CHAPTER III

PRACTICAL γ-RAY IRRADIATION FOR CHITOSAN: AN APPROACH TO CONTROL MOLECULAR WEIGHT

Abstract

The present article shows a range of γ-ray amount for lowering molecular weight of chitosan, which is a guideline to apply for practical production. Molecular weight of chitosan is reduced approximately for 50% under γ-ray amount of 20 kGy in the dry solid-state. The decrease in molecular weight is significant up to 80% when chitosan is suspended in 0.5-2 % aqueous H_2O_2 during γ-ray irradiation. In either condition, the backbone structure of the irradiated products is maintained with only little change in terminal chain. In the cases of (i) chitosan suspended in 2% aqueous $K_2S_2O_8$ and (ii) chitosan in 1% acetic acid, chitosans lose their primary structures and physical properties.

Introduction

Chitin-chitosan is a biocopolysaccharide existed mainly in crustacean shells and exoskeletons 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 its unique properties, which are biodegradability (*1*), biocompatibility (*2*), bioactivity (*3*), and non-toxicity (*4*). Feasible applications are proposed such as water treatment (*5*), papermaking (*6*), pharmaceutics (*7*), biotechnology (*8*), agriculture (*9*), and food processing (*10*).

As seen in the cases of cellulose and other biopolysaccharides, chitin-chitosan possesses a high molecular weight with strong inter- and intramolecular hydrogen bond network. This brings the poor solubility in most organic solvents and chemically inert for derivatization. Up to now, although, various chitin-chitosan derivatives have been proposed (*11*); the commercial productions are limited due to the problems of quantitative reactions.

Molecular weight reduction can be considered as an alternative way to improve basic property about the solubility. For the past decades, several efforts have been done to prepare low molecular weight chitosan (LMWC) and/or oligochitosan such as chemical treatment (*12*), enzymatic degradation (*13*), and photoirradiation (*14-16*). Acid or base hydrolysis is effective, but the change in degree of deacetylation, the chemical waste, and the reproducibility are the main problems. Enzymatic degradation is an approach 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 one step preparation may lead it to a really practical large-scale production. In the past, a number of studies on γ-ray irradiation of chitosan were reported (*15-16*), however, most were dealt with molecular weight reduction in simple conditions such as chitosan in acetic acid or hydrochloric acid or chitosan powder. Those reports gave information in terms of scientific evidences but

rarely pointed out how we can apply the irradiation process to achieve low molecular weight chitosan practically. The utilization of irradiation should be more widely acceptable only if the structural clarification of the irradiated products has been done extensively. This will also bring the answer about whether the γ-ray irradiated product functions as a chitosan and can be used as a starting material for further derivatization. We, then, base our point of view on the optimum amount of γ-ray for practical low molecular weight product under the condition that the backbone structure is retained.

The present work focuses on γ-ray irradiation conditions, i.e., solid, solution, and suspension states with or without radical initiator including the types and concentrations of initiators. The important point is to clarify the chemical structure, morphology, and physical properties of the products including possible mechanisms that might be involved.

MATERIALS AND METHODS

Materials

Chitosan raw material with percent degree of deacetylation (%DD) of 80 (M_v = 11.9 × 10^5 Dalton), 85 (M_v = 8.8 × 10^5 Dalton), and 90 (M_v = 6.7 × 10^5 Dalton) were provided from Seafresh Chitosan (Lab) Company Limited, Thailand, and used as received. Potassium persulfate ($K_2S_2O_8$) and hydrogen peroxide (H_2O_2) were purchased from Aldrich Chemical Company, Inc., USA. Acetic acid was from Lab-Scan, Ltd., IRELAND.

Instruments and Equipment

Structural characterizations were done as follows. Fourier transform infrared spectroscopy (FT-IR) were recorded on a VECTOR 3.0 BRUKER spectrometer with 64 scans at a resolution of 4 cm^{-1} using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of 1 × 10^9 $cm.Hz^{1/2}w^{-1}$. ^{13}C Cross-polarization magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) spectra were taken with a BRUKER DPX-300 (300

MHz) at 23 ± 1°C. ^{1}H Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using a JEOL GSX 400 (400 MHz) at 70 ± 1°C. Chitosan solution was prepared to be 0.2% w/v in 0.04 M CH_3COOH. Ultraviolet spectra (UV) were recorded by a Perkin-Elmer Lamda-10. Chitosan and its irradiated samples were put into electron spin resonance (ESR) tube and sealed in vacuo before exposure to γ-ray at 40 kGy. ESR spectra were taken at 290 K in air and 77 K in liquid N_2 using a Bruker ESP 300 under the conditions of microwave power 1.97 mW, receiver gain 2×10^4, modulation frequency 100.0000 kHz, modulation amplitude 5.080 G, time constant 40.96 msec, sweep time 41.943 sec, and resolution 1024 points. The g-values were calculated with Mn^{2+}/MgO reference under the same conditions as that of the sample, taking g = 2.034 and 1.918, and H = 86.9 G. Morphology studies were carried out using scanning electron microscopy (SEM) technique by a JEOL JSM-5200 at an operating voltage of 25 kV, and X-ray diffraction (XRD) technique by a RIGAKU RINT 2000 with CuKα as an X-ray source at 40 kV, 30 mA having Ni filter in the range of 2θ 5- 50°. Intrinsic viscosity [η] of samples were measured with a calibrated viscometer, Cannon-Ubbelohde No. 2, A149, in 0.2 M CH_3COOH/0.1 M CH_3COONa aqueous solution at 30 ± 0.05°C. Molecular weight was calculated using the Mark-Houwink equation with $K = 7.21 \times 10^{-4}$, 1.69×10^{-3}, and 3.75×10^{-3} mL/g, and a = 1.004, 0.953, and 0.902 for chitosan with %DD 80, 85, and 90, respectively (*17*). Degree of deacetylation (DD) was analyzed by the acid hydrolysis-high performance liquid chromatography (HPLC) method (*18*). The analysis of acetic acid was carried out using a Waters HPLC with a 300 × 7.8 mm column ORH-801 cation exchanged resin (Interaction Chromatography INC.); 1 mM H_2SO_4; flow rate 0.8 mL/min; pressure 1600 psi; He degas rate 15 mL/min; column oven temperature 45°C; sample compartment 25°C; injection volume 30 μL; detection wavelength 210 nm using a Waters™ 486 Tunable absorbance detector. A Dupont thermogravimetric (TGA) analyzer was applied under N_2 flow rate of 20 mL/min and a heating rate of 20°C/min from 30° to 600°C.

Procedures

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. Samples (1.5 g) were irradiated from 10 to 160 kGy at a dose rate of 5 kGy/h. The conditions used for γ-ray irradiation are 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

After γ-ray irradiation, chitosan flakes were changed from yellow to light brown (conditions 1-3). However, in the presence of H_2O_2 (condition 4), the irradiated product turned out to be more pale yellow color as compared to the starting material. Figure 1 confirms that the irradiation induces surface destruction as evidenced from the roughness of flake surface.

Effect of Irradiation on Molecular Weight

In general, when polymer is exposed to the high-energy electrons, both degradation and crosslinking occur depending on the mechanisms and conditions (dispersing medium, concentration, temperature, etc.). Based on the possibility that irradiation gives both degradation and crosslinking, Ulanski and Rosiak (*16*) reported that chitosan showed chain scission after irradiation in solid state either under vacuum or air. The chain scission was more significant under oxygen whereas the crosslinking was hardly proceeded.

Here, we studied on four conditions to observe the chain degradation efficiency among solid, solution, and suspension states. Figure 2 shows that the molecular weight (M_v) of chitosan continuously decreases when the amount of γ-ray is increased. The results also imply that the degradation is prior to the crosslinking.

The molecular weight of the irradiated chitosan was found to be decreased depending on the degree of deacetylation, i.e., 90 > 85 > 80%DD. This might be related to the fact that chitin unit in chitin-chitosan copolymer is rather difficult to degrade due to the more crystallinity than that of chitosan unit (*19*).

For conditions 1 and 2, the molecular weight was decreased for 75-80 % in the first 50 kGy, and slightly decreased even the γ-ray dose 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. This implies that the chain degradation by γ-ray is limited at a certain level. Taking all results in consideration, we speculated that the chain degradation might easily achieve at amorphous region but hardly proceeded at crystalline part due to the strong inter- and intramolecular H-bonds (see more details in Thermal Stability and Packing Structure).

It was reported that water molecules give $OH^{\bullet}$ and $H^{\bullet}$ for chain depolymerization (*20*). Here, chitosan was suspended in water (condition 2) but it was found that the decrease in molecular weight was almost similar to that of condition 1. This might be due to the fact that chitosan flake was stable and insoluble in water.

An attempt to proceed depolymerization in homogeneous system (chitosan solution in 1% acetic acid) was done. Figures 3 shows that at γ-ray amount of 20 kGy, the chemical and packing structures of chitosan are changed (see more details in Thermal Stability and Packing Structure). The decrease in molecular weight is 56% and 84% at γ-ray amount of 20 kGy in the cases of adding $K_2S_2O_8$ (condition 3) and H_2O_2 (condition 4), respectively (Figure 2(C) and (D)). This implies that both initiators induce radical species effectively. It is important to note that the concentration of initiator is slightly effected to the molecular weight of chitosan (Figure 2(C) and (D)). For example, when concentration of H_2O_2 was increased from 0.5 to 2% (for 90%DD), the decrease in molecular weight was raised 6%. In condition 3 when the concentration of $K_2S_2O_8$ is up to 2%, the chemical structure and morphology are changed obviously (Figure 4). This might be due to the severe condition in degrading chitosan (*21*).

In order to answer the question why the chain degradation terminated at a certain level, the re-irradiation onto the irradiated products was carried out. For example, the irradiated product (obtained after 55 kGy irradiation) was further irradiated for 10-160 kGy. Figure 5 shows that there is no significant effect of γ-ray on re-irradiation process since the decrease in molecular weight is similar to that of the single-time-irradiation product. The re-irradiated product was completely dissolved in acetic acid without any particle or gel left. This implied two important points about the degradation, i.e., (i) the amorphous segment was destroyed at the first irradiation, whereas the remained crystalline part was hardly degraded by the re-irradiation, and (ii) the crosslinking was rarely induced by re-irradiation.

Effect of γ-Ray Amount on Degree of Deacetylation (%DD)

The changing of degree of deacetylation before and after irradiation was studied to observe the effect of γ-ray to the chemical structure. The degree of deacetylation can be determined by HPLC technique of which the total amount of acetic acid liberated from hydrolyzed acetamide group of chitin-chitosan in oxalic and sulfuric acids is quantitatively analyzed. Figure 6 shows that 90%DD chitosan maintains its degree of deacetylation even the amount of dose is varied in both conditions 1 and 2. We speculated that $^{\bullet}COCH_3$ was rarely induced by γ-ray irradiation.

Thermal Stability and Packing Structure

All of chitosan starting materials showed thermal stability up to 309°C (T_d) due to the strong hydrogen bond network and high crystallinity. The conditions 3 and 4 gave the significant decrease in molecular weight; thus, it was expected to see T_d of the irradiated products be less than 309°C. Figure 7 illustrates a series of T_d belonging to irradiated chitosan at various doses are constant at 309±2°C. This implies that chain scission mainly occurs at C-O-C glycosidic linkages whereas hydrogen bonds constructed from $NHCOCH_3$, NH_2, and OH groups might be still remained.

The XRD results support this speculation. Generally, chitosan shows three major peaks at 9, 19, and 22°. Here, when the amount of γ-ray was varied, the XRD patterns were maintained for all conditions. Crystal size at 9° 2θ was calculated to evaluate the packing structure (Figure 8) and found to be 17±1Å.

It should be noted that XRD patterns of chitosan obtained from conditions 3 (with 2% $K_2S_2O_8$) and 5 are broaden implying more amorphous-like structure (Figures 3B and 4B). The condition 3 (with 2%$K_2S_2O_8$) reflected that at a certain amount of radical initiator, the packing structure was destroyed. Figure 4C supports this explanation since the T_d of chitosan from condition 3 (with 2%$K_2S_2O_8$) was decreased from 309°C to 180-220°C when the γ-ray dose was only 10-30 kGy.

Effect of Irradiation on Chemical Structure

Figure 9 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 remained even the radiation amount was increased up to 160 kGy.

Figure 10(a) shows ^{13}C CP/MAS NMR spectra of chitosan starting material; 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. The irradiated chitosan (condition 1, 10 kGy) gives the doublet peak of C-4 at 83.7 (Figure 10(b)). Combining with the decreasing in molecular weight results, it should be concluded that free radicals induce the chain scission at either C-1-O or C-4-O to break the C-O-C linkage in the main chain as proposed in Scheme 1.

It is important to point out that the changes in structure were found in the condition 3 (with 2% aq. $K_2S_2O_8$ solution). Figure 4A shows the significant changes after γ-ray irradiation even the dose was as low as 10 kGy (1655 and 1550 cm^{-1} for amide I and amide II, and 1200-900 cm^{-1} for saccharide). Moreover, the irradiated products were not completely dissolved in acetic acid. This insisted that the chemical structure changed and some crosslinking occurred.

For condition 5, it was found that the color of solution was significantly changed from yellow to dark brown. The important point is that the irradiated product could not be reprecipitated; therefore, the solvent was evaporated to get the solid particles. The chemical structure of solid particles was obviously changed at 1525-1275 cm^{-1}, whereas the pyranose peaks at 1200-900 cm^{-1} were disappeared in the case of 90%DD (Figure 3A). The results suggested that the homogeneous system allowed the high degradation leading to the backbone destruction.

In previous, Ulanski *et al.* (*16*) reported about the increasing of carbonyl and carboxyl groups of irradiated chitosan. Here, chitosan and its irradiated products were dissolved in 0.04M CH_3COOH. Our preliminary results confirmed that chitosan after irradiation gave the peak at 290 nm belonging to carbonyl group. Figure 11 illustrates the amount of carbonyl groups increases with an increasing in γ-ray doses. The amount of carbonyl group depended on the conditions in the order of 4>3>2>1. This might be due to $K_2S_2O_8$ and H_2O_2 initiators enhance the radical formation followed by the radical decomposition to result the carbonyl group as speculated in Scheme 1. Here, the carbonyl formation is related to %DD: the higher the %DD, the higher the carbonyl formation. This might be due to the fact the relatively low packing structure of chitosan unit as compared to that of chitin unit (*20*) allows the effective chain scission. The concentration of initiator is another point to be considered. Figure 11(B) shows that chitosan irradiated in 0.1% aq. $K_2S_2O_8$ solution gives the higher absorbance at 290 nm than that in 0.05% aq. $K_2S_2O_8$ solution.

Effect of Irradiation on Radical Formation and Proposed Mechanism

Here, ESR technique was applied to observe free radical species after exposure to γ-ray. Figure 12 illustrates that, at 290 K, unirradiated 90%DD chitosan does not give any signal (Figure 12(a)) due to the lack of radicals whereas the irradiated one (for 40 kGy) gives a peak at 3491 referring to unpaired electrons (Figure 12(b)). In order to stabilize radicals in the system, the measurement at 77 K was attempted. Figure 12(c) shows two homogeneously broad peaks at 3423 and 3353. The result implies that all spins have the same environment and are in polycrystal structure.

In order to declare g-value Mn^{2+}/MgO was applied as a reference. Figure 12(d) illustrates the irradiated 90%DD chitosan (for 40 kGy) with a g-value for 2.0050. Here, a series of the g-values belonged to chitosan irradiated under various conditions were clarified to be in the range of 2.0050 to 2.0109 (Table 1).

Here, the integrated intensity of the ESR signals was applied to evaluate the total energy absorbed by the sample at resonance conditions. Table 1 shows the integral area under the resonance curve to determine the concentration of free radicals in the sample. It should be noted that in dry solid state (condition 1) the amount of radicals formed was larger than those of solution states (conditions 2-4). This might be due to the difficulties in radical combination in the case of dry solid state. This was supported by the low amount of carbonyl group generated in condition 1 as observed from UV. In other words, conditions 2-4 might induce radicals effectively, however, most radicals were terminated by the combination to form carbonyl group significantly (Figure 11). By comparing the concentration of free radicals in different temperatures, it was found that the amount of free radicals at 290 K is higher than that at 77 K for condition 1 (Table 1). This might be due to the more radical mobility at high temperature inducing more radical generation over termination.

In conclusion, the results declared that the γ-ray irradiation on chitosan induced chain scission at 1,4-glycoside linkages. Although, the radical combination generated carbonyl groups at chain ends, it is important to note that even the γ-ray amount was increased up to 160 kGy the overall structure still maintained. In solution state, for example chitosan in acetic acid, the chain degradation occurred continuously even the γ-ray exposure was as low as 20 kGy to result the change in primary structure. Initiator might induce the chain degradation but it requires a certain level of concentration as seen in the case of chitosan suspended in 2% $K_2S_2O_8$. The practical condition for a large-scale production is concluded to be the irradiation of chitosan suspended in H_2O_2 at dose amount 20 kGy to reduce molecular weight for 80% without changing the backbone structure.

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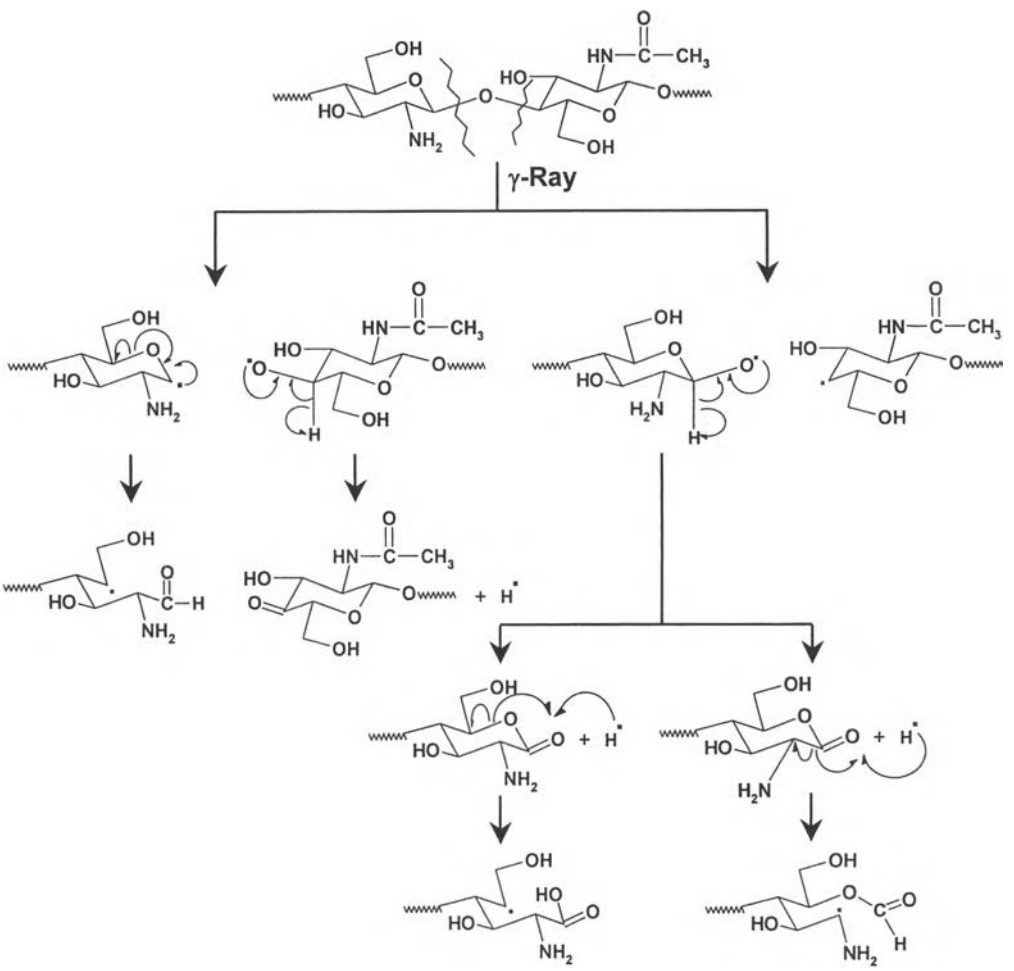

Scheme 1 (Yoksan et al.)

Figure Captions

Figure 1. SEM photographs at 25 kV of 90 %DD chitosan: (A) before exposure (×15,000), and (B) after exposure (×10,000) to γ-ray at the amount of 50 kGy in condition 1.

Figure 2. 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 3. Structural information of (a) 90%DD (without irradiation), (b) 80%DD (condition 5, 20 kGy), (c) 85%DD (condition 5, 20 kGy), and (d) 90%DD (condition 5, 20 kGy) observed by (A) FT-IR, and (B) XRD.

Figure 4. Structural information of 90%DD chitosan (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 observed by (A) FT-IR, (B) XRD, and (C) TGA.

Figure 5. Viscosity average molecular weight of 80%DD (-o-) and 90%DD (-Δ-) chitosan (condition 1): (A) single-time-irradiation, and (B) re-irradiation of chitosan after exposure to γ-ray amount 55 kGy.

Figure 6. Deacetylation degree of 90%DD chitosan after exposure in condition 1 (Δ) and condition 2 (o).

Figure 7. Degradation temperature of 90%DD chitosan: (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 8. Crystal size of 90%DD chitosan evaluated at 2θ 9°: (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 9. FT-IR spectra of 90%DD chitosan (condition 1), using γ-ray dose for (a) 0 kGy, (b) 80 kGy, and (c) 160 kGy.

Figure 10. ^{13}C CP/MAS NMR of 90%DD chitosan (condition 1), using γ-ray dose for (a) 0 kGy, and (b) 10 kGy.

Figure 11. Absorbance at 290 nm of 80%DD (dot line) and 90%DD (solid line) chitosan solution (0.2%w/v in 0.04 M CH_3COOH): (A) condition 1, and condition 2, (B) condition 3, and (C) condition 4.

Figure 12. ESR spectra of 90%DD chitosan (condition 1), using γ-ray dose for (a) 0 kGy measuring at 290 K, (b) 40 kGy at 290 K, (c) 40 kGy and Mn^{2+}/MgO at 290 K, and (d) 40 kGy at 77 K. The peak at 3459 G belongs to ESR tube.

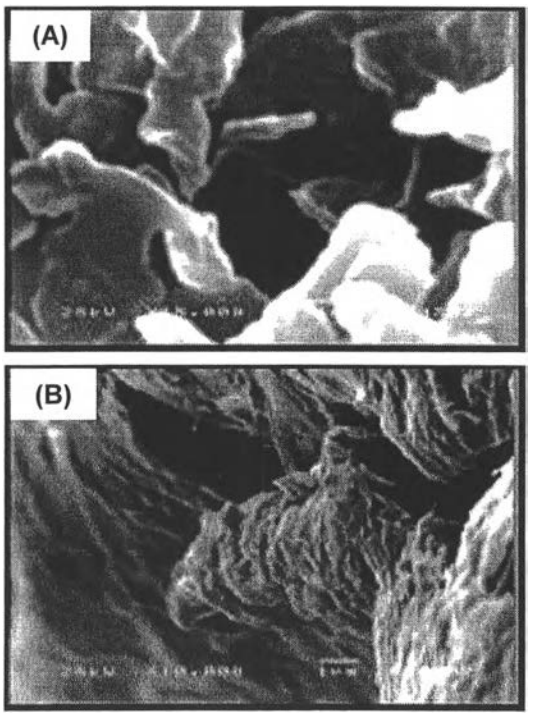

Figure 1. (Yoksan et al.)

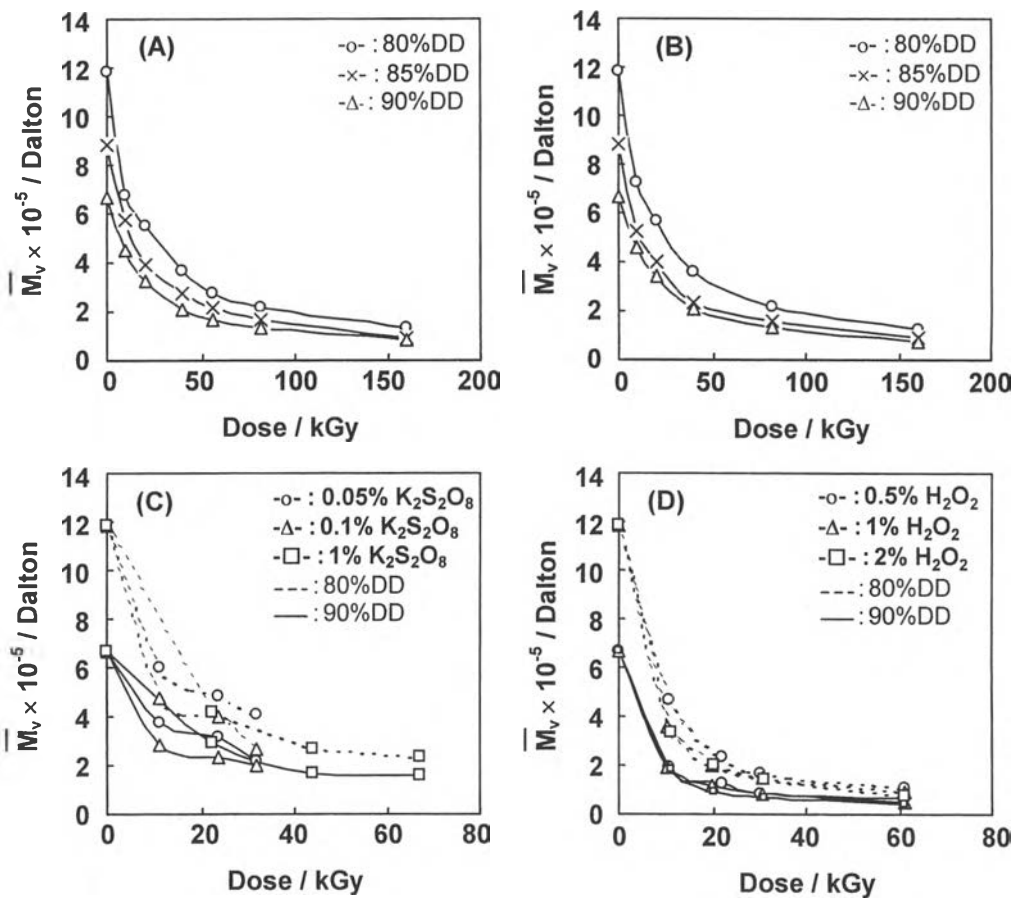

Figure 2. (Yoksan et al.)

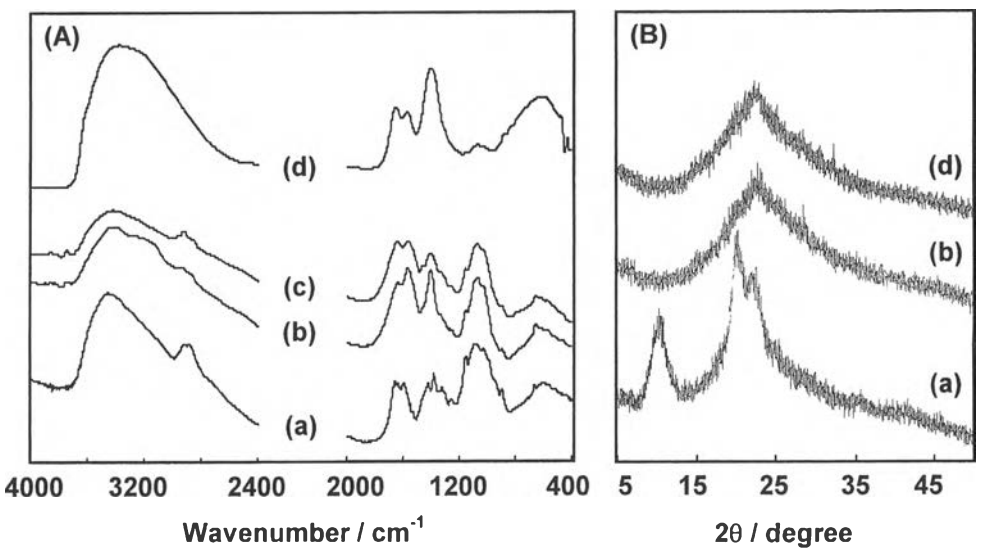

Figure 3. (Yoksan et al.)

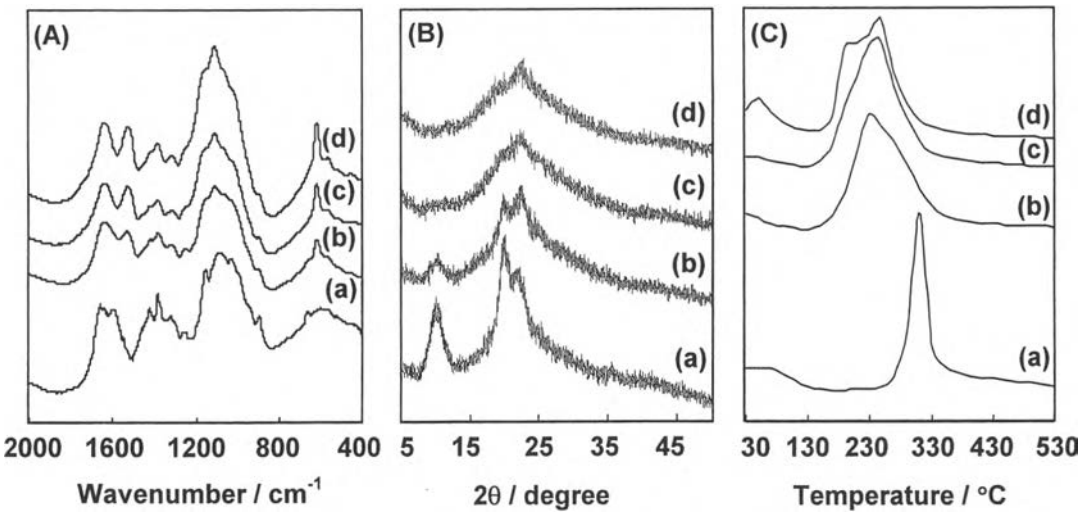

Figure 4. (Yoksan et al.)

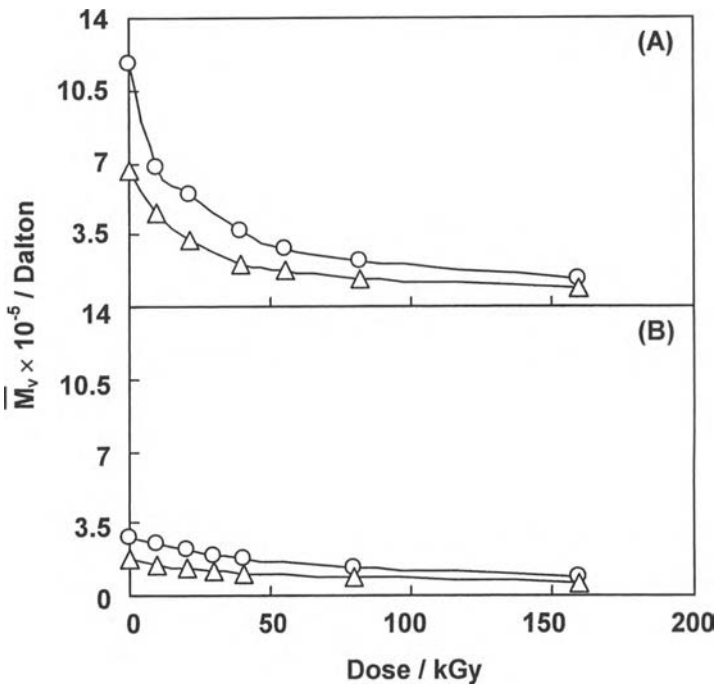

Figure 5. (Yoksan et al.)

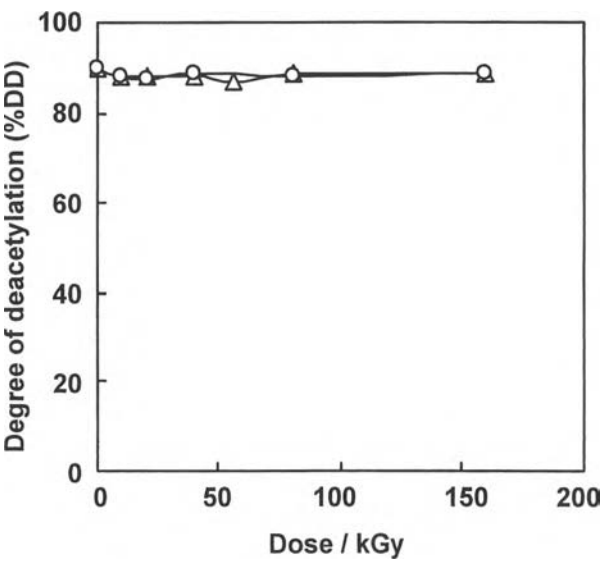

Figure 6. (Yoksan et al.)

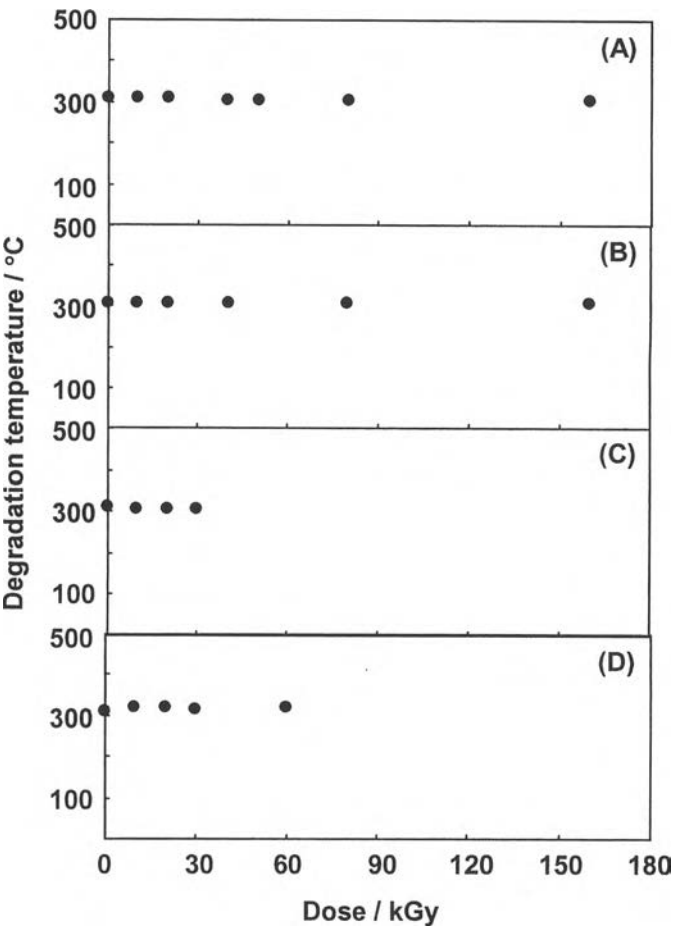

Figure 7. (Yoksan et al.)

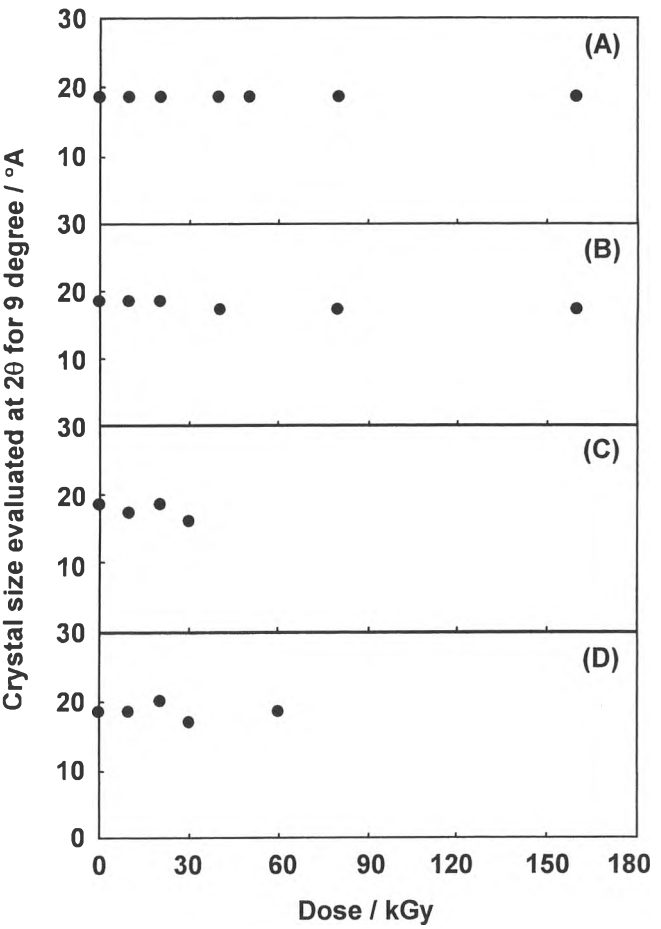

Figure 8. (Yoksan et al.)

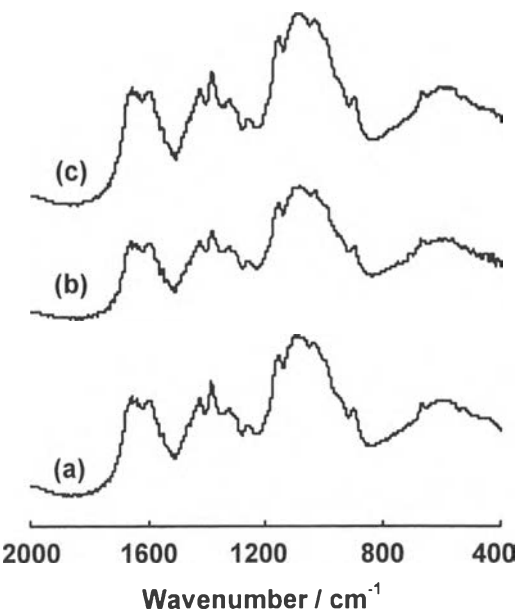

Figure 9. (Yoksan et al.)

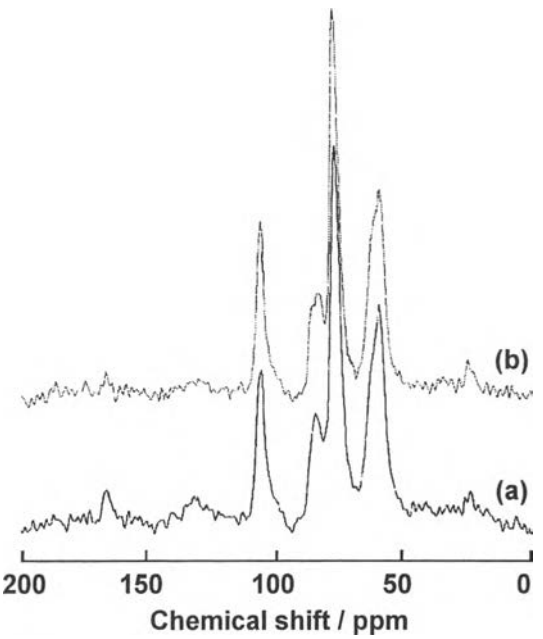

Figure 10. (Yoksan et al.)

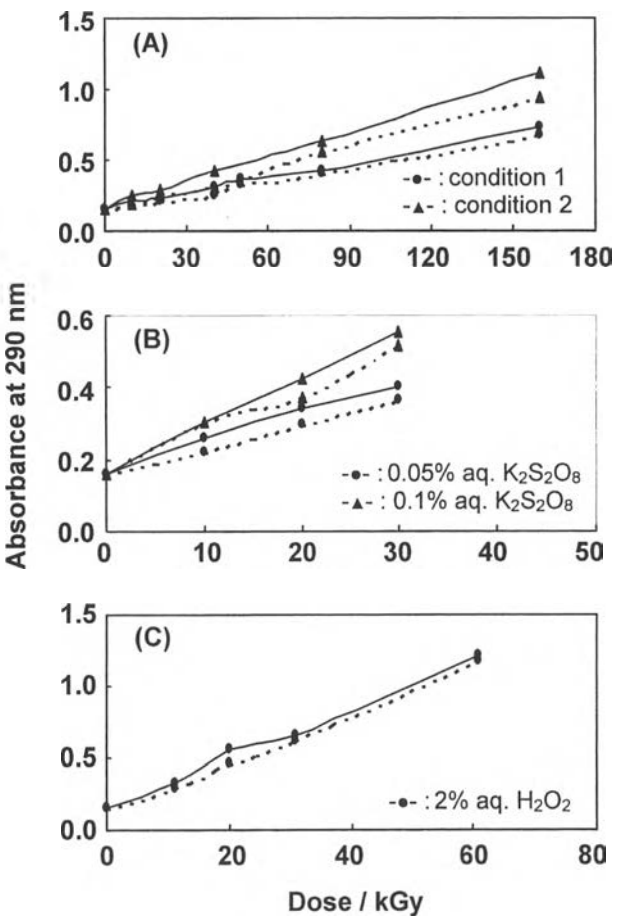

Figure 11. (Yoksan et al.)

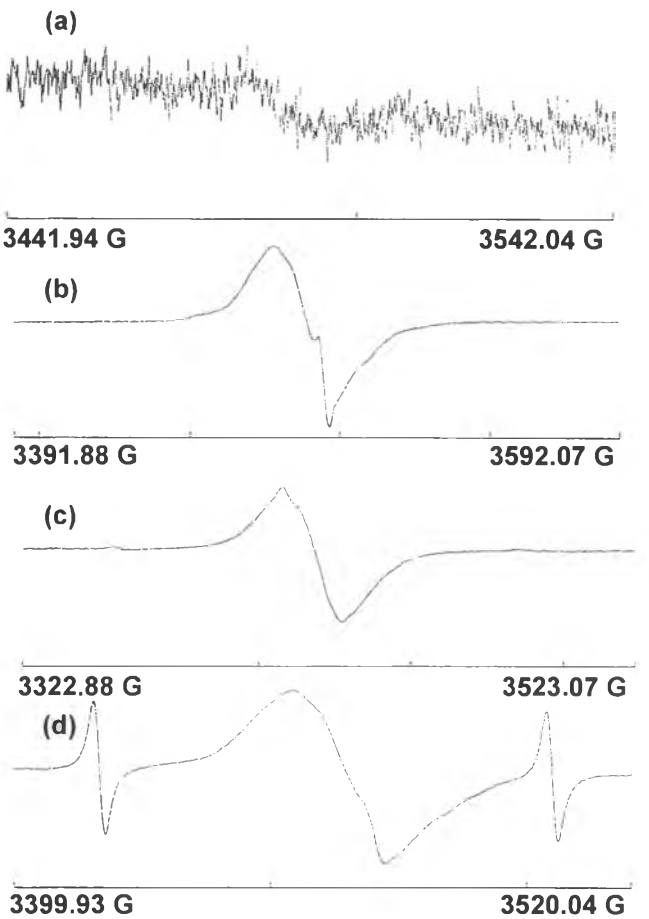

Figure 12. (Yoksan et al.)

Table Caption

Table 1 ESR results of 90%DD chitosan irradiated at 40 kGy under various conditions

Condition	Temperature (K)	Peak integration[a]	g-value
1	290	7.839×10^7	2.0050
	77	5.097×10^7	2.0059
2	77	0.980×10^7	2.0109
3 (1% $K_2S_2O_8$)	77	0.951×10^7	2.0104
4 (1% H_2O_2)	77	1.443×10^7	2.0106

[a]calculated from the difference between total integral and integral of SiO_2

Table 1 (Yoksan et al.)