CHAPTER V
EFFECT OF GAMMA RADIATION ON DILUTE AQUEOUS SOLUTIONS AND THIN FILMS OF *N*-SUCCINYL CHITOSAN

5.1 Abstract

N-Succinyl chitosan (*N*-SC) products with various degrees of substitution were synthesized by a direct reaction between chitosan and succinic anhydride. The susceptibility of the as-synthesized polymers to degradation upon their exposure to γ-ray radiation was investigated. The results were compared with the as-received chitosan. The size exclusion chromatographic results showed that chitosan and *N*-SC products in their dilute aqueous solution state were more subservient to degradation by γ-ray radiation than in their solid film state, despite the much less exposure to the radiation (i.e., 5-30 kGy for the solutions versus 20-100 kGy for the films). Increasing the radiation dose resulted in the rather monotonous decrease in the molecular weights of the polymers. Structural analyses of the irradiated polymers by Fourier-transformed infrared spectroscopy (FT-IR) and UV-visible spectrophotometry indicated the increase in the amount of carbonyl groups with the radiation dose. The formation of the carbonyl groups suggested that the radiolysis of chitosan and *N*-SC products occurred at the glycosidic linkages. In addition, FT-IR, elemental analysis and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) results suggested that γ-ray radiation affected both the *N*-acetyl and *N*-substituted groups on the polymer chains.
(**Key-words**: *N*-Succinyl chitosan; Gamma radiation; Radiolysis)

5.2 Introduction

Chitosan or poly (*N*-acetyl-D-glucosamine-*co*-D-glucosamine) is obtained from deacetylation of chitin or poly(*N*-acetyl-D-glucosamine), a major component of shells of crustaceans, such as crabs, shrimps and crawfish. Due to the presence of the reactive amino, the primary and the secondary hydroxyl groups on the chitosan chains, a variety of water-soluble derivatives of chitosan, which can be obtained

from reductive alkylation, carboxyl alkylation, graft copolymerization and quaternization reactions, can be synthesized. These reactions not only improve the solubility of the resulting polymers, but also their applicability in medical fields due to additional and/or enhanced properties, e.g., antimicrobial, wound healing, mucoadhesivity, antioxidant and mutagenic properties (Chen, *et al.*, 2002; Kogan, *et al.*, 2004; Kotze, *et al.*, 1999; Xie, Xu and Qing, 2001; Yang,Chou and Li, 2004). An example for such derivatives is *N*-succinyl chitosan (*N*-SC), which can be obtained from a simple reaction between chitosan and succinic anhydride. *N*-SC has been found to exhibit several biological properties, such as nontoxicity, biocompatibility and long systemic circulation in mice (Kazuka, Hiraku and Yoshiharu, 1999) As such, *N*-SC has been found its use as a drug carrier (Kato, Onishi and Machida, 2004).

The reduction in the molecular weights of polymers can be induced by several means, some of which are the uses of chemical agents (Kato, Onishi and Machida, 2002), enzymes (Novikov and Mukhin, 2003), sonication (Li, Cai and Fan, 2008) and photo and/or gamma radiation (Wasikiewicz, *et al.*, 2005). Gamma (γ-ray) radiation is capable of ionizing molecules into ionic species. Materials subjected to γ-ray radiation could undergo one or more of the following reactions, e.g., crosslinking, grafting, sterilization and/or degradation, even without the presence of any chemical agent. Interestingly, degradation of natural polymers into oligomers or the low molecular weight materials as induced by γ-ray radiation could enhance certain properties, e.g., antioxidant activity (Feng, *et al.*, 2008), antimicrobial activity, phytoalexin eilicitor activity and plant promotion (Kume, Nagasawa and Yoshii, 2002) of the resulting materials. Yoshii et al. (Yoshii, *et al.*, 2003) showed that carboxymethyl (CM) starch, CM-cellulose, CM-chitin and CM-chitosan could be facilely made into hydrogels by γ-ray radiation of their pastes. More specifically, the exposure of CM-chitin or CM-chitosan aqueous solutions with concentrations >10% (paste-like) to γ-ray radiation resulted in the crosslinking of the polymers to form hydrogels (Zhao, *et al.*, 2003) while degradation would instead occur when the polymers were in their dilute solution or solid forms (Huang, *et al.*, 2007). Compared with other chitosan derivatives, studies on the effect of γ-ray radiation on *N*-SC were limited.

Here, we reported, for the first time, the effect of the exposure of γ-ray radiation to various *N*-SC products, either in the dilute aqueous solution or the solid film form, on their chemical integrity and molecular weights using Fourier-transformed infrared spectroscopy, nuclear magnetic resonance spectroscopy, elemental analysis, size-exclusion chromatography and ultraviolet (UV) spectrophotometry.

5.3 Experimental Section

5.3.1 Materials

Chitosan flakes [M_v = 100,000 g·mol^{-1} and degree of deacetylation (%DD) = 95.6% (^{1}H-NMR)] were purchased from Seafresh Chitosan (Lab) Co., Ltd. (Thailand). Prior to further use, the as-received chitosan flakes were treated with 50% w/w NaOH aqueous solution (KPT Cooperation, Thailand), containing 0.5% w/w of $NaBH_4$ to prevent extensive depolymerization of chitosan, at 110 °C for 1 h in an autoclave. After the treatment, the %DD of chitosan increased to 98.8% (^{1}H-NMR). Succinic anhydride (analytical reagent grade) was purchased from Wako Pure Chemical Industries Co., Ltd. (Japan). All other chemicals were of analytical reagent grade and used without further purification.

5.3.2 Synthesis of *N*-Succinyl Chitosan (*N*-SC)

N-SC was synthesized based on the method of Hirano and Moriyasu (Hirano and Moriyasu, 1981) with a slight modification. First, 1.6 g of the treated chitosan flakes was dissolved in 50 mL of 2% v/v acetic acid. The obtained chitosan solution was then diluted with 300 mL of methanol. Three samples of *N*-SC were synthesized by varying the ratios of succinic anhydride to each glucosamine unit (or per NH_2 group) of 1:1, 3:1 and 5:1, respectively. The obtained samples were hereafter denoted 1 *N*-SC, 3 *N*-SC and 5 *N*-SC, respectively. Succinic anhydride in different amounts, *a priori* dissolved in 95% v/v methanol at 50 °C, was gradually added to the chitosan solution and stirred for 1 h at room temperature. The mixtures were allowed to stand at room temperature overnight, during which time gels were formed. The gels were collected, washed repeatedly with ethanol and dried *in vacuo*

overnight. The obtained products were in H form and did not readily dissolve in water.

To improve the solubility of the products in water, it is necessary to change the obtained products into Na salt form. The original *N*-SC products (in H form) were treated with an excess amount of 0.1 M NaOH aqueous solution at room temperature (3 g/500 mL). *N*-SC product with a low degree of substitution (DS) was obtained by precipitating the 1 *N*-SC sample after it had been treated with the NaOH solution. The product was repeatedly washed with distilled water to remove the excess salt, washed again with ethanol and dried *in vacuo* overnight to obtain yellow solid product. On the other hand, the *N*-SC products with high DS values were obtained from the supernatants of the 3 *N*-SC and the 5 *N*-SC samples after they had been treated with the NaOH solution. The products were first precipitated with ethanol, filtered out, repeatedly washed with ethanol and finally dried *in vacuo*. The *N*-SC products in their Na form (i.e., Na *N*-SC) with high DS values were dissolved and dialyzed (MWCO = 4,000 $g \cdot mol^{-1}$) in distilled water for 3-5 d to remove the excess salt. Finally, the solutions were centrifuged at 10,000 rpm for 10 min and precipitated with ethanol to obtain yellow solid products. The obtained Na *N*-SC products were hereafter denoted as Na-1 *N*-SC, Na-3 *N*-SC and Na-5 *N*-SC, respectively.

5.3.3 Preparation of *N*-SC Solutions and Films

The dilute aqueous solutions of chitosan and the *N*-SC products (1 wt.%) were first prepared. To obtain the films, 70 mL of each of the as-prepared solutions was poured into a polystyrene mold and dried at 40 °C in an oven for 48 h. Two solvent systems were used. For chitosan and Na-1 *N*-SC, 0.5% v/v CH_3COOH was used, while, for Na-3 *N*-SC and Na-5 *N*-SC, distilled water was used. The chitosan and the Na-1 *N*-SC films were later treated with 0.1 M NaOH in 90% v/v methanol to remove the excess acid, while the Na-3 *N*-SC and the Na-5 *N*-SC films were treated with 6 M HCl in 90% v/v methanol (1:50 v/v) in order to change the films from their Na form into H form. The as-prepared films were about 40 μm thick on average.

5.3.4 Radiation of Chitosan and *N*-SC Dilute Solutions and Films

The dilute solutions of chitosan and the *N*-SC products (25 mL) were irradiated with γ-ray from a ^{60}Co source at various dosages of 5, 10 and 30 KGy, whereas the film counterparts, *a priori* packed in polyethylene bags, were irradiated at 20, 50 and 100 KGy (at 10 $KGy \cdot h^{-1}$).

5.3.5 Characterization

5.3.5.1 Structural Characterization

Fourier-transformed infrared spectroscopy (FT-IR) was used to follow any change in the chemical structure of the neat and the irradiated samples of chitosan and all of the *N*-SC products. First, the neat chitosan and the *N*-SC films were characterized for their chemical functionalities. To compare the original and the irradiated samples, chitosan and 1 *N*-SC solutions were first precipitated by 0.1 M NaOH in 90% v/v methanol, while 3 *N*-SC and 5 *N*-SC solutions were precipitated by 6 M HCl in 90% v/v methanol (1:50 v/v). After such treatments, samples were washed repeatedly by a mixture of water and ethanol until of neutral pH. Precipitated samples were dried *in vacuo* and grounded along with KBr powder (1:40 w/w) to finally obtain thin pellet samples. FT-IR spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR at a resolution of 4 cm^{-1} and 4 scans over the wavenumber range of 400-4,000 cm^{-1}.

The chemical structure of chitosan and all of the *N*-SC products (in H form) was analyzed by a JEOL ECA 400 nuclear magnetic resonance (NMR) equipped with the Delta™ analytical software in order to determine the average values of succinyl groups substituted at the *N*-positions along the chitosan chains (i.e., the degree of substitution, DS). CD_3COOD in D_2O (2 wt.%) was used as the solvent to dissolve all samples and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the internal standard (0.0 ppm). The DS values of the *N*-SC products were calculated according to the following equation:

$$DS\ (\%) = \frac{\text{Intergrated area at } 2.5 - 2.7\,\text{ppm}}{4 \times \text{Intergrated area at } 3.15\,\text{ppm}} \times 100, \qquad (1)$$

The *N*-SC products had been dried *in vacuo* and kept in tightly-sealed containers prior to being measured by elemental analysis using a Perkin Elmer 2400 CHN in order to determine the weight percentages of C, H and N

elements in the samples. Such an analysis for chitosan was also carried out as reference.

5.3.5.2 Solubility of N-SC

Solubility of chitosan and the *N*-SC products was evaluated by turbidity measurements based on the method utilized by Sashiwa and Shigemasa (Sashiwa and Shigemasa, 1999) Chitosan and 1 *N*-SC were dissolved in 1 wt.% acetic acid aqueous solution, while 3 *N*-SC and 5 *N*-SC were dissolved in distilled water. The concentration of all the sample solutions was fixed at 0.5 wt.%. The pH values of these solutions were adjusted by 0.1 M HCl or 0.1 M NaOH aqueous solution, followed by the turbidity measurements by a Hitachi U2010 UV-visible spectrophotometer (UV-vis) at 600 nm.

5.3.5.3 Molecular Weight Evaluation

A Hitachi size exclusion chromatograph (SEC) was used to determine the molecular weight information (both M_w and M_n) of the neat and the irradiated samples. First, the as-prepared solutions of chitosan and the *N*-SC products (1 wt.%) were diluted with 0.4 M acetic acid/0.2 M sodium acetate to obtain the solutions at a fixed concentration of 0.5 wt.% (final pH of the solutions ≈ 3.5), while 0.5 wt.% solutions of the corresponding films were prepared by dissolving the films in 0.2 M acetic acid/0.1 M sodium acetate. Each of the sample solutions (48 μL) was then injected into the SEC equipment which was equipped with a Shodex OH PakSB-804HQ column at 40 °C using a reflective index (RI) detector. The flow medium was 0.2 M acetic acid/0.1 M sodium acetate and the flow rate was 0.8 $mL \cdot min^{-1}$. Standard pullulans P82 were used in the calibration. Molecular weights were calculated based on the following universal calibration equation:

$$\text{Log}(M) = -0.4735 V_e + 9.299, \quad (2)$$

where M is weight average molecular weight and V_e is the elution volume or time.

5.3.5.4 UV-vis Measurment

UV-vis was also used to confirm the chemical structure of the neat and the irradiated samples. The aqueous solutions of the *N*-SC products (1 wt.%) were first diluted to 0.1 wt.% using the respective solvent for each *N*-SC

product. The diluted solutions were then measured for UV-vis absorbance over the wavelength range of 200-500 nm.

5.4 Results and Discussion

5.4.1 Chemical Structure of Chitosan and *N*-SC Products

FT-IR spectra of chitosan and all of the *N*-SC films are shown in Figure 5.1 Chitosan showed characteristic peaks at 3445, 1640, 1560 and 1375 cm^{-1}, which can be assigned to O-H and N-H stretching, amide I, amide II and amide III, respectively (Zhang, *et al.*, 2003) In comparison with that of chitosan, the spectra of all of the *N*-SC products showed the characteristic peaks at 1640 and 1560 cm^{-1}, which correspond to C=O stretching and N-H bending of the succinyl groups. These peaks are more pronounced than those observed for chitosan, the result that confirmed the successful substitution of succinyl groups along the chitosan chains.

5.4.2 Degree of substitution of *N*-SC products

Figure 5.2 shows ^{1}H-NMR spectra of chitosan and all of the *N*-SC products. Apparently, the native chitosan showed signals at the chemical shifts of 2.06-2.08 ppm (H of acetyl groups), 3.15 (H2 of glucosamine, GlcN) 3.3-4.3 ppm, (H2 of *N*-acetyl glucosamine, GlcNAc; H3, H4, H5 and H6 of GlcNAc and GlcN) (Zhang, *et al.*, 2003) and 4.90 ppm (H1 of GlcN) (Megia, et al.,2005) On the other hand, all of the *N*-SC products showed additional signals at the chemical shifts of 2.5-2.7 ppm (-NHCOC**H**$_2$C**H**$_2$COOH) and 4.55 ppm (-N**H**COCH$_2$CH$_2$COOH) (Aiping, *et al.*, 2006).The DS values of the *N*-SC products can be determined from the NMR results according to Equation (1) and the results are summarized in Table 5.1. Apparently, the greatest DS value (i.e., 104%) was observed for the 3 *N*-SC sample (i.e., the succinic anhydride to NH_2 ratio of 3:1 mol/mol), followed by those of 5 *N*-SC and 1 *N*-SC samples, respectively (i.e., 73 and 10%, respectively).

To our first approximation, the substitution of the succinyl groups on the chitosan chains (hence, the DS value) should increase with an increase in the succinic anhydride to NH_2 ratio. In the present study, the initial increase in the succinic anhydride to NH_2 ratio from 1:1 to 3:1 resulted in the increase in the DS value from 10 to 104% (i.e., a perfect substitution). At the succinic anhydride to NH_2

ratio of 5:1 however, the value decreased to 73%. It is postulated that, at the ratio of 5:1, the inhomogeneous nature of the mixture could be the reason for the observed decrease in the DS value. Specifically, the gels that were formed in the reaction mixtures of 1:1 and 3:1 were more homogeneous than the one that was formed in the 5:1 mixture. As for the 5:1 mixture, micro-phase separation (i.e., hence the formation of microgels) occurred in the mixture during the time it has been left standing at room temperature overnight. The DS values of the *N*-SC products were investigated further by the elemental analysis. Since the substitution of succinic anhydride occurs more readily at the NH_2 groups along the chitosan chains, upon successful substitution, the amount of C atoms relative to that of N atoms (i.e., C/N) should increase. For the neat chitosan, the C/N value was 5.20. Clearly, with an increase in the succinic anhydride to NH_2 ratio from 1:1 to 3:1, the C/N value increased from 5.34 to 7.26. The C/N value finally decreased to 7.24 when the succinic anhydride to NH_2 ratio was 5:1, which agreed well with the results from the NMR analysis.

5.4.3 Solubility of Chitosan and *N*-SC Products

Solubility of chitosan and the *N*-SC products was assessed by turbidity measurements of the chitosan and the *N*-SC solutions at various pH values. As shown in Table 5.2, 1 *N*-SC can dissolve only in the acidic region (i.e., pH $\leq$ ~6.2), in a similar manner to the neat chitosan. On the other hand, the high DS *N*-SC products (i.e., 3 *N*-SC and 5 *N*-SC) can dissolve in high acidic and high basic conditions (i.e., pH $\leq$ ~3.9 and pH $\geq$ ~7.5, respectively). This is due to the presence of both primary and/or secondary amino and carboxylic groups on the highly-substituted *N*-SC products.

5.4.4 Molecular Weights of Neat and Irradiated Chitosan and *N*-SC Products

After succinylation, the single chain molecular weight ($M_{w,s}$) of chitosan increased from 70,000 to 114,000-117,000 $g \cdot mol^{-1}$ (see Table 5.1). According to the SEC results shown in Figure 5.3, the neat chitosan showed one major peak, located at the higher elution time, and a small peak, located at the lower elution time. Similarly, two peaks located at a low-elution and a high-elution times, were clearly observed for all of the *N*-SC products. The peaks at the low-elution time

should correspond to the aggregated chains with high weight-average molecular weights ($M_{w,a}$) and the high-elution one should correspond to the single chains with low weight-average molecular weights ($M_{w,s}$) (Chen, *et al.,* 2004) Theoretically, chitosan in its solid form possesses both intramolecular hydrogen bond at O3···O5 position across the glycocidic bond in a similar manner with chitin and cellulose and intermolecular hydrogen bond at N2···O6 position that forms a sheet structure (Yui, et al.,1994) In the solution form (when chitosan is dissolved in an acidic aqueous solution, with pH < 4), the destruction of the intermolecular hydrogen bond between adjacent chitosan chains normally occurs due to the repulsion of the protonated amino groups (NH_3^+), resulting in only the formation of intramolecular hydrogen bond (Franca, *et al.,* 2008). In analogy to chitosan, the aggregated chains at the low-elution time of the *N*-SC products should result from the intermolecular hydrogen bonding between carbonyl groups (acting as a proton acceptor) and amino groups (acting as a proton donor) (i.e., OC=O···HN). This led to the aggregation of *N*-SC molecules in a manner similar to those of CM-chitosan, which tend to aggregate when they are in an aqueous solution state due to the interaction between amino and the substitution groups (Aiping, *et al.,* 2006) According to the results shown in Figure 5.3, the intermolecular interaction that occurred in the *N*-SC products was obviously stronger than that observed in the neat chitosan.

The SEC results of the neat chitosan and the *N*-SC products, after their solutions had been exposed to various doses of γ-ray radiation, are shown in Figure 5.4. Interestingly, even at 5 KGy of γ-ray radiation, the peaks associated with the aggregated chains, located at the low elution times, for all of the investigated samples disappeared. With an increase in the dose of the radiation from 5 to 30 KGy, the positions of the peaks shifted towards increasing elution times, the results that indicated the decrease in the $M_{w,s}$'s of the polymers (see Table 5.3). In line with the decrease in the $M_{w,s}$ values of the polymers, the polydispersity indices also decreased with an increase in the radiation dose. In contrast to the dilute aqueous solutions, the neat chitosan and the *N*-SC products in their film form, after having been exposed to various doses of γ-ray radiation, showed slight shifting in the positions of the peaks associated with the single chains of the polymers (see Figure 5.5). Interestingly, the

peaks associated with the aggregated chains for all of the investigated samples did not totally disappear, as in the cases of the dilute solutions, even though the films had been exposed to the radiation as intense as 100 KGy. While the $M_{w,s}$'s of the polymers showed a clear reduction in their values with an increase in the dose of the radiation from 20 to 100 KGy, such trends were not observed for the $M_{w,a}$ values (see Table 5.4). The rather constancy in the $M_{w,a}$ values of the polymers in their film form could be a result of the inability of the reactive radical species to mobilize within the mass of the films, causing some of the radicals to recombine.

To better illustrate the effects of the sample type (i.e., neat chitosan versus *N*-SC products), the form of the samples (i.e., dilute solutions versus films) and the radiation dose on the reduction in the $M_{w,s}$ values of the polymers, the $M_{w,s}$ values are plotted as a function of the radiation dose in Figure 5.7. Evidently, the reduction in the molecular weights of the polymers in their dilute solution form was significant even after the samples had been irradiated with a low dose of 5 KGy. Further increasing the radiation dose only resulted in a slight decrease in their molecular weights and the $M_{w,s}$ values among the various samples were not much different from one another. Comparatively, the reduction in the molecular weights of the film counterparts occurred in a much less extent, with the chitosan film showing the most significant reduction in the $M_{w,s}$ values at all radiation doses investigated (as deduced from the results shown in Figure 5.6). The greater susceptibility of the polymers in their dilute solution form to degradation when being exposed to γ-ray radiation than those in their film counterpart can be explained based on two reasons. The first is the absorption of the radiation energy by a large quantity of water molecules that results in the generation of a number of reactive species, such as e^-_{aq}, H_2O_2, OH•, H•, OH^-, H^+ and H^- (Woods and Pikaev, 1994) that could participate in the abstraction of H atom from the polymer chains, hence the formation of macro-radicals. The instability of the macro-radicals leads to one or more of the following reactions, e.g., H transfer, recombination or chain scission and disproportionation. The second is the high mobility of the polymer chains in the dilute solution state.

The susceptibility of the neat chitosan to degradation by γ-ray radiation (in comparison with all of the *N*-SC products, as deduced from the results

shown in Figure 5.6) should be due to the abundance of the amino groups along the chitosan chains (viz. the number of the amino groups along the *N*-SC chains was lower). The high electron density of the amino groups leads to greater possibility for H-abstraction reaction to occur, which finally leads to the degradation of the chitosan main chains. As reported by Huang et al. (Huang, *et al.*, 2007) CM-chitosan, due to the greater number of free amino groups along its molecules, is more sensitive to decomposition by γ-ray radiation than CM-chitin.

5.4.5 Structural Change of Irradiated Chitosan and *N*-SC Products

As previously shown, the reduction in the molecular weights of chitosan and *N*-SC products that had been subjected to γ-ray radiation occurred more readily in their dilute solution state and in the film form. The structure of the samples in their dilute solution state that had been subjected to γ-ray radiation was then characterized further by FT-IR, UV-vis, elemental analysis and ^{1}H-NMR. As for chitosan, a characteristic peak at 1560 cm^{-1} that was assigned to the protonated amino groups (see Figure 5.1(a)) was shifted to 1590 cm^{-1} (NH_2 bending; see Figure 5.7(a)). Such a shift was due to the effect of NaOH that was used to precipitate the chitosan samples. Additionally, another characteristic peak at 1640 cm^{-1}, which could be disturbed by carbonyl groups of the irradiated chitosan (see Figure 5.1(a)), was gradually shifted to 1622 cm^{-1} (see Figure 5.7(a)), while its intensity increased slightly with an increase in the γ-ray radiation dose from 0 to 30 kGy [based on the relative values of the intensity of this peak to that of the peak at 3440 cm^{-1}, which belongs to the OH groups (see Table 5.5)].

Similar to the results on chitosan, the low DS *N*-SC product (in Na form) showed a characteristic peak at 1650 cm^{-1}, which shifted slightly to 1632 cm^{-1} (see Figure 5.7(b)). The peak around 1620-1630 cm^{-1} could be assigned to carbonyl groups and the shift of this peak to a lower wavenumber could be indicative of the formation of hydrogen bond involving carbonyl groups after irradiation (Feng, *et al.*, 2008) On the contrary to chitosan, the same peak of the low DS *N*-SC product decreased slightly with the radiation dose (see Table 5.5), the result that suggested that some acetyl and/or succinyl groups had been cleaved from its structure. Compared with chitosan and the low DS *N*-SC product, the high DS *N*-SC products

(in H form) exhibited characteristic peaks at 1720 cm^{-1} which could also be ascribed to carbonyl groups (see Figure 5.7(c) and (d)). In comparison with those of the neat samples, the intensities of these two peaks tended to increase, while that of the peak at 1545 cm^{-1} tended to decrease, with an increase in the radiation dose. As shown in Table 5.4, the peak at 1545 cm^{-1}, which corresponded to succinyl groups, decreased slightly with an increase in the radiation dose, a result that was in line with that of the elemental analysis (see later). Moreover, the peak at 1650 cm^{-1} may be related to carbonyl groups which could either decrease due to the abstraction of the succinyl groups or increase due to the degradation of the main chain with an increase in the radiation dose. Interestingly, the irradiated chitosan and *N*-SC products exhibited a peak at about 1375 cm^{-1} that increased in its intensity with an increase in the radiation dose, a result that suggested an increase in the amount of methyl groups in response to the chain scission that occurred to these polymers (Kang, *et al.*, 2007)

Any alteration in the molecular structure of the irradiated samples was also followed by UV-vis (see Figure 5.8). Evidently, no absorption was observed for the neat polymers. For the irradiated chitosan and the irradiated low DS *N*-SC product, two absorption maxima were observed at 255 and 290-295 nm (see Figure 5.8(a) and (b)). The intensities of these peaks increased with an increase in the radiation dose. The obtained results are consistent with those reported by (Ulanski and Rosiak, 1992) who suggested that the two maxima observed for the irradiated chitosan were a result of the absorption by the carbonyl and the carboxyl groups. On the contrary, only one single absorption peak centering around 290-295 nm was apparent in the cases of the irradiated high DS *N*-SC products (see Figure 5.8(c) and (d)). The intensity of the peak also increased with an increase in the radiation dose. Huang et al. (Woods and Pikaev, 1994) reported that CM-chitosan, after its dilute aqueous solution had been exposed to γ-ray radiation, also showed a single absorption peak at around 290 nm, owing to the occurrence of the carbonyl and the carboxyl groups. It was suggested that the position of the absorption peak of the irradiated chitosan depended strongly on the %DD of the polymer.

Elemental analysis was used to further elucidate the effect of γ-ray radiation on the *N*-substituted groups of the irradiated samples. As shown in Table

5.6, the values of the C/N and C/H ratios had a tendency to increase slightly with an increase in the radiation dose for all sample types. In case of the irradiated chitosan, the observed increase in the C/N and C/H values with an increase in the radiation dose suggested that γ-ray radiation might induce the abstraction of some of the *N*-acetyl groups along the chitosan chains without the loss of the free amino groups, resulting in no noticeable change in the %DD of the polymer (Feng, *et al.*, 2008; Zainol, Akil and Mastor, 2009). This postulation was in accord with a previous study on CM-chitosan by Huang et al. (Huang, *et al.*, 2007) who reported that the increase in the C/N value of the irradiated sample with an increase in the radiation dose indicated that the radio-degradation occurred at *N*-position. For all of the irradiated *N*-SC products, even though it was not apparent for some data points, a general trend was observed as the values of the C/N and C/H ratios were found to increase, though very marginally, with an increase in the radiation dose. The results suggested that both of the *N*-acetyl and the *N*-succinyl groups on all types of the *N*-SC chains could be cleaved off upon irradiating the polymers with γ-ray.

To confirm any structural change to the structures of chitosan and *N*-SC products when their dilute solutions had been exposed to γ-ray radiation, ^{1}H-NMR technique was employed. The ^{1}H-NMR spectrum of the neat chitosan is similar to that of the chitosan sample that had been irradiated at 30 kGy (see Figure 5.9), a result that indicated that the integrity of the pyranose ring of the irradiated chitosan was maintained. Notwithstanding, the ratio of the integral area of the peaks at 3.3-4.3 ppm (H3-H6) to that of peak at 3.18 ppm of H2 of the pyranose ring increased from 3.57 for the neat chitosan to 4.36 for the irradiated sample, a result that suggested the abstraction of some of the *N*-acetyl groups from the irradiated sample. As for the ^{1}H-NMR results for the neat and the irradiated 5 *N*-SC products, similar spectra were also observed (see Figure 5.10). However, the observed increases in the ratio of the integral area of the peaks at 2.5-2.7 ppm of the substituted groups to that of the peak at 3.18 ppm of H2 of the pyranose ring from 3.16 of the neat sample to 4.24 of the irradiated sample and in the integral area of the peaks at 3.3-4.3 ppm (H3-H6) from 5.36 to 11.2 suggested that the abstraction of *N*-substitution groups of *N*-SC indeed occurred. The proposed mechanism for the

abstraction of *N*-acetyl groups from chitosan and *N*-succinyl groups from *N*-SC products when their dilute aqueous solutions had been exposed to γ-ray radiation is shown in Figure 5.11.

According to FT-IR and UV-vis results, the exposure of chitosan and *N*-SC products to γ -ray radiation caused the main chains of the polymers to break at the β-(1,4) glycosidic linkages, which later led to the formation of carbonyl groups. Moreover, the results obtained by elemental analysis and ^{1}H-NMR revealed that γ-ray radiation also caused the partial reduction of *N*-acetyl groups of chitosan and *N*-succinyl groups of *N*-SC, resulting in a slight reduction in the DS with an increase in the radiation dose.

5.5 Conclusion

The effect of γ-ray radiation on molecular weight characteristics and molecular structure of *N*-succinyl chitosan (*N*-SC) was investigated by size exclusion chromatography (SEC), Fourier-transformed infrared spectroscopy (FT-IR) and UV-visible spectrophotometry (UV-vis). Comparisons were made against the neat and the irradiated chitosan. SEC results showed that the neat *N*-SC products exhibited two elution peaks of single and aggregated chains, while the neat chitosan showed only one peak of single chains. The aggregation was due to the interaction between C=O and N-H of the substitution groups. In the dilute aqueous solution state, the single chain molecular weights of the polymers systematically decreased with an increase in the radiation dose and the presence of the aggregated chains of the *N*-SC products was practically absent upon their exposure to the γ-ray radiation. In the film state however, the reduction in the molecular weights of the polymer occurred less readily and the γ-ray radiation of as high as 100 kGy was not able to suppress the presence of the aggregated chains of the *N*-SC products. The enhanced degradation via water radiolysis was the main reason for the observed results. FT-IR and UV-vis results on the irradiated chitosan and *N*-SC products in their dilute aqueous solution state confirmed that the amounts of the carbonyl groups along the polymer chains increased with an increase in the radiation dose. The formation of these groups

suggested that the radio-degradation of the polymers occurred at the glysocidic linkages. Additionally, the elimination of some *N*-acetyl groups and *N*-succinyl groups of the irradiated samples readily occurred, which was confirmed by FT-IR, elemental analysis and ^{1}H-NMR results.

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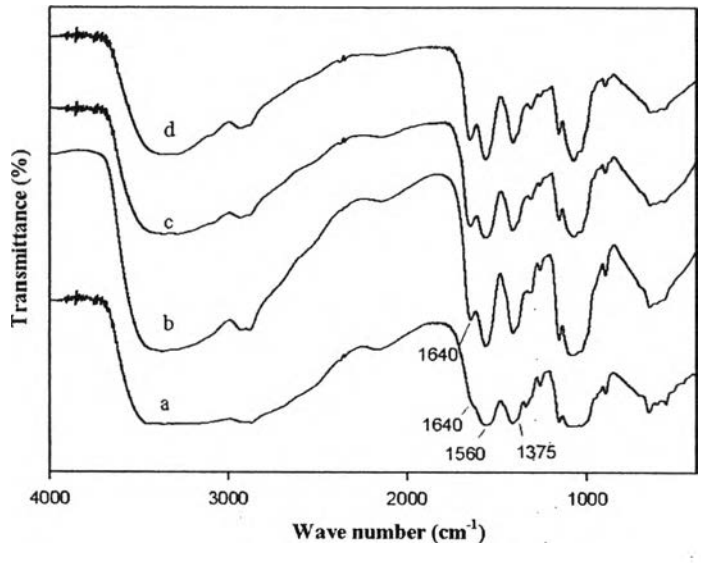


Figure 5.1 FT-IR spectra of (a) chitosan and *N*-succinyl chitosan (*N*-SC) products: (b) 1 *N*-SC, (c) 3 *N*-SC and (d) 5 *N*-SC.

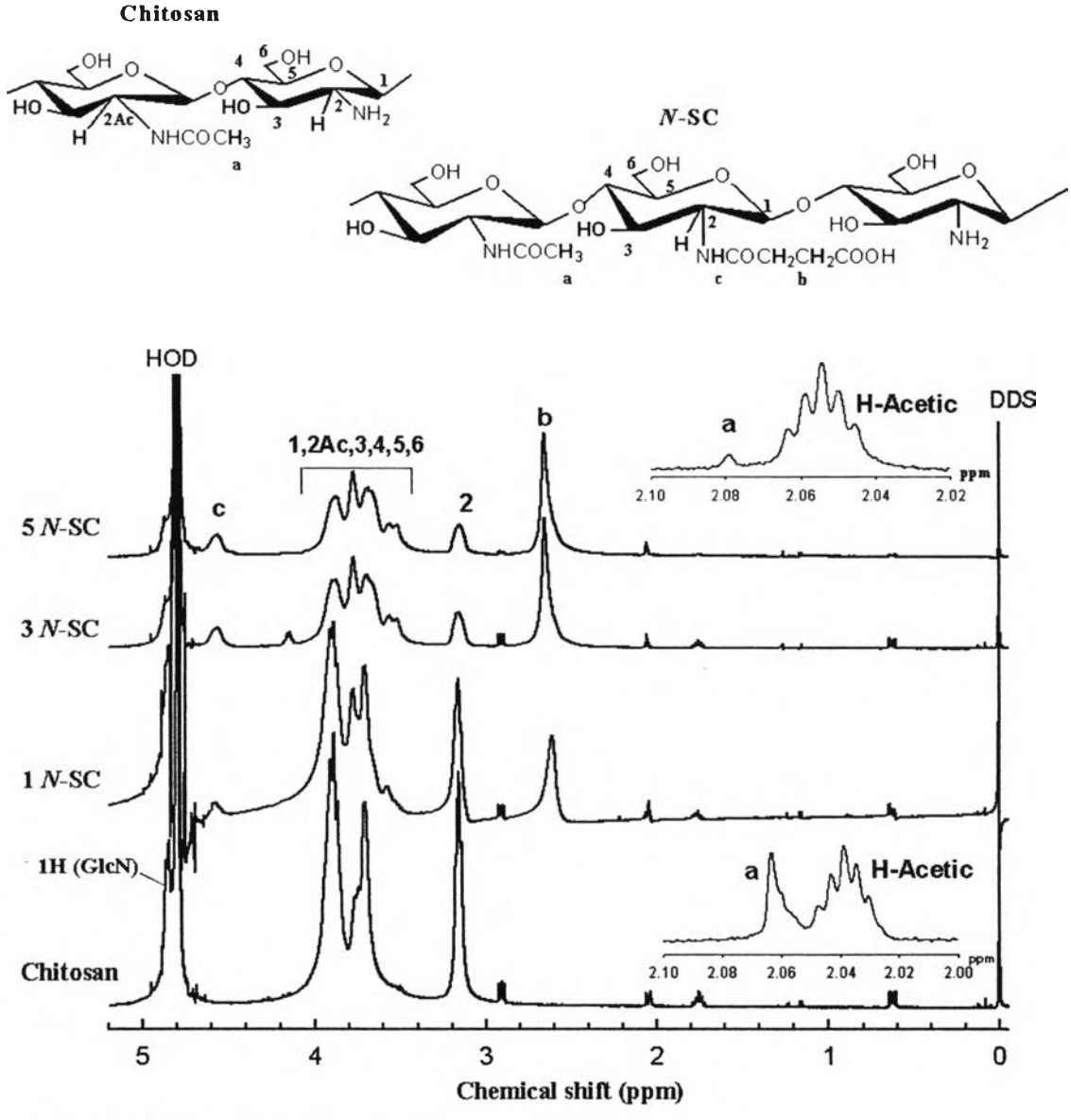


Figure 5.2 ^{1}H-NMR spectra of chitosan and *N*-SC products.

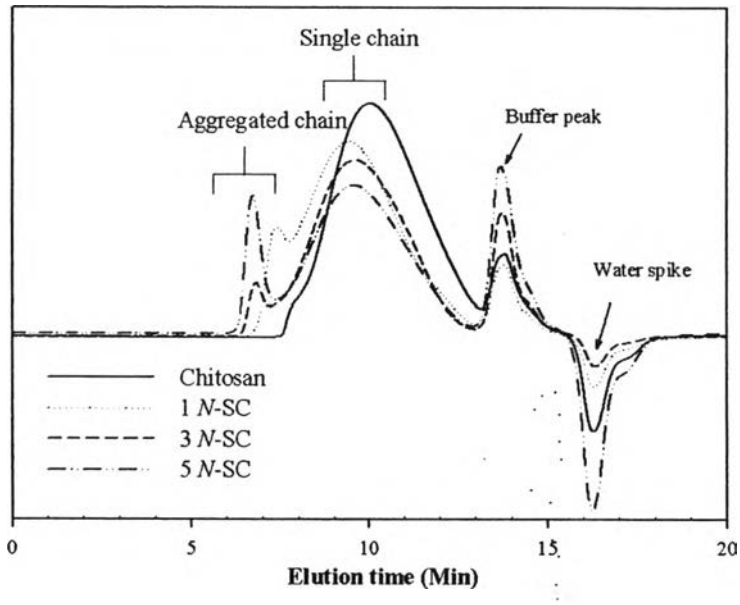


Figure 5.3 SEC chromatograms of chitosan and *N*-SC products.

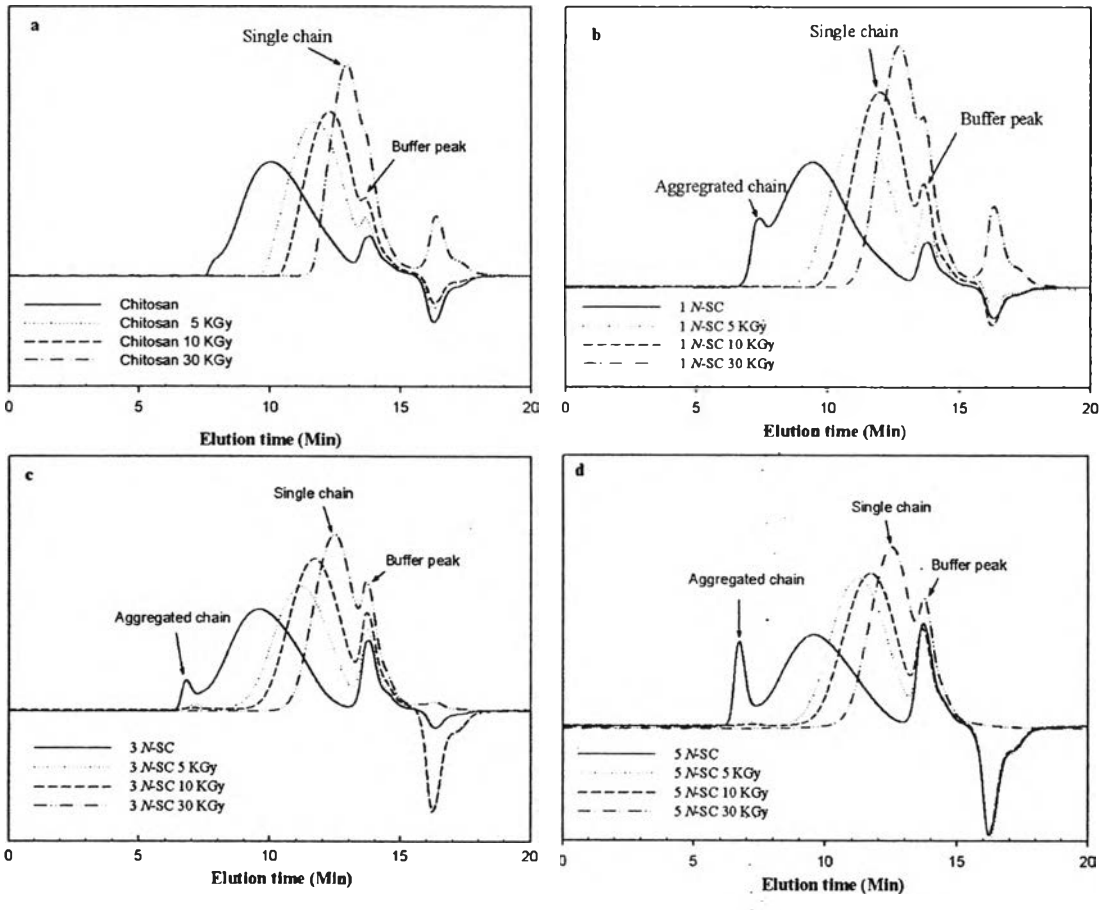


Figure 5.4 SEC chromatograms of (a) chitosan and *N*-SC products: (b) 1 *N*-SC, (c) 3 *N*-SC and (d) 5 *N*-SC, after their dilute aqueous solutions had been exposed to γ-ray radiation of varying doses.

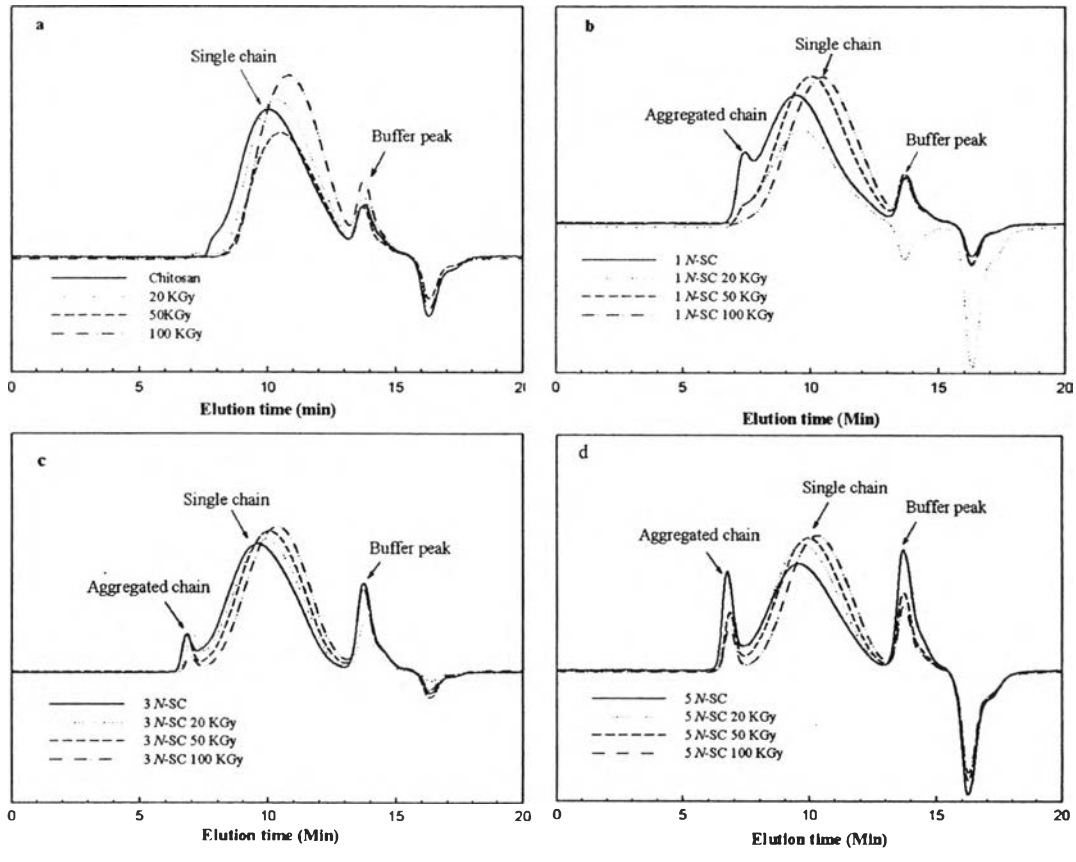


Figure 5.5 SEC chromatograms of (a) chitosan and *N*-SC products: (b) 1 *N*-SC, (c) 3 *N*-SC and (d) 5 *N*-SC, after their films had been exposed to γ-ray radiation of varying doses.

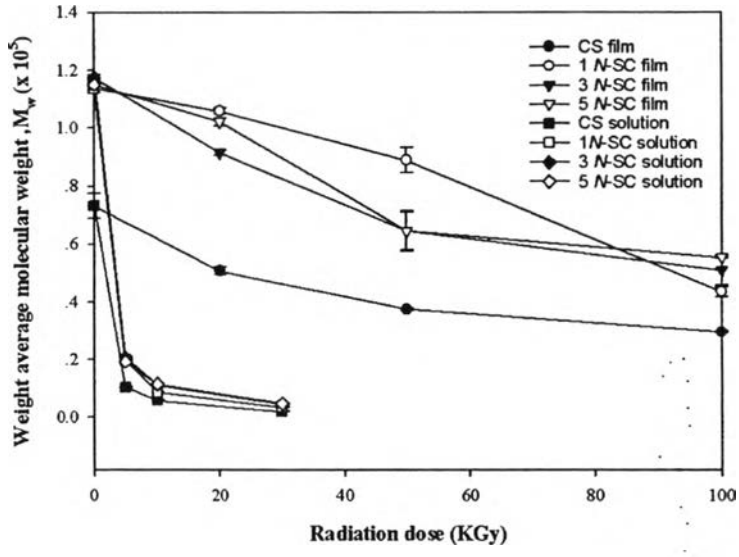


Figure 5.6 Weight-average molecular weights of chitosan and *N*-SC products after their dilute aqueous solutions and films had been exposed to γ-ray radiation of varying doses.

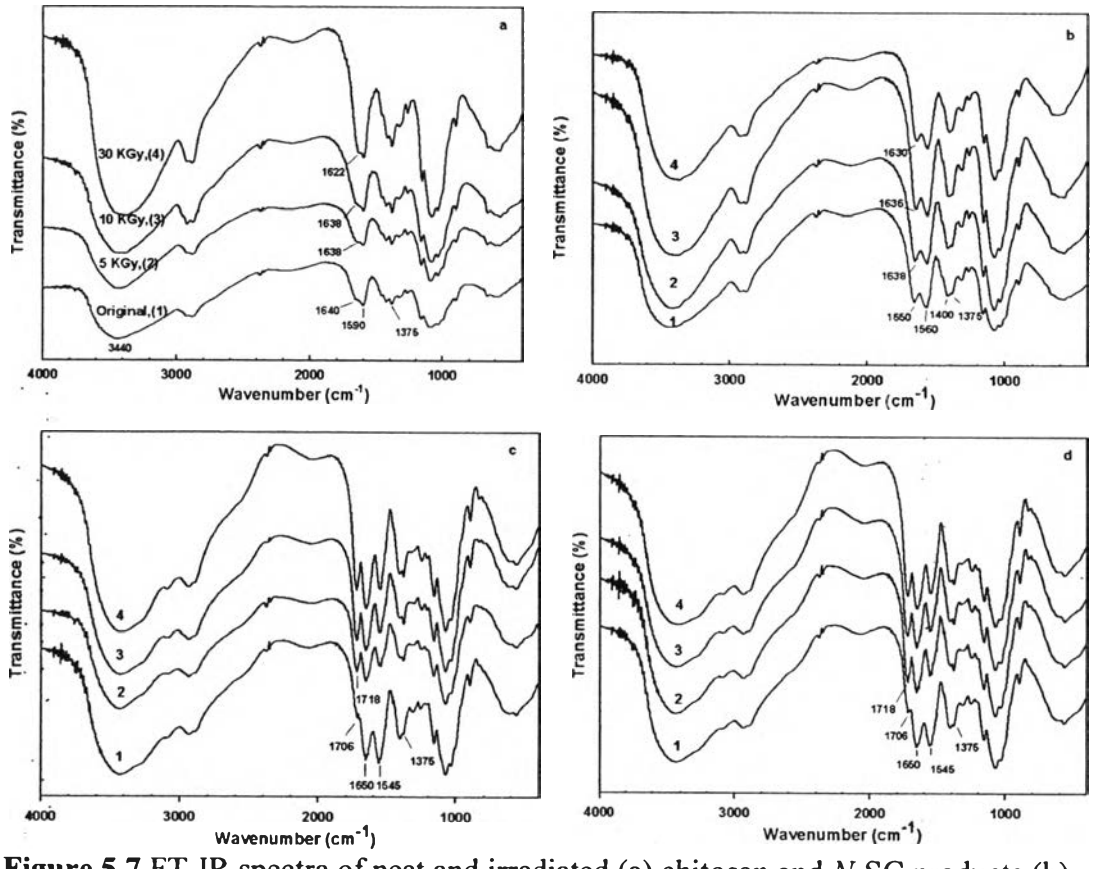


Figure 5.7 FT-IR spectra of neat and irradiated (a) chitosan and *N*-SC products (b) 1 *N*-SC, (c) 3 *N*-SC and (d) 5 *N*-SC. Keys: **1**, neat samples; **2**, 5 KGy; **3**, 10 kGy and **4**, 30 KGy.

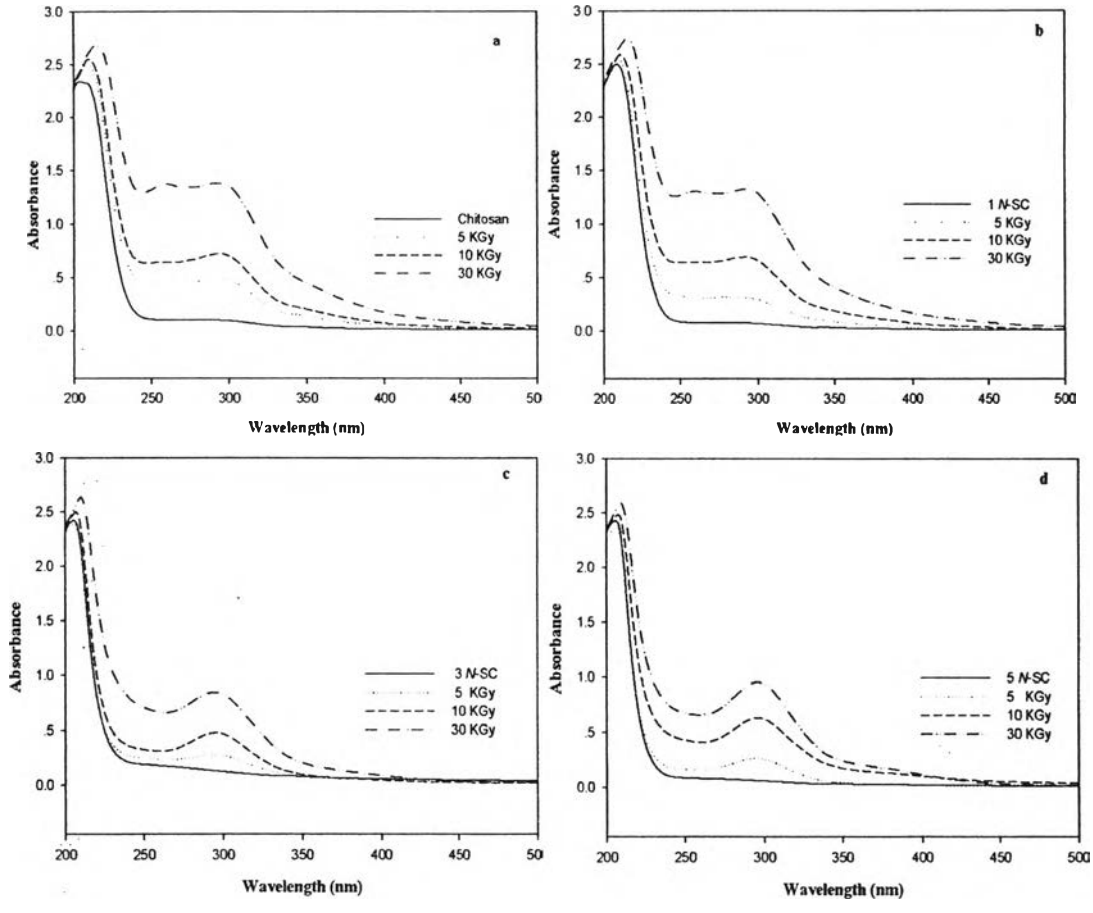


Figure 5.8 UV spectra of neat and irradiated (a) chitosan and *N*-SC products (b) 1 *N*-SC, (c) 3 *N*-SC and (d) 5 *N*-SC.

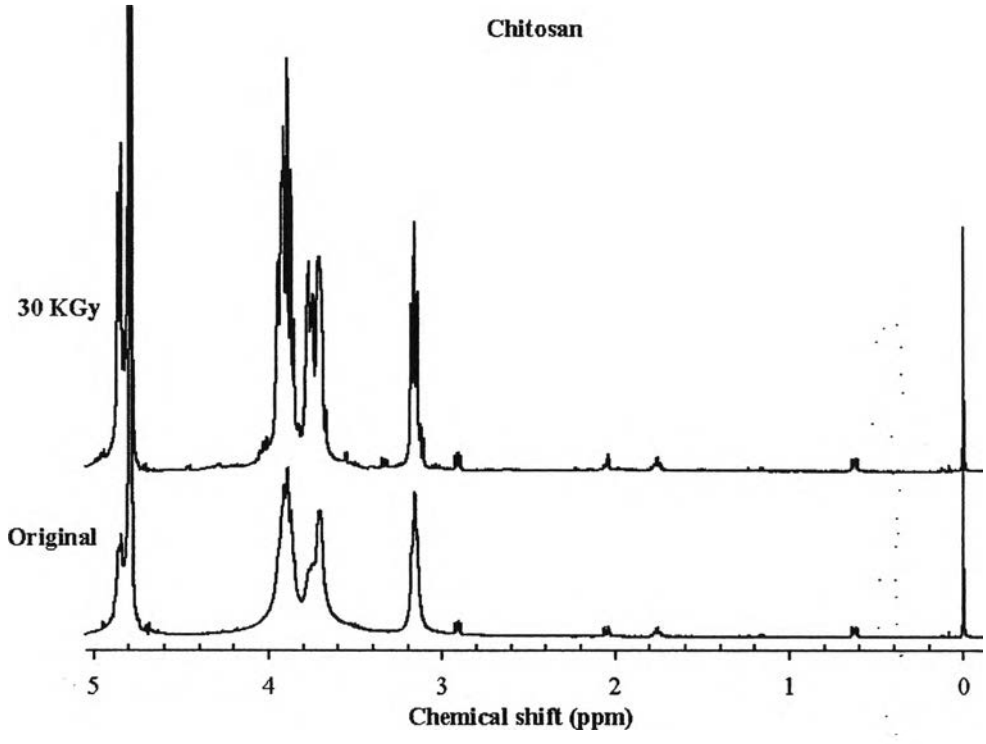


Figure 5.9 H^1-NMR spectra of original and irradiated chitosan (30 kGy, dilute aqueous solution).

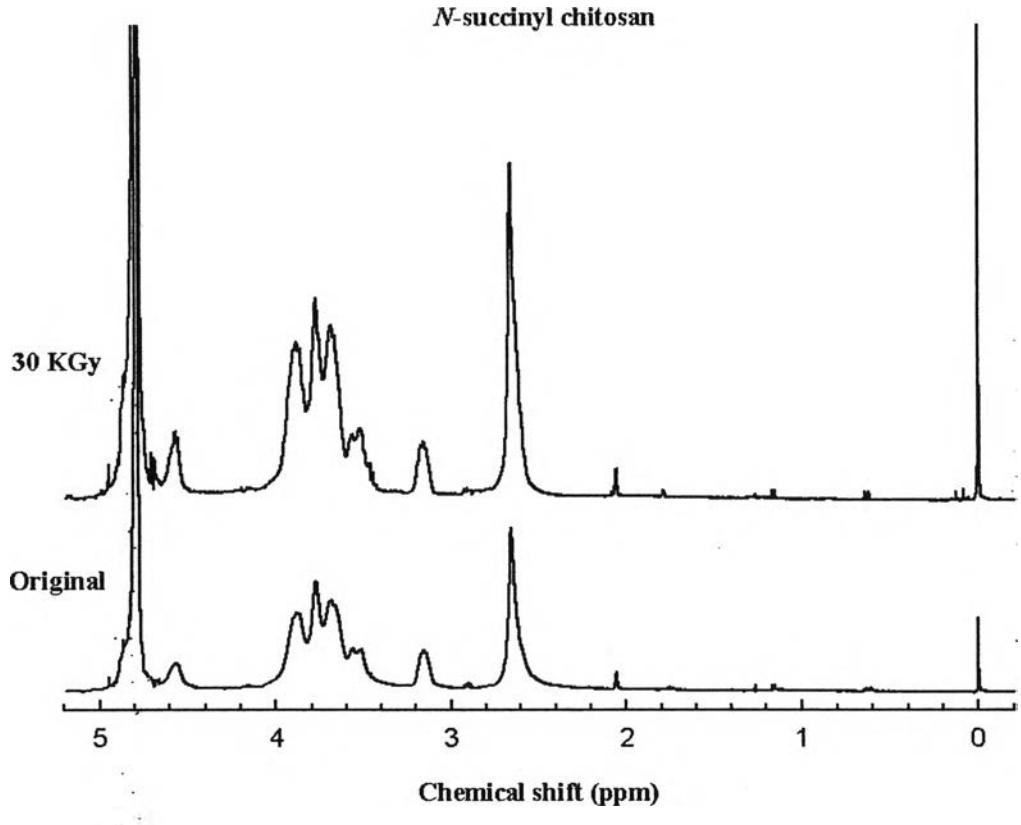


Figure 5.10 H^1-NMR spectra of original and irradiated 5 *N*-SC (30 kGy, dilute aqueous solution).

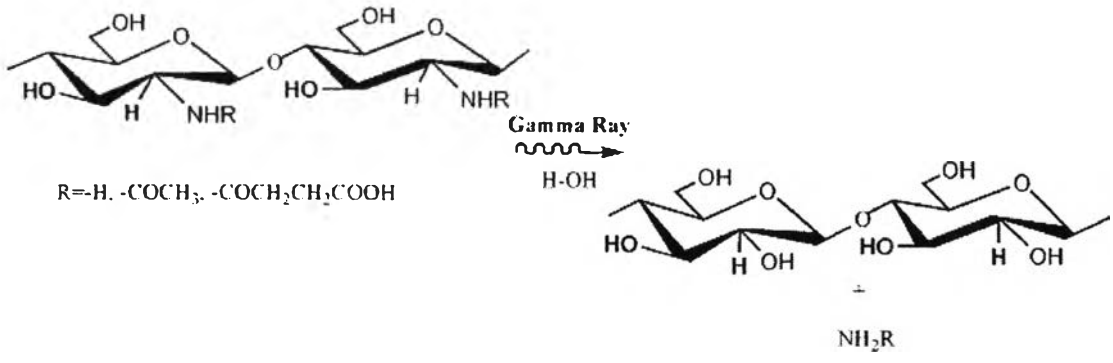


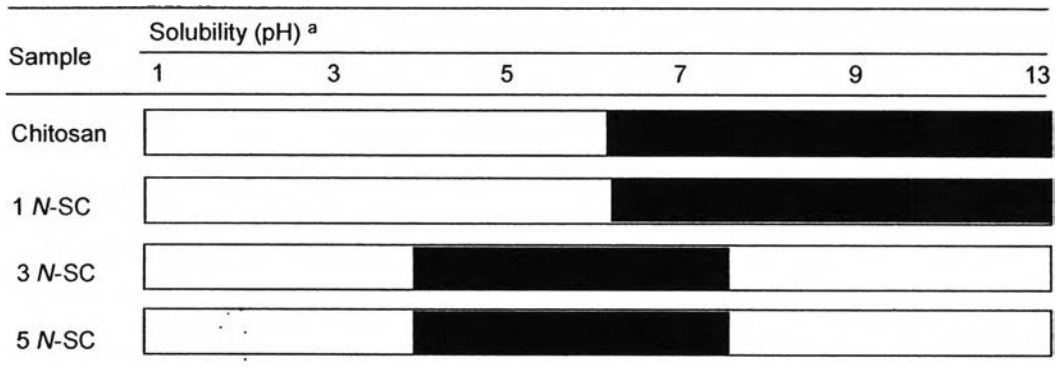
Figure 5.11 Proposed mechanism for *N*-elimination of irradiated chitosan and *N*-SC products in their dilute aqueous solution state.

Table 5.1 Synthesis conditions and characteristics of *N*-succinyl chitosan (*N*-SC) products.

Sample	Succinic anhydride: NH_2 group (mol/mol)	DS[a] (%)	$M_{w,s}$[b] ($\times 10^5$ g·mol^{-1})	Elemental analysis			
				C%	H%	N%	C/N
Chitosan	-	-	0.75	41.90	6.77	7.85	5.34
1 *N*-SC	1:1	10	1.14	41.35	6.38	6.90	5.99
3 *N*-SC	3:1	104	1.17	42.48	6.32	5.85	7.26
5 *N*-SC	5:1	73	1.15	40.91	6.06	5.65	7.24

[a] Degree of substitution, calculated from ^{1}H-NMR; [b] Single chain weigh-average molecular weight, determined by SEC.

Table 5.2 Solubility of chitosan and *N*-SC products at various pH levels.



[a]White bar: water-soluble, Black bar: water-insoluble.

Table 5.3 SEC data of chitosan and *N*-SC products, after their dilute aqueous solutions had been exposed to γ-ray radiation.

Solution (1%w/v)	**Dose** (KGy)	**Time** (min)	**Aggregated molecular weights** ($g \cdot mol^{-1}$)			**Time** (min)	**Single chain molecular weights** ($g \cdot mol^{-1}$)		
			M_n ($\times 10^5$)	M_w ($\times 10^5$)	M_w/M_n		M_n ($\times 10^5$)	M_w ($\times 10^5$)	M_w/M_n
Chitosan	0	-	-	-	-	10.003	0.18	0.73	3.982
	5	-	-	-	-	11.683	0.05	0.10	2.134
	10	-	-	-	-	12.222	0.03	0.06	1.728
	30	-	-	-	-	12.889	0.01	0.02	1.709
1 *N*-SC	0	7.403	7.60	8.06	1.061	9.425	0.29	1.14	3.861
	5	-	-	-	-	11.183	0.09	0.20	2.226
	10	-	-	-	-	11.947	0.04	0.09	2.021
	30	-	-	-	-	12.708	0.02	0.03	1.446
3 *N*-SC	0	6.814	13.59	14.20	1.045	9.583	0.29	1.17	4.072
	5	7.179	9.38	9.86	1.051	11.130	0.09	0.21	2.174
	10	-	-	-	-	11.678	0.06	0.11	1.755
	30	-	-	-	-	12.419	0.03	0.05	1.525
5 *N*-SC	0	6.742	14.08	15.00	1.065	9.594	0.28	1.15	4.083
	5	7.069	10.49	11.22	1.070	11.228	0.09	0.19	2.141
	10	-	-	-	-	11.692	0.06	0.12	1.797
	30	-	-	-	-	12.47	0.03	0.05	1.616

* SD ($n = 3$) < 5%

Table 5.4 SEC data of chitosan and *N*-SC products, after their films had been exposed to γ-ray radiation.

Film**	**Dose** (KGy)	**Time** (min)	**Aggregated molecular weights** ($g \cdot mol^{-1}$)			**Time** (min)	**Single chain molecular weights** ($g \cdot mol^{-1}$)		
			M_n ($\times 10^5$)	M_w ($\times 10^5$)	M_w/M_n		M_n ($\times 10^5$)	M_w ($\times 10^5$)	M_w/M_n
Chitosan	0	-	-	-	-	10.003	0.18	0.73	3.982
	20	-	-	-	-	10.322	0.15	0.51	3.307
	50	-	-	-	-	10.481	0.14	0.38	2.762
	100	-	-	-	-	10.811	0.11	0.30	2.609
1 *N*-SC	0	7.403	7.60	8.06	1.061	9.425	0.29	1.14	3.861
	20	-	-	-	-	10.322	0.11	1.06	9,524
	50	-	-	-	-	10.025	0.21	0.89	4.248
	100	-	-	-	-	10.397	0.15	0.44	2.930
3 *N*-SC	0	6.814	13.59	14.20	1.045	9.583	0.29	1.17	4.072
	20	7.031	10.59	11.19	1.056	9.903	0.24	0.91	3.804
	50	6.886	13.82	14.27	1.032	10.075	0.21	0.65	2.878
	100	6.972	12.00	12.38	1.032	10.367	0.17	0.51	2.951
5 *N*-SC	0	6.742	14.08	15.00	1.065	9.594	0.28	1.15	4.083
	20	6.861	12.48	13.18	1.056	9.756	0.26	1.02	3.909
	50	6.844	13.65	14.16	1.038	9.925	0.22	0.65	2.980
	100	6.830	13.76	14.35	1.043	10.294	0.18	0.55	3.150

(* SD (n = 3) < 10.5%, **an average of film thickness =40 μm)

Table 5.5 Analysis of FT-IR peak ratios of chitosan and *N*-SC products, after their dilute aqueous solutions had been exposed to γ-ray radiation.

Sample	**Dose** (KGy)	**Wavenumber ratio (cm^{-1}/cm^{-1})**					
		1375/1400	**1640,1650/ 1590,1545**	**1720/1545**	**1590,1545/3440**	**1640,1650/ 3440**	**1720/3440**
Chitosan	0	1.015	0.900	-	0.556	0.500	-
	5	1.045	0.972	-	0.444	0.432	-
	10	1.059	1.000	-	0.500	0.500	-
	30	1.091	1.007	-	0.567	0.571	-
1 *N*-SC	0	0.889	1.055	-	0.640	0.674	-
	5	0.902	1.070	-	0.592	0.633	-
	10	0.935	1.029	-	0.633	0.651	-
	30	0.910	0.973	-	0.663	0.646	-
3 *N*-SC	0	0.859	1.070	0.667	0.713	0.762	0.475
	5	1.034	1.469	1.271	0.442	0.650	0.562
	10	1.034	1.400	1.350	0.508	0.711	0.685
	30	0.968	1.377	1.283	0.505	0.695	0.648
5 *N*-SC	0	0.889	1.127	0.794	0.624	0.703	0.495
	5	1.037	1.523	1.477	0.444	0.677	0.657
	10	0.952	1.387	1.330	0.525	0.728	0.698
	30	0.935	1.380	1.352	0.507	0.700	0.685

Table 5.6 Elemental analysis data of chitosan and *N*-SC products, after their dilute aqueous solutions had been exposed to γ-ray radiation.

Sample	**Dose** (KGy)	**%C**	**%H**	**%N**	**C/N**	**C/H**
Chitosan	0	41.90	6.77	7.85	5.34	6.19
	5	42.48	6.95	7.91	5.37	6.11
	10	42.68	6.79	7.70	5.54	6.29
	30	43.22	6.51	7.75	5.58	6.64
1 *N*-SC	0	41.35	6.38	6.90	6.00	6.48
	5	43.89	6.74	7.11	6.17	6.51
	10	42.99	6.46	7.17	6.00	6.66
	30	42.84	6.17	6.80	6.30	6.94
3 *N*-SC	0	42.48	6.32	5.85	7.26	6.72
	5	39.98	5.79	5.68	7.04	6.91
	10	40.83	5.57	5.51	7.41	7.33
	30	41.58	5.62	5.54	7.51	7.40
5 *N*-SC	0	40.91	6.06	5.65	7.24	6.75
	5	40.66	5.99	5.58	7.29	6.79
	10	42.41	6.19	5.44	7.80	6.85
	30	41.45	5.82	5.68	7.30	7.12