

Depolymerization of α - & β -Chitosan by e -Beam Irradiation

Sueng Hwan Jo¹, Changyong Choi², Soo-Kyung Choi³

¹School of Medicine, Chosun University, Gwangju, Republic of Korea

²Petrochemical Process Engineering Department, Hanyeong University, Yeosu, Jeollanam-do, Republic of Korea

³Technical Research Center, LCGen Co., Ltd., Gwangju, Republic of Korea

Email: sookchoi@hanmail.net

How to cite this paper: Jo, S.H., Choi, C.Y. and Choi, S.-K. (2019) Depolymerization of α - & β -Chitosan by e -Beam Irradiation. *Journal of Biosciences and Medicines*, 7, 72-83.

<https://doi.org/10.4236/jbm.2019.710007>

Received: August 30, 2019

Accepted: October 19, 2019

Published: October 22, 2019

Copyright © 2019 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

α - and β -chitosan with molecular weight of 190,000 and 800,000 respectively, were depolymerized by e -beam irradiation with various doses. The radiation yield of scission (G_s) and degradation rate of the chitosans were identified. The synergistic chemical degradation in the presence of hydrogen peroxide is more effective at lower doses. M_w of β -chitosan was dramatically decreased from 800,000 to 21,030 at the irradiation dose 5 kGy, on the other hand, that of α -chitosan was decreased much more gradually from 190,000 to 36,000. The values of G_s at 10 kGy in the solution without H_2O_2 and with H_2O_2 were respectively 6.09×10^{-5} mol/cal and 30.6×10^{-5} mol/cal for α -Chitosan, and 8.18×10^{-5} mol/cal and 43.8×10^{-5} mol/cal for β -chitosan. It was obviously effective on depolymerization by using the combination of e -beam and H_2O_2 . α -Chitosan molecules are likely to adopt a diffuse conformation in the solution and make the different morphologies depending on the concentration.

Keywords

α - and β -Chitosan, Conformation, Depolymerization, e -Beam Irradiation, Hydrogen Peroxide

1. Introduction

Chitosan which is composed of a copolymer of N-acetylglucosamine and glucosamine is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 4)-linkage, deacetylated unit and acetylated unit. The amine group mainly exists in the form of NH_3^+ , making it a charged polycation in the chitosan molecule, which was reported to have either a rigid rod-type structure. The different molecular conformations of α - and β -chitosan influence their antibacterial mechan-

isms, such as the interactions between the protonated amino groups of chitosan and the negatively charged bacterial cell membranes [1] [2] [3].

The low solubility of chitosan exerts its limitation in use, especially in medicine and food science. To overcome the shortcoming, several researchers have attempted to reduce the molecular weight of chitosan with various chemicals such as enzymes, acids, and hydrogen peroxide [4]-[8]. Furthermore, depolymerization by high energy beam radiation has been recently investigated, since this technique provides a useful tool to separate oligo-chitosans without the need of additional processing [4] [5]. However, less attention has been paid to study on electron beam (EB) irradiation [6] [7] [8]. An EB can provide faster processing than a gamma ray [7] from ^{60}Co to irradiate the same radiation doses. It is also not needed to concern the radioactive waste disposal. As we previously described [8], hydrogen peroxide was found to have a synergistic effect in the process of EB-depolymerization of polysaccharides. The combined $\text{H}_2\text{O}_2/\text{EB}$ process for depolymerization of polysaccharide is based on the formation of a reactive hydroxyl radical due to the dissociation of hydrogen peroxide in the presence of e -beam. Generally, chitosan is a charged polycationic molecule and which is reported to have either a rigid rod-type structure. Thus, it can be expected that chitosan can be applied to specific areas as long as the conformation of the molecule is correctly identified. Characteristics of chitosan in solubility and structure of the molecular chain vary according to the factors such as collection origin, processing method, degree of deoxidation and amine distribution [9] [10] [11] [12].

Physical characteristics, such as inherent viscosity and Mark-Houwink (MH) coefficients, are closely related to polymer chain conformation. The dependence of intrinsic viscosity on molecular weight provides information on the conformation and extension of polymers. The simple MH Equation (1) expressing this fact is given by:

$$\ln[\eta] = \ln[K] + a \ln[M_v] \quad (1)$$

where K and a are empirical constants that are valid for a specific polymer-solvent pair within one to two orders of magnitude of molecular weight [13] [14] and M_v is the viscosity average of the molecular weight. The so-called Mark-Houwink constants K and a depend upon the kind of polymer, solvent and the temperature used during the viscosity measurement. The plot of the $\ln[\eta]$ vs. $\ln[M]$ typically gives a straight line with slope a and intercept $\ln[K]$. The slope, a , can vary from 0 (compact sphere) over 0.65 - 0.85 (random coil) to 2.0 (very rigid rod chain) revealing information about the polymer conformation in solution. Conformation of polymers in solution influences strongly macroscopic polymer effects like viscosity.

The conformational studies are essential to control the rheological properties of polymer solutions for semi-flexible and stiff polymer design employed in industries such as pharmaceutical, food and cosmetics. Generally, conformation of polymers is a function of its molecular weight, chain flexibility, solvent proper-

ties, etc. The different intra- and inter-molecular behaviors between α - and β -chitosan could alter the chitosan conformations in the solution, hence, the physical properties of α -chitosan are different from those of β -chitosan in solubility, reactivity, and swelling ability.

In this paper, the depolymerization of chitosan by means of high energy irradiation with various doses in H_2O_2 solution was conducted using an electron beam as an irradiation source. We will discuss the conformation of α - and β -chitosan depolymerized by EB irradiation with/without hydrogen peroxide using a recent advancement in the analysis of the molar mass and size dependencies of radiation yield of scission and the MH coefficients [15].

2. Experimental

To prepare the 1 wt% of α -chitosan (av. M_w ; 190,000 g/mol, ChitoLife Co. Ltd., Korea) and β -chitosan (800,000 g/mol, ChitoLife Co. Ltd., Korea) solution, 1% lactic acid aqueous solution was used as a solvent. The α - and β -chitosan solution were irradiated with electron beam in sealed state with or without hydrogen peroxide. The radiation yield of scission G_s (mol/J) of depolymerized chitosan was calculated by irradiating with 0, 5, 10, 20, 30, 40 kGy at 2.5 MeV energy using E-beam process system (EB Tech, Korea). The electron beam irradiated chitosan solution was precipitated in ethanol, filtered or centrifuged, washed with ethanol, and then dried for analysis. The molecular weight of depolymerized chitosan was determined by GPC-MALLS (WYATT Technology Corporation, Detector-MALLS: DAWN EOS-RI: OPTILAB DSP). Intrinsic viscosities of depolymerized chitosan were measured by using Ubbelohde viscometer at 35°C and the inherent viscosity (η_{inh}) was measured at concentrations of 1.0, 0.5, and 0.25 mg/ml. The morphology of depolymerized chitosan was investigated by TEM with FE-SEM (JEOL, JSM840A), and particle size and zeta potential (ELSZ-2000) were measured.

The radiation yield of scission of chitosan (G_s) was determined in connection with concentrations of chitosan solution. Polymer degradation can be expressed in terms of molecular weight reduction due to polymer chain scission and its efficiency can be estimated by radiation yield of scission G_s (mol/J) [16]. The G_s of chitosan by e -beam irradiation was calculated by the following Equation (2).

$$G_s = \frac{2c}{D \cdot d} \left(\frac{1}{M_w} - \frac{1}{M_0} \right) \quad (2)$$

where G_s is radiation yield of scission (mol/J), D , absorbed dose (Gy), d , solution density (kg/dm³), c , polymer concentration (g/dm³), M_w , M_0 ; weight-average molecular weight of polymer after and before irradiation respectively. The intrinsic viscosities were determined as the average of extrapolating both $\ln(\eta_{sp}/c)$ and $[c]$ to zero concentration. The particle size, size distribution and zeta potential of particles were measured by Zetasizer (Malvern Instruments, UK), based on the dynamic light scattering technique. SEM (Scanning Electron Microscope) and TEM (Transmission Electron Microscope) were used for the observation of

the aggregation of chitosan depolymerized. For MH plot to avoid any accidentally occurrence of aggregated material we started to filter the sample solutions carefully. The morphological examination of samples was conducted using SEM and BIO-TEM (Tecnai G2 spirit Biotwin, FEI, Portland, USA) using an acceleration tension of 100 kV. The diluted samples were placed on carbon coated copper grid for TEM observation.

3. Results and Discussion

The chitosans in different conformation were depolymerized by e -beam irradiation in hydrogen peroxide aqueous solution. **Table 1** & **Table 2** and **Figure 1** display the plots of MH equations for α - (**Figure 1(a)** and **Figure 1(c)**) and β -chitosan (**Figure 1(b)** and **Figure 1(d)**). As illustrated, the molecular weights of the chitosans were decreased rapidly with increasing irradiation dose up to 20 kGy in both the α - and β -chitosan solutions under the condition without H_2O_2 (**Figure 2(a)** and **Figure 2(b)**), and then gradually leveled off as the dose increased. This pattern may be attributed to the fact that the drastic depolymerization occurs in amorphous region in the beginning, and the slow depolymerization at higher doses occurs in crystalline regions. Similar results were also reported at other irradiated polysaccharides [4] [17].

Table 1. Molecular weight (M_w) and intrinsic viscosity of depolymerised Chitosan.

Dose (kGy)	α -Chitosan		β -Chitosan	
	M_w^*	$\ln[\eta]^a$	M_w^*	$\ln[\eta]^a$
0	190,000	5.220	800,000	6.892
5	36,000	5.030	21,030	4.854
10	28,470	4.879	23,910	5.204
20	4950	3.172	7470	-
30	5530	-	8270	4.323
40	5520	3.539	7020	-

*Measured by GPC-MALLS, ^athe intrinsic viscosity was evaluated by Equation (1).

Table 2. Specific viscosities (η_{spc}) of depolymerised α -Chitosan at different concentration.

Dose (kGy)	M_w	η_{spc} of α -Chitosan at different concentration (mg/ml)		
		1.0	0.5	0.25
0	190,000	0.1339	0.0755	0.0441
5	36,000	0.0777	0.0461	0.0348
10	28,470	0.0756	0.0296	0.0318
20	4950	0.0213	0.0058	0.0113
30	5530	0.0192	0.0388	0.0191
40	5520	0.0294	0.0127	0.0091

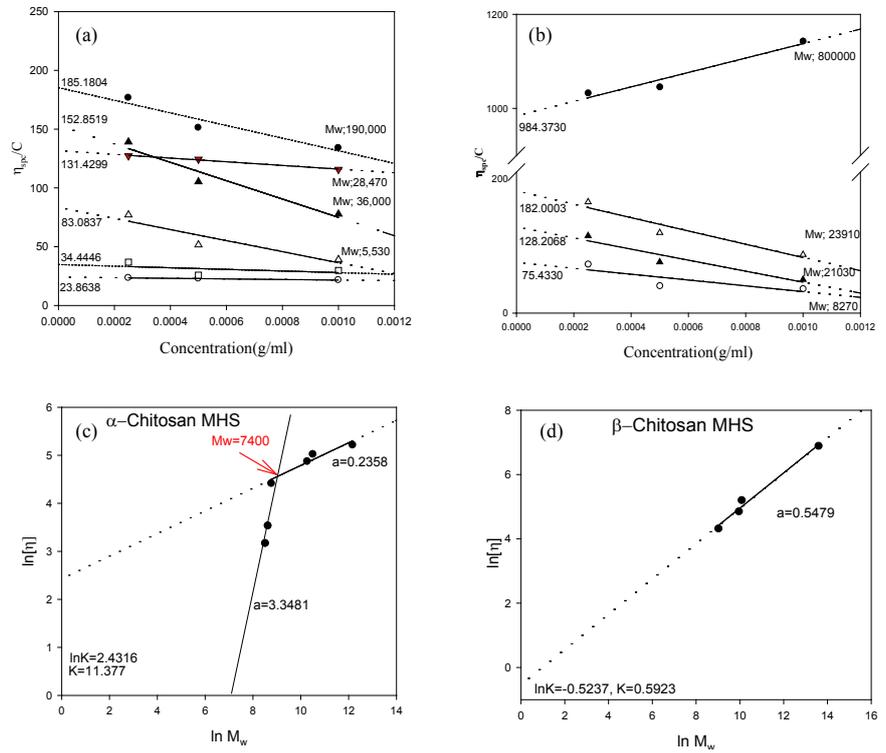


Figure 1. Intrinsic viscosity of (a) α - and (b) β -Chitosan and MHS plots of (c) α - and (d) β -Chitosan.

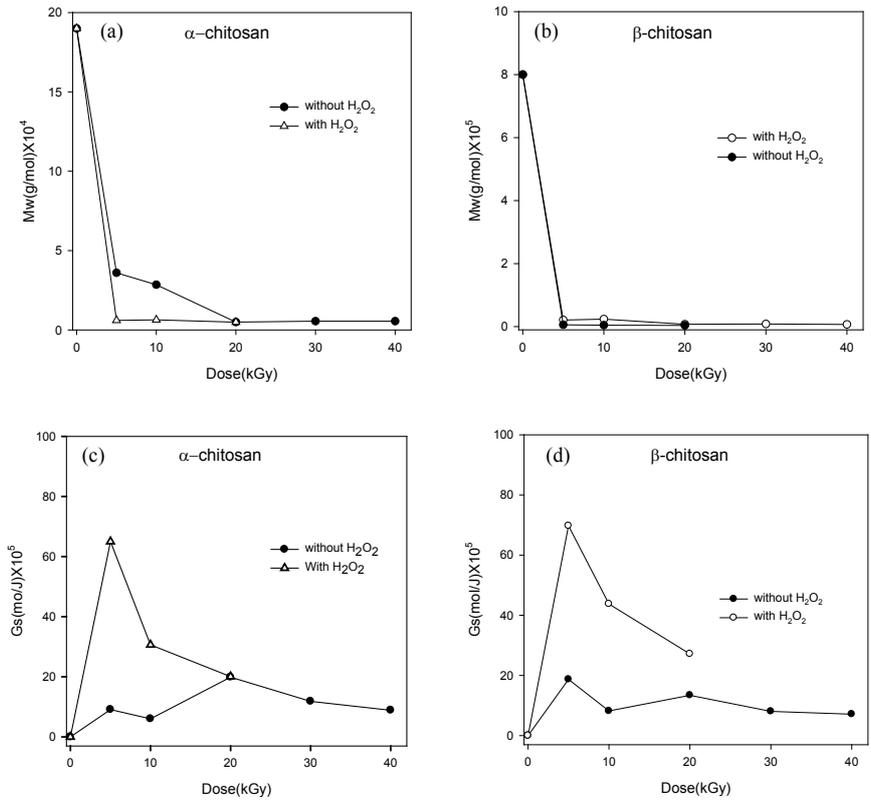


Figure 2. Molecular weight vs. dose of (a) α - and (b) β -Chitosan and G_s of (c) α - and (d) β -Chitosan.

$\ln[\eta]$ was plotted against $\ln[M_w]$ and the MH exponent, “ a ” values were evaluated for the α -chitosan in various concentrations (**Figure 1(c)**). $\ln[\eta]$ of the α -chitosans whose molecular weights are 7400 or higher increased slightly with increasing molecular weight, it gave 0.236 in MH value, a . Whereas $\ln[\eta]$ of α -chitosan whose molecular weights were lower than 7400 showed a pronounced increase of 1.854 in slope. It means that compact sphere changes to very rigid rod chain in the solution at around M_w 7400. This indicates that molecular weight-induced conformational transition occurred in this M_w range. On the other hand, the a for β -chitosan was 0.548 which means random coil in its conformation.

Several reports suggested that irradiation-induced scissions of glycosidic bonds of chitosan caused an inconsistent reduction in molecular weight of the polymers in different conformation [18] [19]. As shown in **Table 1**, the molecular weight of β -chitosan was dramatically decreased from 800,000 to 21,030 at the irradiation dose 5 kGy, on the other hand, that of α -chitosan was decreased much more gradually from 190,000 to 36,000. That means that synergistic degradation in the presence of hydrogen peroxide is rather effective at low doses such as around 5 kGy. It is well known that chitin has two forms, named α and β chitin, in which α -chitin is very stable with intra-chain and inter-sheet hydrogen bonds from the antiparallel sheets along with c -axis in orthorhombic cell, while β -chitin has no hydrogen bonds between two inter-sheets owing to their parallel directions [18] [19] [20].

Kurita reported [21] that α -chitin is more rigid and more crystalline and can be less susceptible to deacetylation compared to β -chitin. Therefore, the polymeric structures of chitosan deacetylated from different forms of chitin may not be identical and β -chitosan can have higher solubility with less crystallinity, thus providing much higher radiation susceptibility than α -chitosan. In order to estimate the radiation sensitivity of the α - and β -chitosan molecules in the presence or absence of H_2O_2 , the radiation yield of scission G_s were calculated (**Table 3** and **Figure 2(c)** and **Figure 2(d)**) in the range from 0 to 40 kGy. The results showed that the depolymerization of chitosan by e -beam irradiation was much faster in the solution with H_2O_2 , than in that without H_2O_2 up to 20 kGy (**Figure 3**). It was obvious that the rate of chitosan molecular weight decreased by the combination of e -beam and H_2O_2 was much higher than that by e -beam irradiation alone. This result showed that the synergistic effect for degradation of chitosan with e -beam and H_2O_2 gained the most effective outcome, especially at the lowest absorbed dose studied of 5 kGy. In both cases, G_s increased dramatically at 5 kGy and then decreased gradually. The values of G_s at 10 kGy in the solution without H_2O_2 and with H_2O_2 were 6.09×10^{-5} mol/cal and 30.6×10^{-5} mol/cal respectively for α -Chitosan, and 8.18×10^{-5} mol/cal and 43.8×10^{-5} mol/cal for β -chitosan. It means that it was obvious that the rate of chitosan molecular weight decreased by the combination of e -beam and H_2O_2 was much higher than that by e -beam irradiation alone as mentioned above. The chain scission yield, G_s seems to be greatly influenced by the presence of H_2O_2 in both chitosan solu-

tions. One G_s value obtained by other authors for chitosan irradiated in aqueous solution with molecular weight of 100 kDa was 0.382×10^{-7} mol/J [22]. The radiation depolymerization yield in chitosan solution is strongly influenced by the presence of hydrogen peroxide, initial molecular arrangement and irradiation conditions such as crystallinity, moisture content, radiation dose [7] [23] [24]. The observation from electron microscope (SEM and TEM) revealed information on the shape and size of the particles. The irradiations of the α -chitosan gave the individual spherical shape. On the other hand, it was difficult to identify the irradiated β -chitosan as a spherical shape. The α and β -chitosan particles in these samples were found in variable shapes (Figure 4 & Figure 5). To investigate the effect of radiation dose and concentration of chitosan during irradiation on the size and size distribution of aggregates, the aggregates from SEM and TEM images were randomly collected and determined for all irradiation conditions. The result indicated that both factors influenced not only aggregate shape but also aggregate size (Figure 4) as well as size distribution (Figure 6). The size distribution plots in Figure 6 clearly show that the e -beam irradiation gave chitosan particles with very narrow size distribution when compared to the original one. The present experimental plots prove that aggregate size distribution is possibly controlled by using e -beam irradiation. Radiation seems to influence the particle size over an extended radiation dose period as observed on the left-shift in the distribution plot. The relationship between radiation dose and aggregate size as shown in Table 4 and Figure 6 confirmed that the higher the radiation dose, the bigger the aggregate size. This is inconsistent with nanoparticles formed from chitosan by high energy irradiation [4] [5] [6] [24].

Table 3. G_s of depolymerised Chitosan with/without hydrogen peroxide.

Dose (kGy)	α -Chitosan		β -Chitosan	
	Without H ₂ O ₂	With H ₂ O ₂	Without H ₂ O ₂	With H ₂ O ₂
	$G_s(\times 10^5)$	$G_s(\times 10^5)$	$G_s(\times 10^5)$	$G_s(\times 10^5)$
0	0.0000	0.0000	0.0000	0.0000
5	9.0959	65.0168	18.7084	69.7559
10	6.0320	30.6579	8.1958	43.7562
20	19.8848	20.0597	13.4017	27.1489
30	11.8282	-	8.0548	-
40	8.8828	-	7.1255	-

Table 4. Average diameters and zeta potential of α -Chitosan at different concentration.

Dose (kGy)	Ave. Diameter (nm) at different concentration (mg/ml)			Zeta potential (mV) at different concentration (mg/ml)		
	1.0	0.5	0.25	1.0	0.5	0.25
	0	137.6	-	-	2.87	-
10	305.8	-	-	14.8	-	-
20	666.8	-	1188.7	13.0	-	5.07
30	1287.4	1119.7	1027.1	16.2	18.03	2.52

Data from Particle size analyzer.

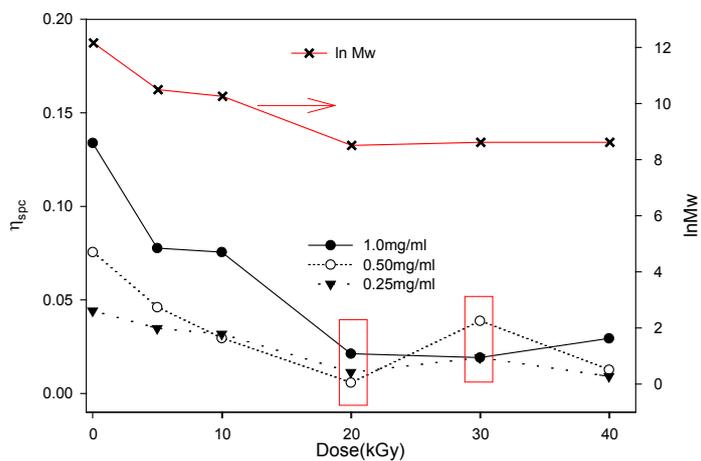


Figure 3. Molecular weight and specific viscosity of depolymerized α -Chitosan with different dose.

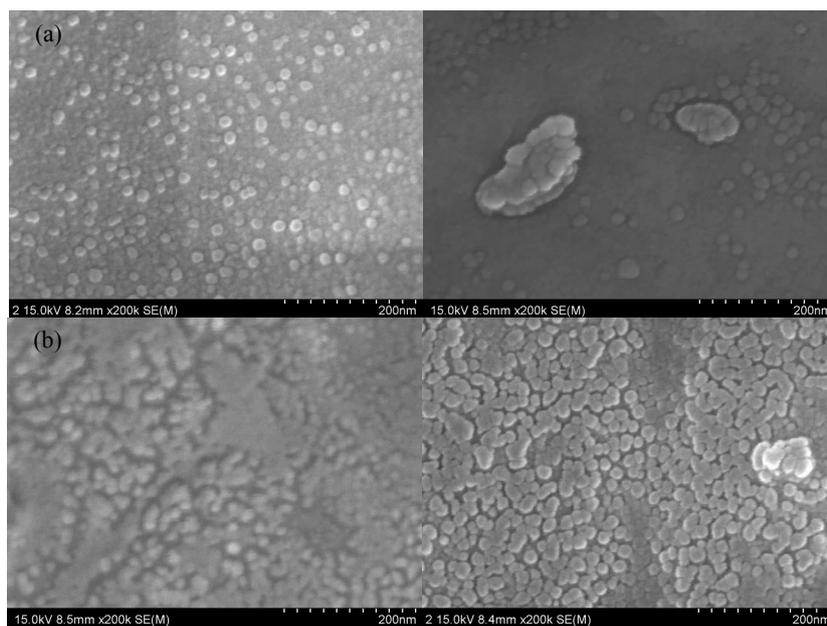


Figure 4. SEM images of depolymerised α -Chitosan (30 kGy) at (a) 1.0 mg/ml (b) 0.5 mg/ml concentration.

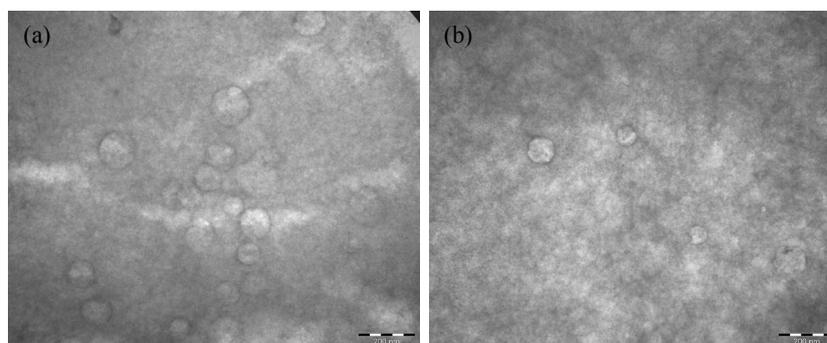


Figure 5. TEM images of depolymerised α -Chitosan (30 kGy) at (a) 1.0 mg/ml (b) 0.5 mg/ml concentration ($\times 100$ K magnification).

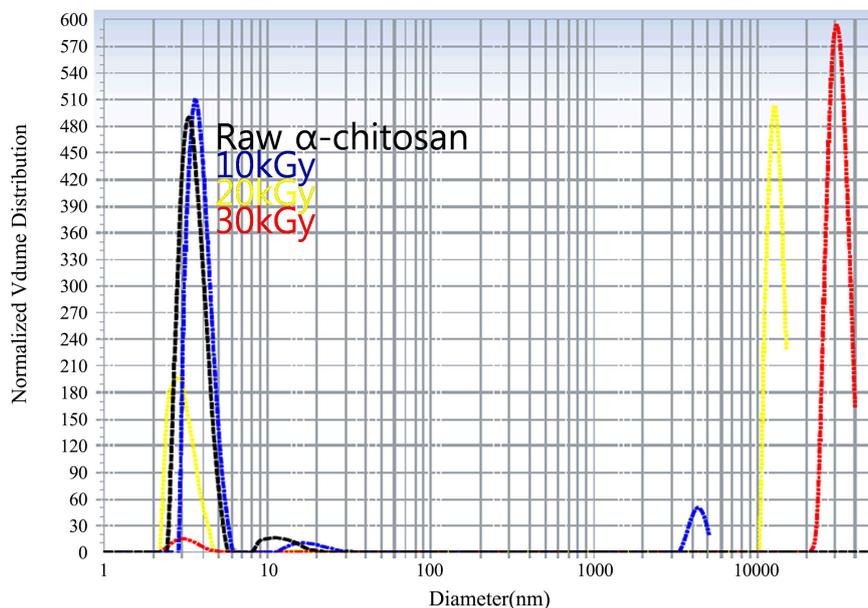


Figure 6. Particle size of raw α -Chitosan (black), depolymerised α -Chitosan at 10 kGy (Blue), 20 kGy (Yellow), and 30 kGy (Red) at 0.5 mg/ml concentration.

This pattern obtained from α -chitosan may be due to the formation of ionic clusters based on ion-ion interaction which could give much bigger aggregates. Additionally, the irradiation of the chitosan seems to bring the aggregate size bigger. The particle sizes are up to more than 100 nm, when using e -beam dose as low as 5 kGy.

The particle still formed significant circle-like shape as expected, especially in spherical form. TEM images implied that α -chitosan irradiated at low concentration (0.5 mg/ml) gave a smaller particle size with individual spherical shape than that from high concentration (1.0 mg/ml). SEM images have shown the morphological properties and surface appearance of aggregates. The aggregates have nearly spherical shape, smooth surface and size range of about 20 - 80 nm (**Figure 4**).

SEM imaging of the two solutions are consistent with the size profiles, mono-dispersed nanoparticles and agglomerates of several hundred nanometers were visible in the solution. In the present study, the results obtained by Zetasizer revealed that the zeta potential of the α -chitosan nanoparticles can greatly influence their stability in suspension by means of electrostatic repulsion between the particles. Our results demonstrated respective zeta potentials of α -chitosan nanoparticles of 2.5 and 28.7 mV. It is supposed that the α -chitosan molecules depolymerized by e -beam radiation are not uniform. Chitosan molecules are likely to adopt a diffuse conformation in the solution because of electrostatic repulsion force existing between amine groups along the molecular chain [8] [10] [23]. The carboxyl groups on the surface of a large protein molecule may form hydrogen bonds with amine groups at certain sites along the chitosan chain [23].

Moura *et al.* (2008) investigated the possibility of producing and controlling

chitosan nanoparticles with different sizes via electrostatic interaction of chitosan and methacrylic acid [25]. The mechanism for the synergistic effect was presented by Kang, Dai, Zhang, and Chen (2007) [5]. Particularly, the degradation of chitosan by the gamma-ray irradiation alone can be ascribed to the direct action of radiation on the chitosan chains. In the present study the effect of different chitosan concentrations (0.25, 0.5, 1.0 mg/mL) on molecular weight and viscosity was evaluated (Table 2) and measured the size and zeta potential by Zetasizer (Table 4, Figure 6). Our results showed that by increasing the chitosan concentration from 0.25 to 1.0 mg/mL in the absence of hydrogen peroxide, the size of nanoparticles increased (Table 1). Table 2 also shows that specific viscosity of chitosan decreased with decreasing chitosan molecular weight in solutions. For chitosan of the same molecular weight, specific viscosity decreased with increasing solution concentration. Zetasizer revealed that the size of the aggregates of α -chitosan was increased from 138 nm to 1287 nm with increasing radiation dose from 0 to 30 kGy, possibly due to Zeta potential of chitosan particles can greatly influence their stability in suspension by means of electrostatic repulsion between the particles [26]. The results indicate that the chain flexibilities of higher molecular weight chitosans were higher than those of lower molecular weight ones.

However, strong pre-peaks in the detector responses (especially in the light scattering detector) and an increase or curvature of the $\ln[M_w]$ distribution over the retention volume indicate the presence of aggregates. These effects were completely absent for the samples measured. Yanagisawa *et al.* [27] showed that the absence of aggregates is an important prerequisite for a reliable conformational analysis. For MH plot to avoid any occurrence of aggregates we filtered the sample solutions carefully. Chitosan molecules are likely to adopt a diffuse conformation in the solution because of electrostatic repulsion force existing between amine groups along the molecular chain.

4. Conclusion

The chitosan with two different molecular weights in different conformation was depolymerized by e -beam irradiation in aqueous solution. Radiation sensitivity was evaluated by the determination of radiation yield of scission G_s and degradation behavior. The e -beam irradiation, used as a method to reduce the molecular weight of chitosan, showed even greater synergy when used with H_2O_2 and its synergistic degradation is more effective at lower irradiation doses. The zeta potentials of the chitosans demonstrated the molecules depolymerized by e -beam are not uniform

Acknowledgements

This work was supported by the NRF and WISSET Grant (2019-333) funded by the MIST under the Program for Returners into R & D and also NRF grant (2014R1A1A 2058323).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Rinaudo, M. (2006) Chitin and Chitosan: Properties and Applications. *Progress in Polymer Science*, **31**, 603-632. <https://doi.org/10.1016/j.progpolymsci.2006.06.001>
- [2] Paul, W. and Sharma, C.P. (2000) Chitosan, a Drug Carrier for the 21st Century: A Review. *STP Pharma Sciences*, **10**, 5-22.
- [3] Brugnerotto, J.D.J., Heux, L., Mazeau, K. and Rinaudo, M. (2001) Overview on Structural Characterization of Chitosan Molecules in Relation with Their Behavior in Solution. *Macromolecular Symposia*, **168**, 1-20. [https://doi.org/10.1002/1521-3900\(200103\)168:1<::AID-MASY1>3.0.CO;2-W](https://doi.org/10.1002/1521-3900(200103)168:1<::AID-MASY1>3.0.CO;2-W)
- [4] Ahn, K.-J., Choi, W.-S., Lee, D.-W., Byun, M.-W. and Park, H.-J. (2002) Preparation of Chitosan Oligomers by Irradiation. *Polymer Degradation and Stability*, **78**, 533-538. [https://doi.org/10.1016/S0141-3910\(02\)00226-4](https://doi.org/10.1016/S0141-3910(02)00226-4)
- [5] Kang, Y.D.D.B., Zhang, H.Q. and Chen, D. (2007) Synergetic Degradation of Chitosan with Gamma Radiation and Hydrogen Peroxide. *Polymer Degradation and Stability*, **92**, 359-362. <https://doi.org/10.1016/j.polymdegradstab.2006.12.006>
- [6] Kim, H. (2013) Molecular Weight Control of Chitosan Using Gamma Ray and Electron Beam Irradiation. *Journal of Radiation Industry*, **7**, 51-54. <https://www.ksri.kr/0304/view/page/25/id/293>
- [7] Tahtat, D., Mahlous, M., Benamer, S., Khodja, A.N. and Youcef, S.L. (2012) Effect of Molecular Weight on Radiation Chemical Degradation Yield of Chain Scission of γ -Irradiated Chitosan in Solid State and in Aqueous Solution. *Radiation Physics and Chemistry*, **81**, 659-665. <https://doi.org/10.1016/j.radphyschem.2012.02.036>
- [8] Jo, B.W. and Choi, S.-K. (2014) Degradation of Fucoidans from Sargassum Fulvellum and Their Biological Activities, Carbohydrate Polymers. *Carbohydrate Polymers*, **111**, 822-829. <https://doi.org/10.1016/j.carbpol.2014.05.049>
- [9] Ulanski, P. and Rosiak, J.M. (1992) Preliminary Studies on the Radiation-Induced Changes in Chitosan. *Radiation Physics and Chemistry*, **39**, 53-57. [https://doi.org/10.1016/1359-0197\(92\)90171-B](https://doi.org/10.1016/1359-0197(92)90171-B)
- [10] Nagasawa, H.M.N., Yoshii, F. and Kume, T. (2000) Radiation-Induced Degradation of Sodium Alginate. *Polymer Degradation and Stability*, **69**, 279-285. [https://doi.org/10.1016/S0141-3910\(00\)00070-7](https://doi.org/10.1016/S0141-3910(00)00070-7)
- [11] Wu, W.J., Huang, K.S., Chen, J.B. and Lian, H.S. (2008) Application of Low-Molecular-Weight Chitosan in Durable Press Finishing. *Carbohydrate Polymers*, **73**, 254-260. <https://doi.org/10.1016/j.carbpol.2007.11.023>
- [12] Diep, T.B., Hai, L., Nagasawa, N., Yoshii, F. and Kume, T. (2003) Radiation Depolymerization of Chitosan to Prepare Oligomers. *Nuclear Instruments and Methods in Physics Research B*, **208**, 466-470. [https://doi.org/10.1016/S0168-583X\(03\)01181-9](https://doi.org/10.1016/S0168-583X(03)01181-9)
- [13] Bohdanecky, M. and Kovar, J. (1982) Viscosity of Polymer Solutions. Elsevier Scientific Publishing Company, Amsterdam.
- [14] Anthonsen, M.W., Vürum, K.M. and Smidsrod, O. (1993) Solution Properties of Chitosans: Conformation and Chain Stiffness of Chitosans with Different Degrees of N-Acetylation. *Carbohydrate Polymers*, **22**, 193-201.

- [https://doi.org/10.1016/0144-8617\(93\)90140-Y](https://doi.org/10.1016/0144-8617(93)90140-Y)
- [15] Wang, W., Bo, S., Li, S. and Qin, W. (1991) Determination of the Mark-Houwink Equation for Chitosans with Different Degrees of Deacetylation. *International Journal of Biological Macromolecules*, **13**, 281-285. [https://doi.org/10.1016/0141-8130\(91\)90027-R](https://doi.org/10.1016/0141-8130(91)90027-R)
- [16] Chemat, F., Teunissen, P.G.M., Chemat, S. and Bartels, P.V. (2001) Sono-Oxidation Treatment of Humic Substances in Drinking Water. *Ultrasonics Sonochemistry*, **8**, 247-250. [https://doi.org/10.1016/S1350-4177\(01\)00084-0](https://doi.org/10.1016/S1350-4177(01)00084-0)
- [17] Aliste, A.J., Vieira, F.F. and DelMastro, N.L. (2000) Radiation Effects on Agar, Alginates and Carrageenan to Be Used as Food Additives. *Radiation Physics and Chemistry*, **57**, 305-308. [https://doi.org/10.1016/S0969-806X\(99\)00471-5](https://doi.org/10.1016/S0969-806X(99)00471-5)
- [18] Lamarque, G., Cretenet, M., Viton, C. and Domard, A. (2005) New Route of Deacetylation of α - and β -Chitins by Means of Freeze-Pump Out-Thaw Cycles. *Biomacromolecules*, **78**, 1380-1388. <https://doi.org/10.1021/bm049322b>
- [19] Dweltz, N.E. (1961) The Structure of β -Chitin. *Biochimica et Biophysica Acta*, **51**, 283-294. [https://doi.org/10.1016/0006-3002\(61\)90169-X](https://doi.org/10.1016/0006-3002(61)90169-X)
- [20] Minke, R. and Blackwell, J. (1978) The Structure of α -Chitin. *Journal of Molecular Biology*, **120**, 167-181. [https://doi.org/10.1016/0022-2836\(78\)90063-3](https://doi.org/10.1016/0022-2836(78)90063-3)
- [21] Kurita, K., Yoshino, H., Nishimura, S.-I. and Ishii, S. (1993) Preparation and Biodegradability of Chitin Derivatives Having Mercapto Groups. *Carbohydrate Polymers*, **20**, 239-245. [https://doi.org/10.1016/0144-8617\(93\)90095-L](https://doi.org/10.1016/0144-8617(93)90095-L)
- [22] Hien, N.Q., Phu, D.V., Duy, N.N. and Lan, N.T.K. (2012) Degradation of Chitosan in Solution by Gamma Irradiation in the Presence of Hydrogen Peroxide. *Carbohydrate Polymers*, **87**, 935-938. <https://doi.org/10.1016/j.carbpol.2011.08.018>
- [23] Pasanphan, W., Choofong, S., Rimdusit, P. and Piroonpan, T. (2010) Systematic Fabrication of Chitosan Nanoparticle by Gamma Irradiation. *Radiation Physics and Chemistry*, **79**, 1095-1102. <https://doi.org/10.1016/j.radphyschem.2010.04.003>
- [24] Pillai, C.K.S., Paul, W. and Sharma, C.P. (2009) Chitin and Chitosan Polymers: Chemistry, Solubility and Fiber Formation. *Progress in Polymer Science*, **34**, 641-678. <https://doi.org/10.1016/j.progpolymsci.2009.04.001>
- [25] De Moura, M.R., Aouada, F.A. and Mattoso, L.H.C. (2008) Preparation of Chitosan Nanoparticles Using Methacrylic Acid. *Journal of Colloid and Interface Science*, **321**, 477-483. <https://doi.org/10.1016/j.jcis.2008.02.006>
- [26] Wu, Y., Yang, W., Wang, C., Hu, J. and Fu, S. (2005) Chitosan Nanoparticles as a Novel Delivery System for Ammonium Glycyrrhizinate. *International Journal of Pharmaceutics*, **295**, 235-245. <https://doi.org/10.1016/j.ijpharm.2005.01.042>
- [27] Yanagisawa, M., Kato, Y., Yoshida, Y. and Isogai, A. (2006) SEC-MALS Study on Aggregates of Chitosan Molecules in Aqueous Solvents. *Carbohydrate Polymers*, **66**, 192-198. <https://doi.org/10.1016/j.carbpol.2006.03.008>