purify low molecular weight of chitosan as product from the solution.

In our work we tried to reduce molecular weight of chitosan in heterogeneous system (without acetic acid as solvent) but it was still in low concentration of chitosan. The aim of this work is to explore and explain the influence of sonication temperature and time on the alteration in the physicochemical properties of chitosan residue which were monitored by its intrinsic viscosity, $[\eta]$, and Fourier transform infra-red (FTIR) spectrum. The degradation kinetic of the chitosan was also studied.

2. Experimental

Material used was medical grade chitosan and purchased from Biotech Surindo (Cirebon, Indonesia). Chitosan as initial material was used directly without any treatments. The degree of deacetylation (DD) and viscosity-average molecular weight (Mv) of chitosan are 80 % and 3,783 kDa. Water used was medical grade for injection purposes and purchased from Ika Pharmindo Putramas, Pharmaceutical Laboratories (Jakarta, Indonesia)

The experiment was carried out as follows: chitosan (2 g) was dispersed in 200 mL water. All the experiments were conducted in a 400 mL pyrex glass as reactor at different time periods (10 – 120 min) and temperature (20, 40, and 60 °C). Sonication was performed with a high-intensity ultrasonic processor VCX 500 Sonics and Materials Inc, USA (500 W, 20 kHz, 50 % Amplitude) equipped with Titanium Alloy probe transducer. The converter was made of piezoelectric lead zirconate titanate crystals. The tip of the probe was 1.3 cm in diameter and was immersed 1 cm above the bottom of reactor. The reactor was immersed into a water bath equipped with circulating water thermostat model 9102 (PolyScience, USA) to maintain temperature processes. Treated samples were filtered and dried before subjected to Fourier Transform Infra Red and viscosimetry.

The content of the total organic carbon (TOC) in the liquid phase was utilized to determine the amount of organic carbon dissolved after sonication. TOC was analyzed by a SHIMADZU-TOC-VC PN. TOC liquid were analyzed from the slurry after sonication. Before subjected to be analyzed, slurry was centrifuged and filtered to separate the solid residue.

Fourier Transform Infrared Spectroscopy (FTIR 8400S Shimadzu, Japan) was used to examine the signal variations of the degraded chitosan with the KBr disc technique. Chitosan residue was mixed with dry KBr (at ratio 1:100) and pressed into a transparent disc. FTIR spectroscopic was conducted in range 4000 – 400 cm^{-1} . All spectra were recorded at room temperature at resolution of 4 cm^{-1} and 45 scans.

The intrinsic viscosities were determined using an Ubbelohde viscosimeter by capillary viscometry Schott Gerate type 53210 (Germany) at 25 °C. Prior to measurement of flow times, dried chitosan residues were dissolved in 1 % v/v acetic acid solution at various concentration. The intrinsic viscosities $[\eta]$ were calculated according to the following equation:

$$[\eta] = \lim_{c \rightarrow 0} \ln \frac{\eta_r}{c} \quad (1)$$

The rate of decrease in viscosity was calculated according to the following equation:

$$R(\%) = \frac{[\eta_0] - [\eta_t]}{[\eta_0]} \times 100\% \quad (2)$$

3. Results and Discussion

The FT-IR spectra of the parent and degraded chitosan on various temperature of sonication as displayed in Figure 1 were recorded to investigate any structural changes during the sonication process. Generally, there were no significant differences in functional group between the residues of chitosan before and after sonication, unless there were reducing in absorption peaks in the range of spectra. The IR spectrum of chitosan showed bands assigning to the saccharide structure due to stretching of C-O-C at 1000 – 1200 [V.K.Mourya et al, 2011]. The band around 1424 cm⁻¹ was attributed to –CH₂- bending and orientation of the primary hydroxyl group in the polysaccharides. The absorption band at 1596 cm⁻¹ assigned the amino deformation mode, and the absorption bands at 1650 dan 1324 cm⁻¹ were referenced as amide I (C=O) bending and amide III bands. The band 1324 cm⁻¹ corresponds to the GlcNAc residues. Compared to original chitosan, there was a decrease in the intensity of the peak or had disappeared in the FT-IR spectra confirming association of depolymerization with lowering degree of acetylation [Focher, 1992].

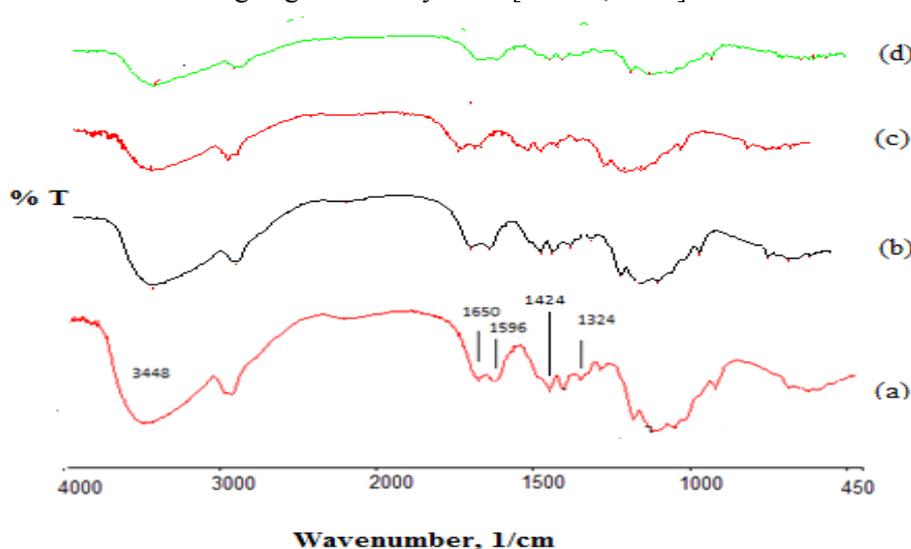


Figure 1. FT-IR spectra of chitosan : (a) original chitosan and degraded chitosan by means of sonication for a hour at temperature (b) 20 °C, (c) 40 °C and (d) 60 °C

The peak at 3340 – 3570 and 3230 - 3310 cm⁻¹ was –NH₂ bond stretching overlapping with valence vibration of H-bonded O-H, indicating intermolecular and intramolecular hydrogen bonding of chitosan molecule. Those bands of parent chitosan had a strong intramolecular and intermolecular O-H band, but those of degraded chitosan for all temperature were weaker. This indicated that sonication for all temperature gave significant alteration to chitosan structure by decreasing inter and intra molecular hydrogen bonding. The lowest of hydrogen bonding was shown by spectrum at 60 °C. The intermolecular hydrogen band becomes smaller than the intramolecular hydrogen band at 20 and 40 °C. It can also be seen that no band is observed between 1650 and 1900 cm⁻¹, which allows us to conclude that groups such as the carboxylic or aldehyde groups do not exist in chitosan residue. Moreover, the C–H binding vibration of the b-pyranose is manifested through the peak at 900 cm⁻¹. All these indicate that the conditions of this reaction system do not result in the ring-opening of chitosan repeating units. The results showed that an increase in temperature gave a significant degradation effect by lowering hydrogen bonding of chitosan. Hydrogen bonding in chitosan is one of factors that is responsible on the strong chitosan structure. The results is suitable with Price *et al* (1992) that maximum temperature can be reached from cavitation process in sonication system is proportional with ambient temperature of sonication system. The higher temperature can be produced from cavitation, the degradation rate is also increase because of increasing radical formation that responsible on breaking glycosidic bond in chitosan [Garreth J.Price and Paul F. Smith, 1993].

Similar spectra of FTIR were also obtained for various time period of sonication. The significant changes on the development of absorption peaks indicated the inter and intramolecular

hydrogen bond and no new band bands in the spectra. Reducing inter and intramolecular hydrogen bonding because chitosan molecular chain degrade into small parts and it decreases hydrogen bond of chitosan.

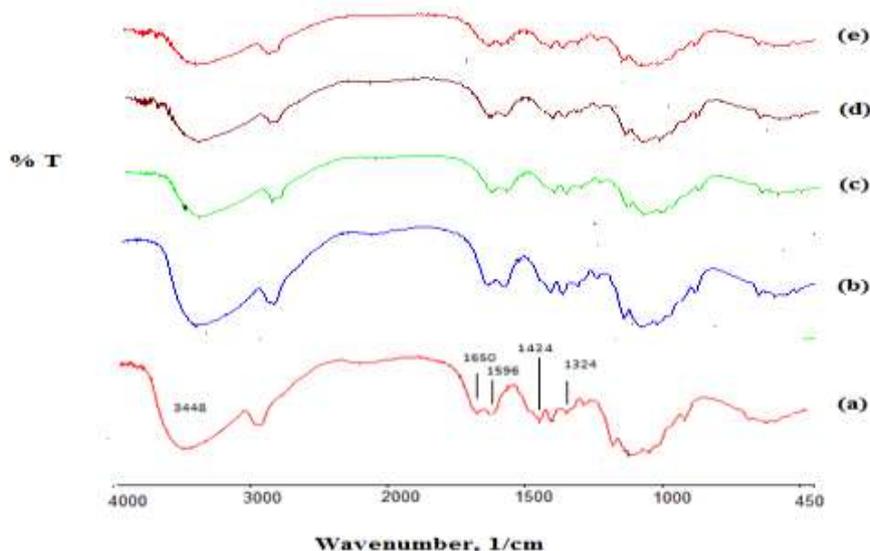


Figure 2. FT-IR spectra of chitosan : (a) original chitosan and degraded chitosan by means of sonication at 40 °C for sonication time period (b) 30 min, (c) 60 min, (d) 90 min, and (d) 120 min

When chitosan is subjected to high frequency of sonication, it undergoes degradation (main chain scission). This manifests itself as a decrease in molecular weight. We followed degradation by direct determination of changes in intrinsic viscosity by viscosimetry. The intrinsic viscosities of initial chitosan were 27 dL/g in all cases. These results are shown in Figure 3. Within the investigated time range, the intrinsic viscosity decreases with sonication time. When the initial chitosan was exposed to sonication, the intrinsic viscosity of chitosan was decreased to 22-23 dL/g for 120 min sonication. This is fully understandable, since more of OH radicals that are formed locally in the cavitation bubbles and are present in the immediate vicinity of these bubbles at high concentrations contact with chitosan for longer time than shorter time of sonication, it would yield more scissions in chitosan molecules. Moreover, the reaction becomes gradually less efficient with sonication time, what is expected for mechanochemical effects. Only long chains are vulnerable to breakage by the action of hydrodynamic forces, while chains below some limiting critical size cannot be degraded in this way (Chen et al., 2000), this limiting size, presumably under pure mechanochemical conditions.

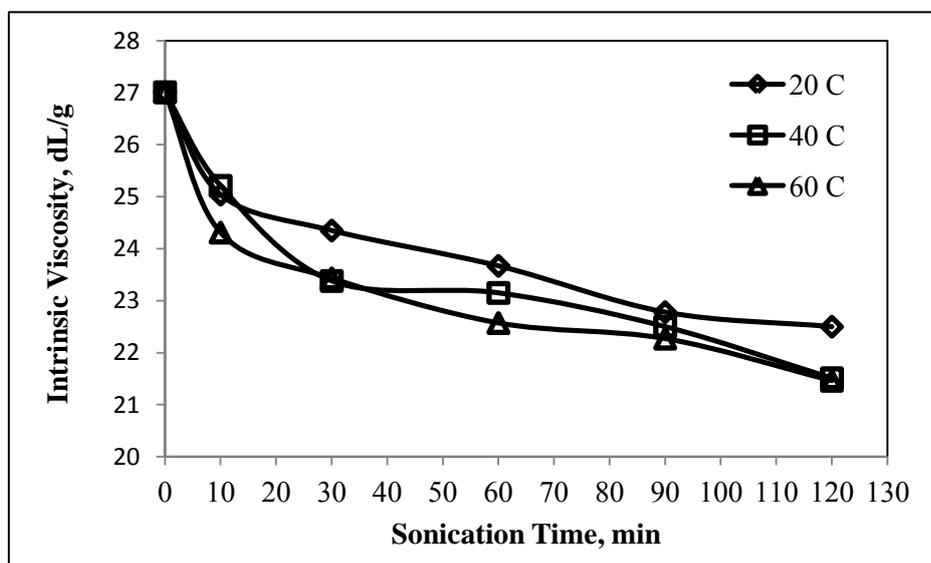


Figure 3. The intrinsic viscosities versus sonication time at different temperatures

Increasing temperature also gave effects on decreasing intrinsic viscosity, although there were no significant different between sonication temperature at 20, 40 and 60 °C compared to time of sonication. As state previously, ambient temperature of sonication affects the maximum temperature that could be reached of local cavity that responsible on mechanochemical effect on sonication. Higher ambient temperature produce higher maximum temperature of cavitation. The chemical reactions are occurring; an optimum operating temperature might exist. This is attributed to the fact that there is always a possibility that the higher concentration of chemical species is present in the cavitating bubble due to higher vapor pressure at higher operating temperature and this generates much higher amounts of free radicals in the system leading to higher reaction rates [Vinayak, Parag , 2009].

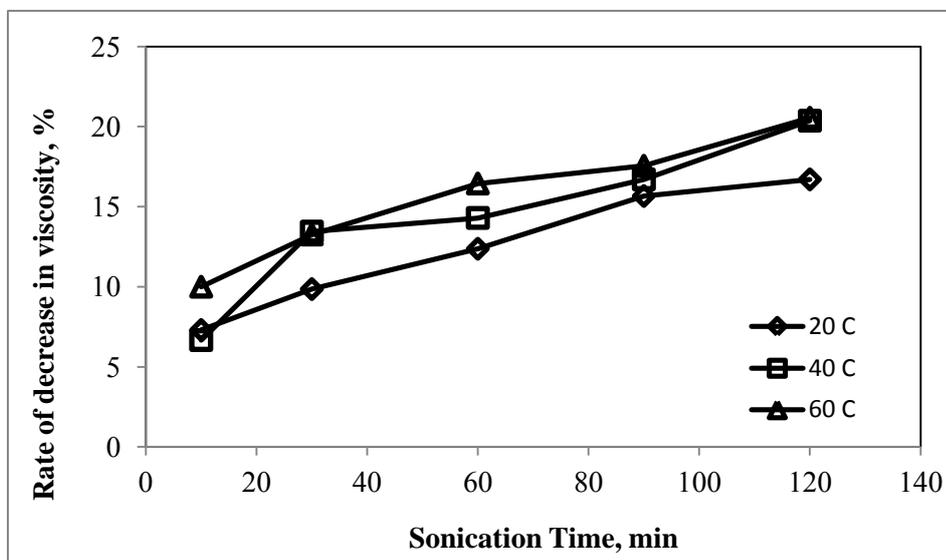


Figure 4. The rate of decrease in viscosity versus sonication time at different temperatures

The rate of decrease in viscosity of chitosan was increased to 17 - 20 dL/g at temperature 20, 40, 60 °C and 120 min of sonication as shown in figure 4. The increase of the rate tendency is similar with the decrease of intrinsic viscosity. Higher temperature and longer time of sonication increase the rate of decrease in viscosity.

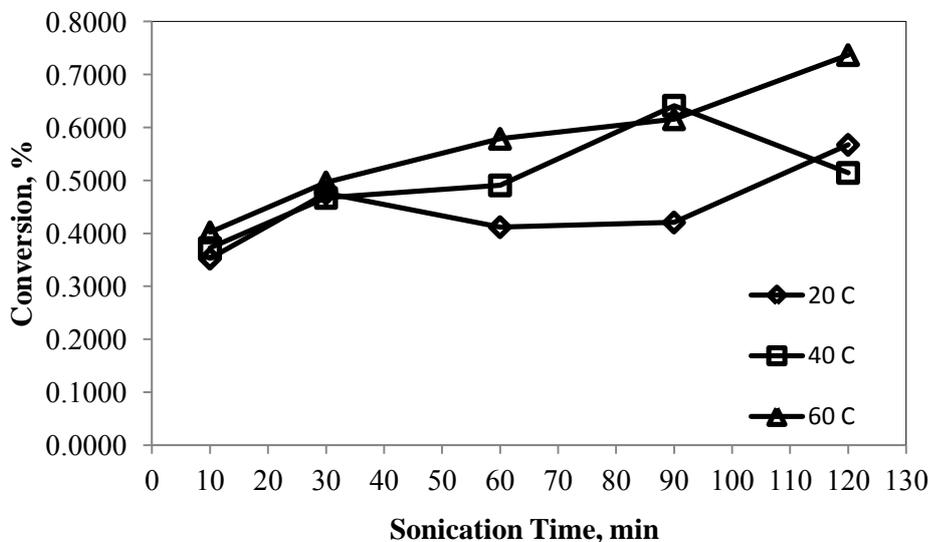


Figure 5. The conversion of degraded chitosan dissolved in water

Figure 5 shows that conversion of degraded chitosan dissolved in water was still below 1 % for time of sonication 10 – 120 min at 20 – 60 °C. Low conversion can be probably caused by several reasons such as, firstly sonication degrade polymer up to some limiting critical size as the nature of mechanochemical process of sonication. Secondly, degraded chitosan produced in the range operating condition still had high intrinsic viscosity, it meant that the molecular weight of chitosan was still high. According to Kasaai *et al* (2008) that sonication can decrease high molecular weight to

moderate molecular weight, not low molecular weight or monomer. Third, homogenous system sonication was still better performance than heterogenous system.

4. Conclusion

Sonication under the conditions, degradation is caused by OH radicals and mechanochemical effects. The hydrogen bonding (inter and intramolecular) and intrinsic viscosity of degraded chitosans in the time range of 10 – 120 min at temperature 20, 40 and 60 ° C tend to decrease up to 22 dL/g. Unfortunately, it has been demonstrated on chitosan that treatment with high intensity of ultrasound in heterogeneous system is an inefficient procedure for production of low molecular weight or monomer of chitosan. But, sonication can change the molecular structure of chitosan.

Acknowledgement(s)

This work has been financed by University of Surabaya

Nomenclature

x conversion, ratio ($\text{TOC}_{\text{dissolved substance}}/\text{TOC}_{\text{chitosan}}) \times 100 \%$

t sonication time (min)

$[\eta]$ Intrinsic viscosity (dL/g)

η_r Relative viscosity

R rate of decrease in viscosity (%)

$[\eta_0]$ Intrinsic viscosity of initial chitosan (dL/g)

$[\eta_t]$ Intrinsic viscosities of chitosan degraded solution at different times (dL/g)

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