A new approach for the preparation of chitosan from $\gamma$-irradiation of prawn shell: effects of radiation on the characteristics of chitosan

Taslim Ur Rashid, a Mohammed Mizanur Rahman, a Shahriar Kabir, a Sayed M Shamsuddin a and Mubarak A Khan b

Abstract

Chitosan is a biodegradable polymer composed of randomly distributed $\beta$-(1,4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimps) and the cell walls of fungi. In the work reported, we developed a facile technique for the preparation of chitosan by irradiating prawn shell at various intensities from 2 to 50 kGy. It was observed that $\gamma$-irradiation of prawn shell increased the degree of deacetylation (DD) of chitin at a relatively low alkali concentration during the deacetylation process. Among the various irradiation doses applied to prawn shell, a dose of 50 kGy and 4 h heating in 50% NaOH solution yielded 84.56% DD while the chitosan obtained from non-irradiated prawn shell with the same reaction conditions had only 74.70% DD. In order to evaluate the effect of $\gamma$-irradiation on the various physicochemical, thermomechanical, and morphological properties, the chitosan samples were again irradiated (2–100 kGy) with $\gamma$-radiation. Molecular weight, DD, thermal properties with differential scanning calorimetry and thermogravimetric analysis, particle morphology by scanning electron microscopy, water binding capacity (WBC), fat binding capacity (FBC) and antimicrobial activity were determined and the effects of various $\gamma$-radiation doses were assessed. The DD, WBC, FBC and antimicrobial activity of the chitosan were found to improve on irradiation. It was obvious that irradiation caused a decrease of molecular weight from 187 128 to 64 972 g mol$^{-1}$ after applying a radiation dose of 100 kGy which occurred due to the chain scission of chitosan molecules at glycosidic linkages. The decrease of molecular weight increased the water solubility of the chitosan, the extent of which was explored for biomedical applications.

Keywords: prawn shell; chitosan; $\gamma$-irradiation; fat binding capacity; antimicrobial activity

INTRODUCTION

Chitin and chitosan (deacetylated form of chitin) polymers are natural aminopolysaccharides having unique structures, multidimensional properties, highly sophisticated functions and wide-ranging applications in biomedical and other industrial areas. 1, 2 The difference between chitin and chitosan lies in the degree of deacetylation (DD), usually defined as the ratio of the amount of glucosamine to the total amount of N-acetylglucosamine and glucosamine, being the most important parameter determined for chitosan and chitin. 3 The positive attributes of excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity with versatile biological activities such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development. 4–8

Despite its huge annual production and easy availability, chitin still remains an underutilized resource primarily because of its intractable molecular structure. The non-solubility of chitin in almost all common solvents has been a stumbling block in its appropriate utilization, although chitin fibres stand apart from the other biodegradable natural fibres in many inherent properties. 9 With the vast amount of shellfish waste, applications requiring large amounts of chitosan are now possible. Chitosan is used in many traditional and potential applications like in water and beverage clarification and purification, wound dressings, drug delivery, contact lenses, bandages, cholesterol reducing agent, cosmetics and personal care, etc. 3 Interest in all forms and levels of purity continues to expand tremendously.

Much research has been done using chitosan in various fields. 10–16 Isolation of chitosan from crustacean shell wastes consists of four basic steps: deproteinization for protein separation, demineralization for calcium carbonate separation, decolouration for pigment separation and deacetylation for removal of acetyl groups. 17 The experimental conditions set up by Kurita et al. 18 and Broussignac 19 were compared by Tolaimate et al. 20 to determine optimum N-deacetylation conditions which can be used to obtain chitosan with high molecular weights and with a large range of degrees of N-deacetylation. The influence

* Correspondence to: Mohammed Mizanur Rahman, Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering and Technology, University of Dhaka, Dhaka 1000, Bangladesh. E-mail: mizan607@gmail.com

a Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering and Technology, University of Dhaka, Dhaka 1000, Bangladesh

b Institute of Radiation and Polymer Technology (IRPT), Atomic Energy Research Establishment (AERE), PO Box 3787, Dhaka 1000, Bangladesh
of alkaline concentration, temperature, and time and chitin to solution ratio on N-deacetylation was investigated by Methacanona et al.21

Bangladesh processes and exports large quantities shrimp every year and the prawn processing plants produce large quantities of shells that remain unused. The shells become waste, although this prawn shell waste is high in natural polymer, thus creating environmental pollution. Extraction of chitosan from prawn shell and its utilization in fields such as agriculture, water purification, pharmaceuticals as excipients, medicinal use, fat reduction, textiles, etc., will add new dimensions in terms of economics.

The investigation reported here dealt with the preparation of chitosan from γ-irradiated prawn shells and the comparison of molecular weight, alkali concentration for deacetylation, reaction time and DD of the chitosan obtained from γ-irradiated prawn shells with those of chitosan prepared using the conventional method. Ionizing radiation such as γ-radiation can produce electronic excitation as well as ions and may also produce ions and free radicals in excited states. Thus, it is important that possible chemical effects due to electronically excited groups as well as to ionized groups may occur. The most important chemical changes that may occur during the irradiation of chitosan are those of crosslinking and degradation.22 From the viewpoint of mechanical properties of polymer materials, crosslinking of polymers leads to increasing hardness, tensile strength, elastic modulus and softening temperature, and to decreasing elongation to failure and solubility in solvents.23 On the other hand, degradation of chitosan due to main-chain scission leads to the opposite effects on the mechanical properties, and eventually produces soft, gummy or tar-like materials. When chitosans are irradiated, both crosslinking and degradation often occur simultaneously. Irradiation also brings about significant changes in physicochemical, thermal and morphological properties of chitosan which provides a great potential for many applications. So another purpose of the research reported here was to evaluate the effects of γ-irradiation on the physicochemical, thermal and morphological properties of chitosan which provides a great potential for many applications.

EXPERIMENTAL

Materials

Chitin, the source of chitosan, was produced from prawn shell. The prawn shell was collected from freshwater prawn (Macrobrachium malcolmsoni) cultivated in the coastal region of Bangladesh. The amount of chitin in the prawn shell was 18–20% (w/w). A 60Co gamma source was used to irradiate prawn shell and chitosan. The γ-beam was loaded with source GB5-98 that comprised 36 double-encapsulated capsules of type T-T252 loaded with 60Co pellets. It was located in the Institute of Food and Radiation Biology (IFRB) at the Atomic Energy Research Establishment (AEERE), Savar, Dhaka, Bangladesh. Sodium hydroxide (Merck, Germany; purity > 97%), fuming hydrochloric acid (Merck, Germany; 37% solution), glacial acetic acid (BDH Chemicals Lt, UK), sodium chloride (UNI-Chem., China; purity 99.8%), potassium bromide (JHD, Germany; purity > 99%) and soybean oil (Ruchandha pure soybean oil, Bangladesh edible oil supplier) were used in the extraction of chitosan and for the various analytical methods.

Methods

Extraction of chitosan from waste prawn shell

Chitosan was extracted from waste prawn shell using a modified method of No and Meyers.27 The waste prawn shell was boiled in water for 1 h and then washed thoroughly with hot water several times. Then the prawn shell was dried in a gravity convection oven (Memmert UM series) at 105 °C for 24 h. The dried prawn shell was treated with 3–4% solution of aqueous NaOH at a ratio of 1 : 16 (w/w) for 3 h to remove the protein. The mixture was washed with water several times and placed in the gravity convection oven at 105 °C for 24 h. The dried sample was treated with 1 N HCl at a ratio of 1 : 16 (w/w) with stirring for 3 h. The mixture was washed and dried in the gravity convection oven at 105 °C for 24 h and chitin was obtained.

The chitin was deacetylated by heating under boiling conditions (150–160 °C) with 50% (w/w) NaOH solution at a ratio of 1 : 20 (wt%) for 3 h. The mixture was then washed thoroughly with distilled water to remove the NaOH completely from the product. The chitosan thus produced was dried in a gravity convection oven for 72 h at 60 °C. Two other batches of chitosan were also produced with 2 h and 4 h of heating during the deacetylation process.

Extraction of chitosan from γ-irradiated prawn shell

Prawn shell was γ-irradiated at various intensities (2–50 kGy at a rate of 1000 krad h⁻¹). Chitosan was extracted from irradiated prawn shell following the same procedure as described above.

Irradiation of chitosan with γ-radiation

The chitosan produced using the procedure described above was dried in a gravity convection oven for 1 h at 105 °C and collected in polyethylene bags, 10 g in each. Then the bags were sealed and irradiated with γ-radiation at doses of 2–100 kGy at a rate of 1000 krad h⁻¹. After irradiation the samples were stored in the dark at room temperature and were subjected to various characterization tests in order to evaluate the effect of radiation on sample properties.

Determination of molecular weight of chitosan using viscometric method

Solutions of 0.2–0.8% irradiated and non-irradiated chitosan in 0.1 mol L⁻¹ acetic acid/0.2 mol L⁻¹ NaCl (1 : 1 v/v) were prepared. The solutions were passed through a filter (Whatman no. 4) to remove insoluble materials. An Ostwald viscometer was used to measure the passage time of the solutions flowing through a capillary in a constant-temperature water bath at 25 °C. The intrinsic viscosity was obtained by extrapolating the reduced viscosity versus concentration data to zero concentration. The viscosity-average molecular weights of chitosan solutions were calculated using the Mark–Houwink equation which provides the relationship between intrinsic viscosity and molecular weight:

\[
[\eta] = K M_w^a
\]

where K and a are constants for a given solute–solvent system and temperature. Values of K and a were taken as 1.81 × 10⁻³ and 0.93, respectively.24,25
Determination of DD of chitosan using Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of chitosan (with KBr pellets) were obtained for both qualitative and quantitative analysis. KBr discs were prepared according to the method of Sabnis and Block \(^{26}\) with slight modifications. Approximately 2 mg of dried chitosan powder and 200 mg of KBr were blended and triturated with an agate mortar. The mixture was compacted using an IR hydraulic press at a pressure of 6 tons for 60 s. The spectra of chitosan samples (in the form of KBr discs) were obtained using an FTIR spectrometer (T60U model, PerkinElmer, UK) with a wavenumber range of 400–4000 cm\(^{-1}\). The DD of the chitosan samples was calculated using the baseline of Baxter et al.\(^ {27}\). The computation equation for the baseline is

\[
DD = 100 - \left( \frac{A_{1655}}{A_{3450}} \times 115 \right)
\]

where \(A_{1655}\) and \(A_{3450}\) are the absorbance at 1655 cm\(^{-1}\) of the amide-I band as a measure of the \(N\)-acetyl group content and 3450 cm\(^{-1}\) of the hydroxyl band as an internal standard to correct for differences in chitosan.

DSC and TGA of irradiated chitosan

A differential scanning calorimeter (DSC-60, Shimadzu Corp., Japan) was used to determine the thermal behaviour of irradiated chitosan. The samples were scanned from 30 to 500 °C at a rate of 10 °C min\(^{-1}\). The flow rate of nitrogen gas was 20 mL min\(^{-1}\). Thermograms of non-irradiated and irradiated chitosans were obtained. TGA of the chitosan was also carried out using a thermogravimetric analyser (model TGA-50, Shimadzu, Japan) within the temperature range 30–600 °C.

Morphological analysis of chitosan using SEM

The surface morphology of chitosan was investigated using a JSM-6490 (JEOL-JSM-6490LA, Japan) high-resolution SEM instrument at 3.0 nm and an accelerating voltage of 10 kV. Freeze-dried samples of non-irradiated chitosan, and chitosan irradiated at 20 and 50 kGy were examined using SEM.

Determination of WBC and FBC of chitosan

The WBC of chitosan was measured using a modified method described by Wang and Kinsella.\(^ {28}\) WBC was initially determined by weighing a centrifuge tube containing 0.5 g of sample, adding 10 mL of water, and thoroughly mixing in a vortex mixer (VM-2000, Digsystem Laboratory Instruments Inc., Taiwan) for 1 min. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s and then centrifuged (Z383K, HERMLE-Labortechnik, Germany). The supernatant was decanted, the tube was weighed again. WBC was calculated using following equation:

\[
WBC (\%) = \frac{\text{Water bound (g)}}{\text{Initial sample weight (g)}} \times 100
\]

The FBC of chitosan was also measured using the same method as described by Wang and Kinsella.\(^ {27}\) The method was similar to that for WBC determination, except soybean oil was used instead of water for FBC measurement:

\[
FBC (\%) = \frac{\text{Fat bound (g)}}{\text{Initial sample weight (g)}} \times 100
\]

Antimicrobial activity of irradiated chitosan

The antimicrobial activity of chitosan was determined for Bacillus sp. following the standard method.\(^ {29}\) A saline solution of chitosan sample was incubated at 37 °C for 72 h on a medium of nutrient broth and agar powder. After 24 h and after 72 h, the colonies were marked off with a felt-tipped pen on the outer surface of the plate and counted.

RESULTS AND DISCUSSION

Effect of \(\gamma\)-radiation on prawn shell for extraction of chitosan

Chitosan is generally characterized by its extent of \(N\)-acetylation, which affects not only its physicochemical characteristics, chain morphology and molecular weight but also its biomedical applications, biodegradability and immunological activity.\(^ {30,31}\) The DD of chitosans (produced from both non-irradiated and irradiated prawn shell) was determined using an FTIR spectroscopic method with slight modifications as mentioned by Sabnis and Block.\(^ {26}\)

The results (Table 1) reveal that the DD of chitosan increases gradually with increasing radiation dose on prawn shell. At higher radiation doses the DD of chitosan is significantly higher than that of non-irradiated samples. Furthermore, the heating time in alkaline solution during deacetylation also influences the DD. The increase in DD on irradiation is interpreted as a result of a reduction in molecular weight and decrease in crystallinity of chitin which provides the possibility of \(N\)-deacetylation of chitin into chitosan under mild reaction conditions. The DD of chitosan obtained from prawn shell \(\gamma\)-irradiated at 50 kGy and heated for 4 h in alkali solution was 84.56%, thus indicating the superior quality of the chitosan.

Effect of \(\gamma\)-irradiation on the properties of chitosan

Polysaccharides, including chitosan, are typical degradable materials due to ionizing radiation.\(^ {32,33}\) The effect of \(\gamma\)-radiation (2–100 kGy) on the physicochemical, biological and thermal properties of chitosan were studied.

Effect on molecular weight of chitosan

The viscosity-average molecular weight of chitosan was determined using a viscometric method through the Mark–Houwink relationship:

\[
\text{Viscosity-average molecular weight} = K [\eta] ^{a}
\]

where \(K\) and \(a\) are the Mark–Houwink constants. The values of \(K\) and \(a\) were determined using a Ubbelohde type viscometer at 30 °C. The dependence of the viscosity-average molecular weight of chitosan on \(\gamma\)-irradiation is presented in Table 1.
Chitosan from γ-irradiation of prawn shell

Figure 1. Variation of molecular weight of chitosan with irradiation dose.

The intrinsic viscosity and molecular weight of non-irradiated chitosan are 144.8 mL g⁻¹ and 187 128.43 g mol⁻¹, respectively. However, irradiation causes a significant decrease of both intrinsic viscosity and molecular weight of the chitosan. Figure 1 shows the decrease in molecular weight of chitosan with increasing radiation dose. The decrease of molecular weight occurs due to the chain scission of chitosan molecules at glycosidic linkages under high-energy ionizing γ-radiation.22 Fig. 1 also reveals that a significant decrease in molecular weight takes place from 2 to 20 kGy. A further increase in radiation intensity has an insignificant effect on molecular weight as only a small change in molecular weight is observed.

Effect on DD of chitosan

Table 2. Determination of DD of irradiated chitosan

<table>
<thead>
<tr>
<th>Irradiation dose on chitosan (kGy)</th>
<th>Absorbance at 1655 cm⁻¹</th>
<th>Absorbance at 3450 cm⁻¹</th>
<th>DD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0614</td>
<td>0.2696</td>
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<tr>
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<td>0.2542</td>
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</tr>
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<td>78.88</td>
</tr>
<tr>
<td>50</td>
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<td>79.50</td>
</tr>
<tr>
<td>100</td>
<td>0.0215</td>
<td>0.1235</td>
<td>79.99</td>
</tr>
</tbody>
</table>

Effect on thermal stability of chitosan

DSC scans of chitosan samples were obtained to compare the thermal stability and miscibility of chitosan before and after irradiation. A comparison of the thermograms (Fig. 3) of irradiated chitosan reveals that the exothermic peaks (decomposition temperature) within the region 290–310 °C slightly shifts to the left with increasing radiation dose, i.e. the decomposition temperature decreases with increasing radiation dose. Similar trends are observed for the glass transition temperature (endothermic peak in the middle) within the region 230–260 °C and water removal temperature (broad endothermic peak) within the region 70–90 °C. The changes are more visible in the case of glass transition temperature and decomposition temperature than in the case of water removal temperature. The degradation of absorbance at 1655 cm⁻¹ ($A_{1655}$; due to stretching of C=O of amide groups) to absorbance at 3450 cm⁻¹ ($A_{3450}$; due to O–H stretching overlapping N–H stretching) decreases with increasing irradiation dose. This confirms the increase of DD of chitosan as described by Baxter et al.27 The detailed results for the DD of chitosan as a function of irradiation dose are summarized in Table 2. We observe that DD increases with increasing radiation dose. The increase of DD due to irradiation can be explained by the fact that hydrolysis of acetamide to amine occurs in the presence of bound moisture caused by ionizing radiation, and thus DD is increased.34

Effect on DD of chitosan

Figure 2 shows significant differences in the FTIR spectra of non-irradiated and irradiated chitosan at 3450–3500 and 1650–1655 cm⁻¹. From the spectra, it is noticed that the ratio of absorbance at 1655 cm⁻¹ ($A_{1655}$) to absorbance at 3450 cm⁻¹ ($A_{3450}$) decreases with increasing irradiation dose. This confirms the increase of DD of chitosan as described by Baxter et al.27 The detailed results for the DD of chitosan as a function of irradiation dose are summarized in Table 2. We observe that DD increases with increasing radiation dose. The increase of DD due to irradiation can be explained by the fact that hydrolysis of acetamide to amine occurs in the presence of bound moisture caused by ionizing radiation, and thus DD is increased.34

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chitosan molecules by $\gamma$-radiation may be responsible for such effects on the thermal properties. It is found that low-molecular-weight chitosan degrades at lower temperature than high-molecular-weight chitosan. Furthermore the degree of crystallinity of chitosan also decreases which contributes to a decrease in the glass transition temperature of chitosan.$^{35–39}$

TGA of the chitosan samples was also carried out within the temperature range 30–600 $^\circ$C. The thermograms show a weight loss at 40–150 $^\circ$C due to moisture vaporization. The other weight loss at 200–300 $^\circ$C is due to the degradation of chitosan. In the degradation zone, the peak at a temperature of 255 $^\circ$C indicates the glass transition temperature of the chitosan.

Effect on morphology of chitosan
Surface morphologies of non-irradiated and irradiated chitosan were studied using SEM. The images are shown in Fig. 4. It can be seen that the non-irradiated chitosan particles have a uniform network and are agglomerated. After irradiation at 100 kGy the chitosan chains are ruptured and irregular arrangements of the molecules are observed. The figure also reveals that irradiation causes the chitosan molecules to degrade more severely and the surface structure of the chitosan particles is observed as blistered and scattered. A comparison of the micrographs reveals that chitosan molecules are degraded with the application of $\gamma$-radiation.

Effect on WBC and FBC of chitosan
The WBC of chitosan was measured and the results are presented in Fig. 5. The WBC for non-irradiated chitosan is 627.32%; however, $\gamma$-irradiation at 2 and 100 kGy leads to WBC of 837.98 and 1210.28%, respectively. The WBC is generally involved with the molecular weight, DD and degree of crystallinity of chitosan. The lower the molecular weight, the higher the WBC; the higher the DD, the higher the WBC; the lower the crystallinity, the higher the WBC; all these facts mean that the WBC increases with increasing radiation dose.$^{40,41}$ Moreover the surface area increases due to the decomposition of chitosan; as a result, the area for binding with the $–$OH groups, $–$NH$_2$ groups and end groups also increases. The increased DD due to $\gamma$-irradiation provides more $–$NH$_2$ groups to bind water and the decrease in crystallinity increases the penetration of the water molecules.$^{40}$

The FBC of chitosan is also shown in Fig. 5. The results show a similar pattern to that of WBC. In addition it depends mainly on the DD. The fat molecules are glycerides of fatty acid bound with the amine group of the chitosan, and one molecule of chitosan...
can absorb 8 to 10 times its weight of fat molecules.\textsuperscript{42} We studied the effect of radiation on the FBC of chitosan with soybean oil, worldwide the most popular edible oil. The FBC of non-irradiated chitosan is 574.03%; however, chitosan irradiated at 100 kGy has an FBC of 1573.35%. The increase in DD with increasing radiation dose causes such a substantial increase of FBC.

Effect on antimicrobial activity of chitosan
Antimicrobial tests of irradiated chitosan show a zero count for \textit{Bacillus} sp. on appropriate media after incubation at 37°C for 72 h, whereas the control (non-irradiated chitosan) shows 8 counts (Fig. 6). This reveals that the irradiated chitosan exhibits strong microbial growth inhibition properties. Several studies have shown that the biological activity of chitosan depends on its molecular weight and degree of acetylation. Both parameters affect the antimicrobial activity of chitosan independently, though it has been suggested that the influence of the molecular weight on the antimicrobial activity is greater than that of the degree of acetylation.\textsuperscript{43} Lower molecular weight chitosan shows better results against microbial growth while the antimicrobial effectiveness of chitosan is improved as the degree of acetylation is decreased or DD is increased.\textsuperscript{44–047}

CONCLUSIONS

The high cost of extraction of chitosan can be minimized by irradiating the starting raw materials, e.g. prawn shell, by γ-radiation. Chitosan with higher DD can be prepared by applying γ-radiation, thus minimizing the use of concentrated NaOH solution and heating time during the deacetylation process. It was also confirmed by this study that the molecular weight and DD of chitosan decrease with increasing radiation dose. The molecular weight of chitosan can be minimized below 64 000 g mol\textsuperscript{-1} when it is subjected to an irradiation dose of 100 kGy. In addition, γ-irradiation of chitosan leads to an improvement of various properties such as solubility, WBC, FBC and antimicrobial activity. All of these properties provide good potentiality of chitosan for many applications in a wide range of fields like plant growth promotion, fat reduction, antifungal applications, water purification, desalination of seawater in coastal regions, wound dressing, drug delivery systems, etc.

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REFERENCES


Figure 6. Microbial test plates after 24 h of incubation for chitosan irradiated at (a) 2 kGy, (b) 5 kGy, (c) 30 kGy, (d) 50 kGy and (e) 100 kGy; (f) non-irradiated chitosan.