การประยุกต์รังสีแกมมาในระดับที่เหมาะสมสำหรับไคโตซานมวลโมเลกุลต่ำ

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บทคัดย่อ

งานวิจัยนี้เสนอระดับรังสีแกมมาเพื่อการใช้จริงสำหรับการลดมวลโมเลกุลไคโตซานในสภาวะที่เป็นของแข็ง และในน้ำ มวลโมเลกุลไคโตซานลดลงได้มากถึง 80% เมื่อใช้ปริมาณรังสี 50 kGy ในกรณีที่มีตัวกระตุ้นฟรีราดิคอล ปริมาณรังสีที่ใช้สำหรับการลดมวลโมเลกุลในระดับเดียวกันเหลือเพียง 20 kGy โครงสร้างไคโตซานเปลี่ยนไปอย่างเด่นชัดเมื่ออยู่ในสภาวะสารละลายกรดอะซีติก 1% หรือไคโตซานในน้ำที่มีสารละลายโปตัสเซียมเปอร์ซัลเฟต 2%

Practical γ-Ray Level for Low Molecular Weight Chitosan

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Abstract

The present work proposes a practical level of γ-Ray to lower the molecular weight of chitosan irradiated in solid state and water. The molecular weight reduction is up to 80% at γ-ray amount of 50 kGy. The same level of reduction can be achieved by only 20 kGy in the presence of initiator ($K_2S_2O_8$ or H_2O_2). The structure is significantly changed in the case of chitosan-acetic acid solution or chitosan dispersed in water with 2% aq. $K_2S_2O_8$ solution.

Keywords: chitosan, γ-ray irradiation, low molecular weight chitosan, radical formation

Introduction

Chitin-chitosan is a biocopolysaccharide existed mainly in crustacean shell and exoskeleton of insects, consisting of β-(1,4)-2-acetamido-2-deoxy-β-D-glucose and β-(1,4)-2-amino-2-deoxy-β-D-glucose units linked by glycoside bonds. Up to now, many researchers have been interested in chitin-chitosan due to the biodegradability[1a], biocompatibility[1b], bioactivity[1c] and non-toxicity[1d]. The expected applications are, for examples, water treatment[2a], papermaking[2b], pharmaceutics[2c], biotechnology[2d], agriculture[2e], and food processing[2f].

As seen in the cases of cellulose, chitin-chitosan possesses a high molecular weight with a strong inter- and intra-molecular hydrogen bond network to stabilize polymer chain. In order to develop chitin-chitosan for practical and advanced applications, it is important to overcome the limitations about the poor solubility in most organic solvents. Low molecular weight species is an alternative way to activate chitosan. For the past decades, several efforts have been done to prepare low molecular weight chitosan (LMWC) and/or oligochitosan such as chemical treatment[3], enzymatic degradation[4], and photoirradiation[5,6]. Chemical treatment can be easily done, but chemical waste and reproducibility are the main problems. Enzymatic degradation is an effective way to achieve specific cleavage under mild conditions, however it requires multi-steps for enzyme preparation and product purification. In the case of photoirradiation, although the expertise and operating system are needed, the simple process and the effectiveness in degradation are attractive. Previously, the studies on γ-ray irradiation on chitosan are mainly dealt with radiation conditions, mechanisms, and initiators whereas the optimum amount of γ-ray for practical low molecular weight production under the retaining of chitosan structure are still questioning.

Herein, we stand on the viewpoint to apply γ-ray for producing low molecular weight chitosan under the simple, but effective conditions. The present work focuses on comparative studies about solid-state, dispersion state and solution conditions with and without radical initiator, type and concentration of initiators as well as other related factors.

Methods

The γ-irradiation of chitosan samples was carried out in a γ-cell (Co-60) by a courtesy of Office of Atomic Energy for Peace, Ministry of Science and Technology, Thailand. Chitosan (%DD of 80, 85, and 90 provided by Seafresh Chitosan (Lab) Company Limited, Thailand) were irradiated from 10 to 160 kGy at a dose rate of 5 kGy/h. The conditions used for γ-ray irradiation were chitosan flake in solid state (condition 1), chitosan flake dispersed in water (condition 2), chitosan flake dispersed in 0.05, 0.1, 1, and 2% $K_2S_2O_8$ solution (condition 3), chitosan flake dispersed in 0.5, 1, and 2% H_2O_2 solution (condition 4), and chitosan solution in 1% acetic acid (condition 5).

Results and Discussion

It was found that the color of chitosan was changed from a pale yellow flake to dark yellow as a result from the γ-ray irradiation (conditions 1-3). In the presence of H_2O_2 (condition 4), the irradiated product turns to be yellowish.

Effect of Irradiation on Molecular Weight In general, when polymer is exposed to the irradiation, both degradation and crosslinking will occur depending on the mechanism and conditions (dispersing medium, concentration, temperature, etc.). Based on the theory of radiation degradation and crosslinking, Ulanski and Rosiak[6] found that the chain scission of chitosan occurred in solid state either under vacuum or air. The chain scission was the most significant under oxygen whereas the crosslinking was hardly proceeded. Figure 1 shows the comparative studies in the different conditions (conditions 1-4). For each condition, the molecular weight (M_v) of chitosan continuously decreases when the amount of γ-ray is increased. Figure 1 also implies that the degradation is prior to the crosslinking. The 90%DD chitosan was found to give the lowest molecular weight as compared to those of 85 and 80%DD. This might be due to the higher crystalline structure of chitin than that of chitosan.

For conditions 1 and 2, the molecular weight is significantly decreased (about 75-80 %) in the first 50 kGy, and slightly decreased even the γ-ray amount was kept increasing. The decrease in molecular weight reached the equilibrium at 50 kGy for 1.8×10^5 dalton in the case of 90%DD chitosan.

Ulanski and Rosiak reported that water molecules give $OH^\bullet$ and $H^\bullet$ for chain

depolymerization in pulse hydrolysis. Here, chitosan was suspended in water (condition 2) to enhance the depolymerization by water molecules.   However, it was found that the decrease in molecular weight was almost similar to the condition 1. This might be due to the fact that chitosan flake was stable and insoluble in water even under the irradiation condition.

Another attempt to proceed depolymerization in homogeneous system was done. Chitosan solution in 1% acetic acid (condition 5) was irradiated to find that at γ-ray amount of 20 kGy the chemical structure of chitosan were changed as observed from FT-IR (Figure 2). When $K_2S_2O_8$ was added (condition 3), the decrease in molecular weight was achieved for 56% at only γ-ray amount of 20 kGy. For H_2O_2, the decrease in molecular weight was more obvious to be 84% at 20 kGy. This implied $K_2S_2O_8$ and H_2O_2 were effective initiators due to radical induction. It should be noted that, in all cases, chemical structure and morphology were rarely changed, however, in the case of $K_2S_2O_8$, when the concentration is increased to 2%, the chemical structure was largely changed (Figure 3).

Effect of Irradiation on Thermal Stability and Packing Structure   All of chitosan starting materials show thermal stability up to 309°C (T_d) due to strong hydrogen bonds network and high crystallinity. The conditions 3 and 4 showed the significant decrease in molecular weight, thus, it was expected that T_d of the irradiated products should be less than 309°C. Figure 4 ((a)-(d)) illustrates that T_d of irradiated chitosan at various doses is constant at 309$\pm$2°C. This implies that chain scission mainly occur at C-O-C glycosidic linkages whereas hydrogen bonds constructed from $NHCOCH_3$, NH_2, and OH groups still remain.

Effect of Irradiation on Chemical Structure   Figure 5 illustrates FT-IR spectra of chitosan and its irradiated products under condition 1. It was found that the major peaks of chitosan at 895, 1087, 1598, 1653, and 3439 cm^{-1} belonging to pyranose ring, glucoside, amino, acetamide, and hydroxyl groups, respectively, still remain in the structure even the radiation amount was increased up to 160 kGy. Figure 6 shows ^{13}C CP/MAS NMR spectra of chitosan starting material and the one from condition 1; the signals due to carbons of hexosamine residues, methyl group, and carbonyl acetamide are assigned at 58.4-105.4, 23.5, and 166.5 ppm, respectively. In the cases of chitosan obtained from condition 1, using γ-ray amount for 10 and 40 kGy, only the C-4 singlet peak at 83.7 was splitted to doublet suggesting that the chain scission and new functional group formation most probably occur around C-4. Combining with the decreasing in

molecular weight results, it should be concluded that γ-ray irradiation induces the chain scission at β-(1,4) glycosidic bond, where free radicals would attack at either C-1-O or C-4-O to break the C-O-C linkage in the main chain (Scheme 1). Here, the peak shift at C-1 is expected, but it was not significant as observed in ^{13}C CP/MAS NMR spectrum.

It is important to note that in the conditions 3 (with 2%$K_2S_2O_8$ aq. solution) and 5, the changes in structure were found. Figure 3 shows that the peaks at 1655, 1550, and 1200-900 cm^{-1} belonging to amide I, amide II, and saccharide, respectively were shifted and changed after γ-ray irradiation even the dose is as low as 10 kGy. Moreover, the irradiated products gave gel after dissolving in acetic acid. These indicated that the chemical structure of chitosan after exposure to γ-ray under this condition was changed and some crosslinking was occured.

For condition 5, chitosan was homogeneously dissolved in 1% acetic acid before irradiation at 20 kGy. It was found that the color of solution was significantly changed from yellow to dark brown. The chemical structure of irradiated chitosan was obviously changed at 1525-1275 cm^{-1} and the pyranose peak at 1200-900 cm^{-1} disappeared for the case of 90%DD (Figure 2). This might be due to the function of γ-ray via the homogeneous system lead to high degradability.

Chitosan and its irradiated products were dissolved in 0.04M CH_3COOH to observe the change in chemical structure by UV spectroscopy. Chitosan solution shows the main peak at 290 nm belonging to carbonyl group. Figure 7 shows that the amount of carbonyl groups increases with the increasing in γ-ray doses in all conditions; however, the increasing rate and the amount of carbonyl group are depending on the condition. The increasing rate is significant for the conditions 4, 3, 2, and 1, respectively. This might be due to $K_2S_2O_8$ and H_2O_2 initiators enhance the radical formation on chitosan chain, and the decomposition induces carbonyl group formation as shown in Scheme 1. The carbonyl formation was found to relate to %DD; the higher the %DD is, the higher the carbonyl formation will be. This might be due to the fact that amino units decrease the chain packing of chitosan and brings the amorphous region to the structure; as a result, the penetration of γ-ray is occurred effectively. In the case of initiator, the higher the concentration of initiator is, the higher the carbonyl group formation will be. This result is markedly in the case of $K_2S_2O_8$ (Figure 7(b)).

Conclusion

The γ-ray irradiation could be practically applied for a large-scale production of low molecular weight chitosan. Irradiated condition 4 is the most optimum condition since the degradation could be accomplished by using half of γ-ray energy as comparing to other conditions to give the molecular weight reduction for 80%. The chemical structures of irradiated chitosan were rarely changed as observed from FT-IR, and NMR, except in the cases of 2% aq. $K_2S_2O_8$ solution and 1% aq. acetic acid solution.

Acknowledgement

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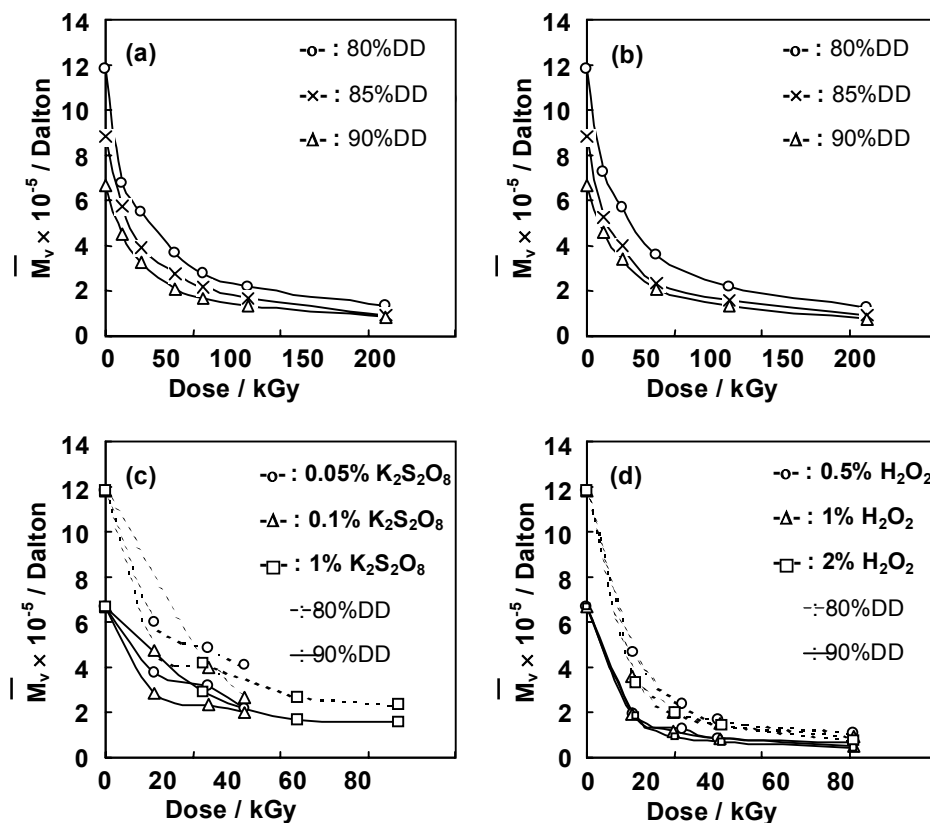
Figure 1. Viscosity average molecular weight of chitosan as a function of dose: (a) condition 1, (b) condition 2, (c) condition 3, and (d) condition 4.
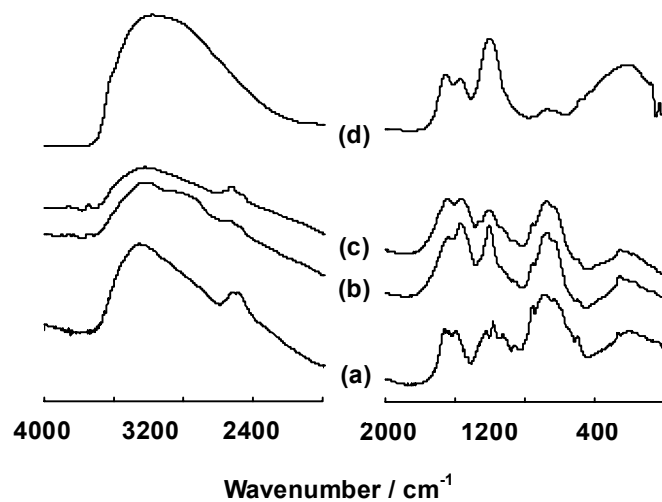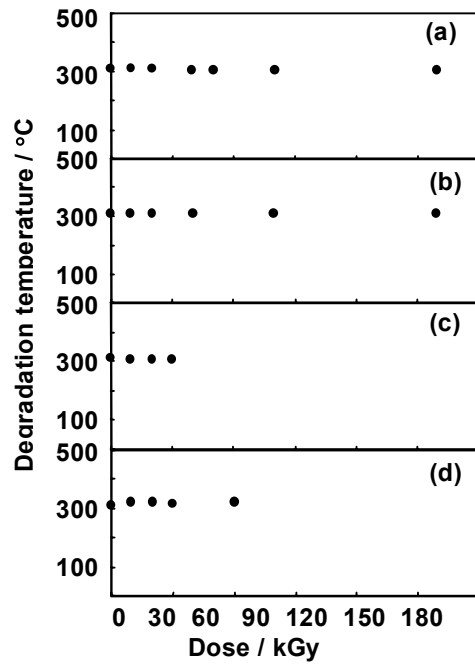
Figure 2. FT-IR spectra of (a) 90%DD chitosan, (b) 80%DD chitosan obtained from condition 5, using γ-ray dose for 20 kGy, and (c) 85%DD chitosan obtained from condition 5, using γ-ray dose for 20 kGy, and (d) 90%DD chitosan obtained from condition 5, using γ-ray dose for 20 kGy.
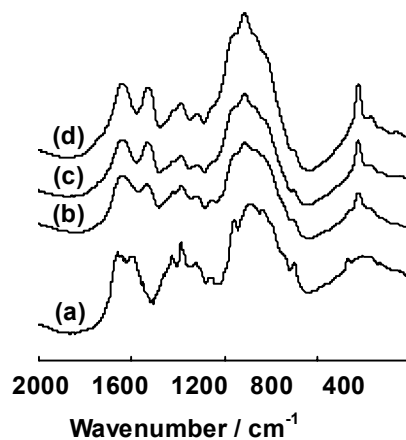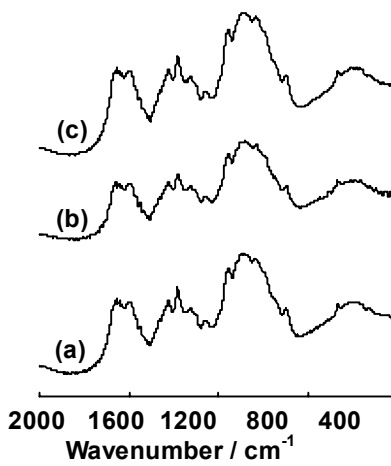

Figure 3. FT-IR spectra of 90%DD chitosan obtained from condition 3 (with 2% aq. $K_2S_2O_8$ solution), using γ-ray dose for (a) 0 kGy, (b) 10 kGy, (c) 20 kGy, and (d) 30 kGy.
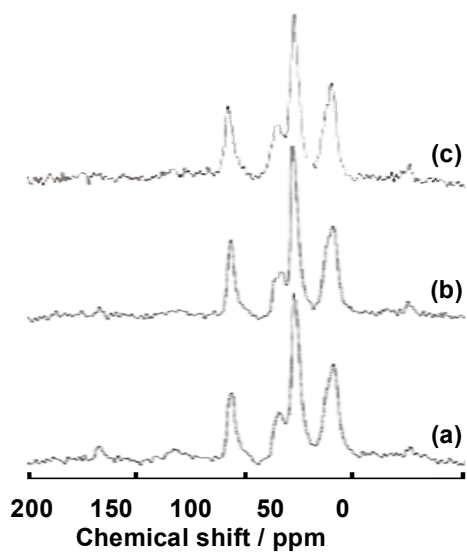
Figure 4. Degradation temperature of 90%DD chitosan as a function of dose: (a) condition 1, (b) condition 2, (c) condition 3 (0.1% aq. $K_2S_2O_8$ solution), and (d) condition 4 (1% aq. H_2O_2 solution).
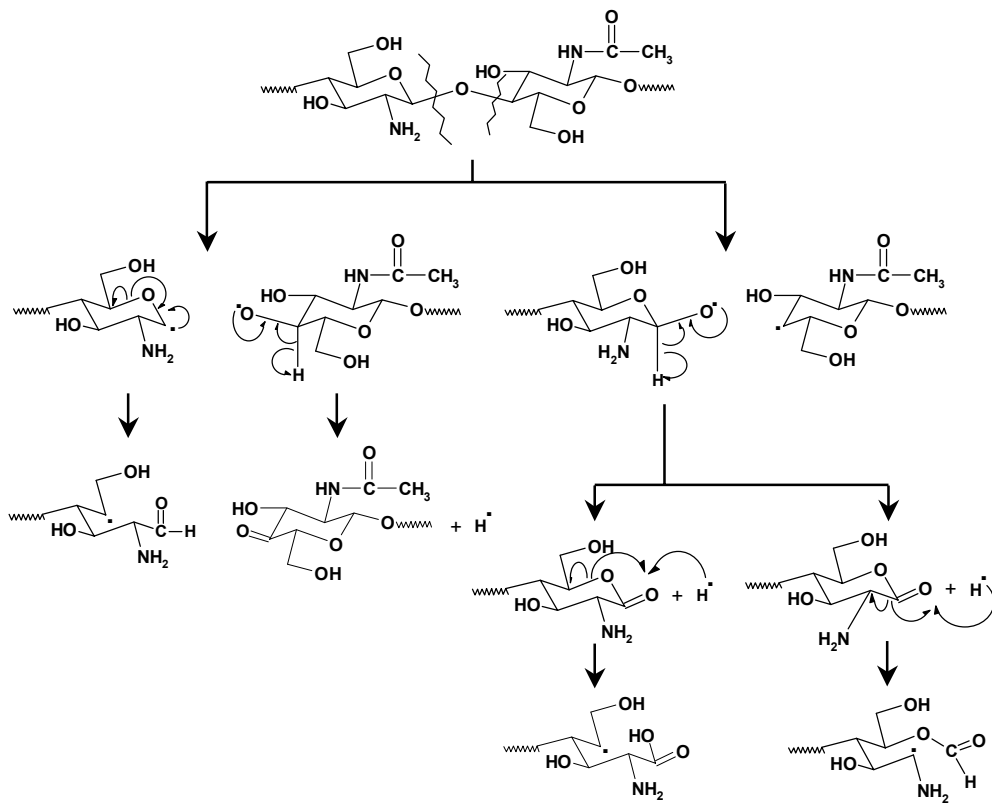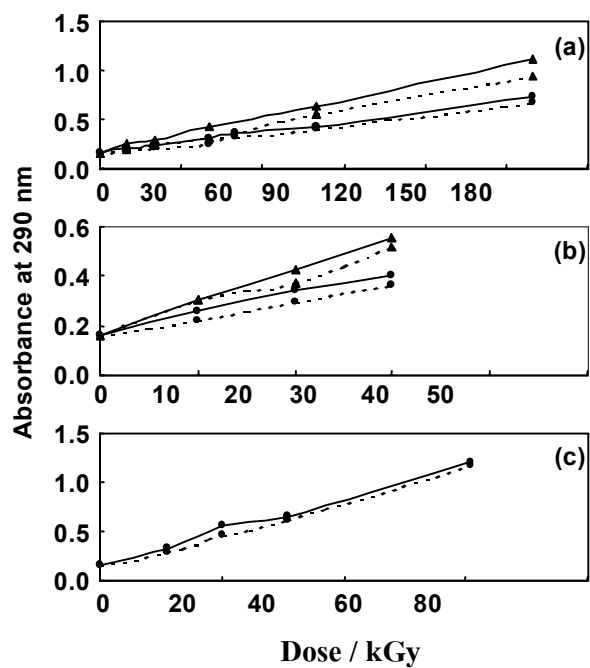

Figure 5. FT-IR spectra of 90%DD chitosan obtained from condition 1 irradiated at (a) 0 kGy, (b) 80 kGy, and (c) 160 kGy.

Figure 6. ^{13}C CP/MAS NMR of 90%DD chitosan obtained from condition 1 irradiated at (a) 0 kGy, (b) 10 kGy, and (c) 40 kGy.

Scheme 1.

Figure 7. Absorbance at 290 nm of 80%DD (dot line) and 90%DD (solid line) chitosan solution (0.2% in 0.04 M CH$_3$COOH) as a function of dose: (a) condition 1, ● and condition 2, ▲, (b) condition 3 (0.05% aq. K$_2$S$_2$O$_8$ solution, ● and 0.1% aq. K$_2$S$_2$O$_8$ solution, ▲), and (c) condition 4 (2% aq. H$_2$O$_2$ solution).