Preparation of Crosslinked Chitosan by Electron Beam Irradiation in the Presence of CCl₄

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ABSTRACT: In this article, we report the synthesis of crosslinked chitosan using 8 MeV electron beam (EB) irradiation in the presence of carbon tetrachloride. The crosslinked chitosan is characterized by dissolution, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), differential scanning colorimetry (DSC), and nanoindentation studies. The insolubility of irradiated films in acetic acid indicates that chitosan has undergone crosslinking reaction. FTIR analysis also confirms the crosslinked structure of chitosan. Mechanical properties such as elastic modulus and hardness are calculated from the nanoindentation data. Modulus and hardness of chitosan increase with increase in the irradiation dose due to the increase in the crosslinking. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 1063–1068, 2009

Key words: electron beam irradiation; crosslinking; chitosan; carbon tetrachloride; nanoindentation

INTRODUCTION

Chitin is a natural polysaccharide composed mainly of β-(1,4) linked 2-deoxy-2-acetamido-D-glucopyranose and partially of β-(1,4) linked 2-deoxy-2-amino-D-glucopyranose[1–5] shown in Figure 1(A). It is usually obtained from the shells of shellfish, crab, lobster, or shrimp. The limited solubility of chitin in common solvents limits its commercial applications. However its deacetylated form, chitosan [See Fig. 1(B)], has attracted attention because of several important advantages such as biocompatibility, biodegradability, high mechanical strength, nontoxicity, and availability in large amount.[1,2] Solubility of chitosan in acids is due to the presence of amino group. The solubility of chitosan in acid medium has several advantages for processing in various commercial applications, such as applications in medicine, cosmetics, textile, paper, food, and many other industrial branches.[6] However, for applications such as actuators and effluent treatment, it is necessary to crosslink chitosan to make it insoluble in the entire pH range. It can be chemically crosslinked by an aqueous solution of glutaraldehyde, chlorohydrin, etc.[5]

Ionizing radiation is highly effective in the modification of polymers.[7] Irradiation-induced modifications in the polysaccharide material depends on the polymer, parameters of irradiation, and state of material under irradiation. The final properties of irradiated polymer is determined mainly by two reactions: (i) main chain scission, leading to reduction in molecular weight of macromolecules and (ii) crosslinking, the opposite process to degradation, resulting in the formation of macroscopic insoluble material (gel).[8] Chemical modifications of polysaccharide has been widely studied, but only a very few reports on radiation crosslinking of polysaccharides are available. Radiation crosslinking of polysaccharide has been reported by Yoshii et al. for the first time.[9] They showed that water-soluble polysaccharides derivatives, such as carboxymethylcellulose (CMC), carboxymethylstarch (CMS), carboxymethylchitin (CMCT), and carboxymethylchitosan (CMCTS), lead to radiation crosslinking at high concentrated aqueous solution. Recently γ-ray induced crosslinking of chitosan in the powder form in the presence of CCl₄ has been reported by Ramnani et al.[4] In this article, we report the crosslinking of chitosan films using EB radiation and the results are compared with gamma crosslinked chitosan.
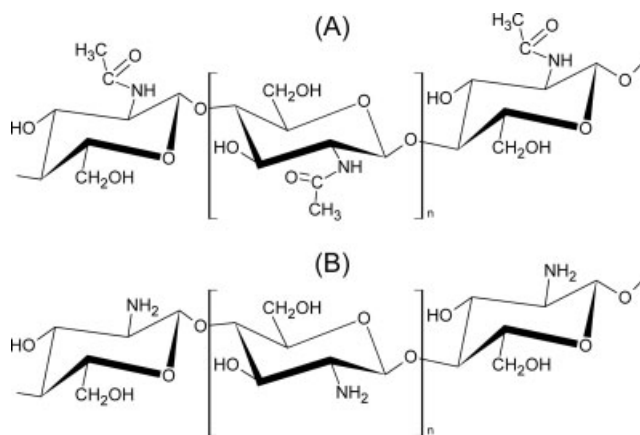
Figure 1 Structure of (A) chitin and (B) chitosan.

EXPERIMENTAL

Materials and chemicals

Chitin from HIMEDIA, Mumbai, with molecular weight 400,000 g/mol, CCl_4 and Methanol (AR grade) from SRL (Mumbai, India).

Synthesis of crosslinked chitosan film

Chitin is converted into chitosan by alkali hydrolysis using 50% NaOH. So obtained chitosan is 87% deacetylated, and viscosity average molecular weight is found to be 3.76×10^5 g/mol. Films of chitosan were cast on glass plate using 4% chitosan solution in 2% acetic acid and dried at 40°C. The films were peeled off and treated with 10% NaOH solution. These films were washed with water until it is free from NaOH and then with acetone followed by drying at 50°C. The thickness of the film is found to be $\approx$ 300 μm. The chitosan films were soaked in a mixture of CCl_4, water, and methanol (5 : 35 : 65) overnight. These soaked films were irradiated with electron beam at Microtron center, Mangalore University, and with γ-ray at BARC Mumbai. Specifications of electron beam used for irradiation is as follows: beam energy 8 MeV, beam current 25–30 mA, pulse repetition rate 50 Hz, pulse width 2.5 μs, dose rate of 1 kGy/min (Fricke dosimetry), and sample is kept at a distance of 30 cm. Gamma irradiation was carried out using Co^{60} gamma chamber having radiation dose rate of 5 kGy/h determined using Fricke dosimetry. Films were taken out and first washed with methanol to remove any traces of CCl_4 and other radiolytic product formed during the irradiation, followed by washing with water. The resultant films were yellow in color and it was treated with NaOH to remove chlorine. So obtained brown-colored films were washed with water followed by acetone and dried at 50°C.

Physical characterization

Chitosan film was used for recording FTIR spectra in transmittance mode using PerkinElmer FTIR spectrophotometer. Dissolution studies were done using acetic acid. XRD measurements were carried out using a Bruker D8 Advance X-ray diffractometer. The angular range was from 10° to 30° (2θ). The operating voltage and current of the tube were kept at 40 kV and 20 mA, respectively, throughout the entire course of investigation. DSC analysis was carried out using SHIMODZ DSC-50. Analysis was carried out in inert nitrogen atmosphere and at a scanning rate of 10°/min. SEM photographs of cross section and surface of films coated with gold were taken using scanning electron microscope (Model: JEOL JSM 5800CV). Nanoindentation was carried out for chitosan films having thickness $\approx$ 300 μm, using Nanoindentation machine with 5 μm conical tip.

RESULTS AND DISCUSSION

When chitosan in solid state is irradiated with ionizing radiation, many free radicals are produced on the back bone of the chain. These radicals generated preferably on the position 1 and 4 of the 2-deoxy-D-glucose residue results in chain scission at the 1,4 glucosidic bond, thus leading to degradation. But when chitosan is exposed to ionizing radiation in the presence of CCl_4, following changes are noticed:

1. the irradiated chitosan films swell in 1% aqueous acetic acid solution in which chitosan otherwise dissolves.
2. the product formed is dark yellow compared to unirradiated chitosan.
3. After hydrolysis with 1% NaOH, it becomes brown in color and still it swells in 1% acetic acid.

The insolubility of irradiated films in 1% acetic acid solution indicates that either chitosan has undergone a crosslinking reaction or NH_2 groups of chitosan are involved in some interaction during irradiation. However, FTIR spectrum of crosslinked chitosan is similar to that of chitosan showing the presence of NH_2 group (Fig. 2). Further presence of NH_2 group is confirmed by the estimation of ionic capacity of chitosan samples. The ionic capacity of uncrosslinked and crosslinked chitosan is almost same for all samples (Table I). Hence second possibility is ruled out. It is well known that any polymer undergoing crosslinking reaction swells in the solvent in which, it otherwise dissolves. The dark yellow color of crosslinked chitosan suggests the incorporation of chlorine in crosslinked chitosan, as
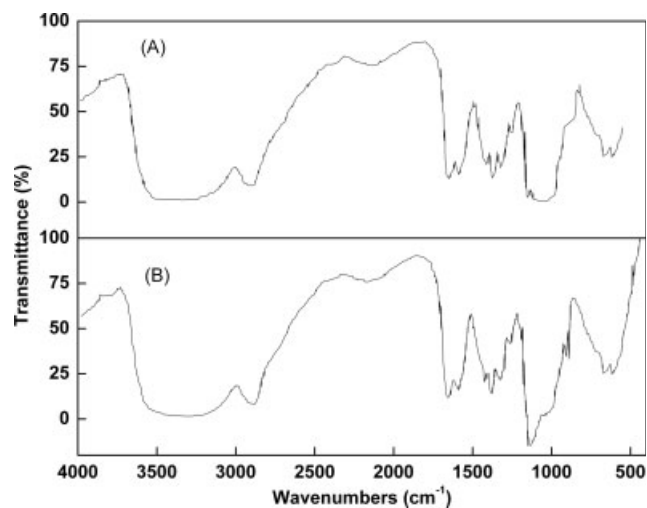
Figure 2 FTIR spectrum of (A) chitosan and (B) crosslinked chitosan.

organochloride compounds. Qualitative analysis also showed the presence of chlorine in crosslinked chitosan. When unhydrolyzed crosslinked chitosan was treated with 1% NaOH and filtrate was acidified with aqueous nitric acid, the filtrate gave white precipitate with silver nitrate, where as when the same procedure was repeated with uncrosslinked chitosan, did not give white precipitate with silver nitrate. This clearly indicates the incorporation of Cl in the crosslinked chitosan. The presence of chlorine also indicates the role of CCl_4 in the crosslinking of chitosan. Third observation, i.e. swelling of crosslinked chitosan in acetic acid even after hydrolyzing with 1% NaOH suggest that during hydrolysis chlorine atoms present in the crosslinked chitosan are removed and even after the removal of Cl atoms the crosslinking structure is retained.

Since all chains may not get crosslinked, there is always some soluble fraction known as sol and crosslinked portion known as gel. The percentage of sol fraction in the crosslinked chitosan can be determined by allowing a known weight of crosslinked chitosan to swell in 1% acetic acid solution by stirring the mixture for 24 h. During this process, soluble fraction goes in to solution and only the crosslinked portion remains insoluble. The resultant

TABLE I
Ionic Capacity of Different Chitosan Samples

Dose (kGy)	Ionic capacity (meq g^{-1})
0	4.75
25	4.76
50	4.71
70	4.80

TABLE II
Comparison of Gel Fraction of Gamma Ray and Electron Beam Crosslinked Chitosan

	Crosslinked fraction (%)	
Dose (kGy)	Gamma ray	Electron beam
25	16	29
50	55	67
70	62	76

gel was filtered and allowed to dry. Generally polysaccharides are prone to acid hydrolysis. To ensure this in the case of chitosan, drop in molecular weight with time is determined for uncrosslinked chitosan for 48 h. It is observed that there is no significant hydrolytic cleavage of chitosan.

The weight of dry gel was determined and percentage of sol was calculated from the following relation:

$$\%sol = [W_0 - W_1/W_0] \times 100 \tag{1}$$

where W_0 is the weight of chitosan before swelling and W_1 is the weight of dry gel. The percentage of gel fraction, which is the percentage of chitosan that has undergone crosslinking, was calculated using the following relation.

$$\%gel = 100 - \%sol \tag{2}$$

From the above relation, percentage of crosslinked fraction (gel fraction) of crosslinked chitosan irradiated with gamma ray and electron beam were calculated and are presented in Table II. From the table it is clear that for lower electron beam dose, the percentage of crosslinked portion is higher compared to gamma ray crosslinked chitosan. This may be due to the difference in the dose rate. During irradiation, chitosan undergoes crosslinking in the presence of CCl_4, but at the same time it undergoes oxidative degradation due to the free radicals produced. If we compare the dose rate of EB and γ-rays, EB delivers dose in seconds where as γ-rays takes several hours to deliver the same dose. This long residence time in the case of gamma rays leads to more oxidative degradation. Hence extent of crosslinking is less in the case of gamma ray crosslinked chitosan compared to electron beam crosslinked chitosan.

XRD and SEM studies were carried out for the crosslinked as well as uncrosslinked films. XRD pattern of chitosan shows characteristics peak at $\sim 2\theta = 20°$. The XRD pattern of chitosan and EB crosslinked chitosan are shown in the Figure 3. The results shows peak at $\sim 2\theta = 19.7°$ for both chitosan and for crosslinked chitosan, but intensity of peak for crosslinked chitosan decreases and gets broadened with irradiation dose. This clearly indicates that the crystalline structure of chitosan changes during
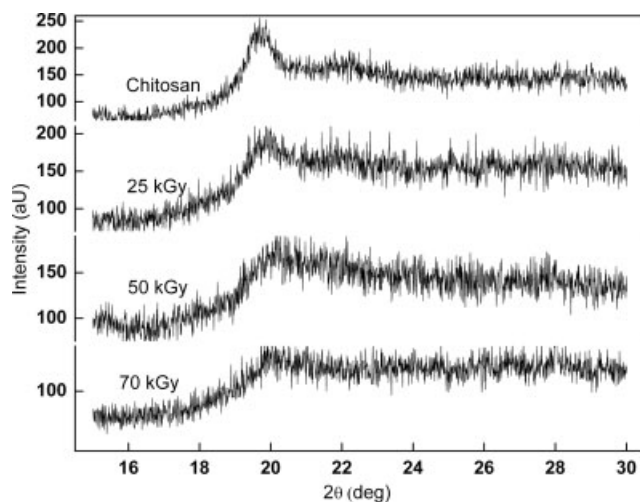
Figure 3 XRD of chitosan and crosslinked chitosan.

irradiation in the presence of methanol, CCl₄, and water mixture. This is further supported by SEM (Fig. 4) of irradiated chitosan samples. Surface and cross section view of uncrosslinked chitosan is shown in Figure 4(A,C), respectively, which shows very tightly bound structure with few voids. Surface and cross section view of crosslinked chitosan is shown in Figure 4(B,D), respectively. Crosslinked chitosan shows interlinked structure with more voids.

Figure 5 shows the DSC themograms of chitosan and crosslinked chitosan. Chitosan shows one endo-

thermic peak at 60°C, due to the presence of moisture in the sample and a small endothermic peak at ∼ 205°C, which can be attributed to T_g of chitosan. This peak vanishes in the case of crosslinked sample. In the case of chitosan, the endothermic peak corresponding to T_g is small due to the presence of the rigid 2-amino-2-deoxy-D-glucopyranose or glucosamine residue[10] and on crosslinking, motion of these chains at T_g is further hindered. So in the case of crosslinked chitosan peak corresponding T_g is not observed.

The mechanical properties of EB crosslinked chitosan were characterized by nanoindentation measurement. In the nanoindentation measurement, a pyramidal shaped diamond tip was progressively pressed onto the sample surface with a μN scale force, which initiated the loading cycle. After reaching the maximum indentation depth, the tip was gradually removed from the sample, which initiated the unloading cycle, leaving behind an indentation on the sample surface. The AFM image of this indent is shown in Figure 6(A).

Figure 6(B) shows the load versus displacement curve. The displacement decreases as the irradiation dose increases. It is due to the increase in crosslinking density. Elastic modulus and hardness of crosslinked chitosan are calculated from the data obtained from Figure 6. The apparent plastic hardness (H) is given by
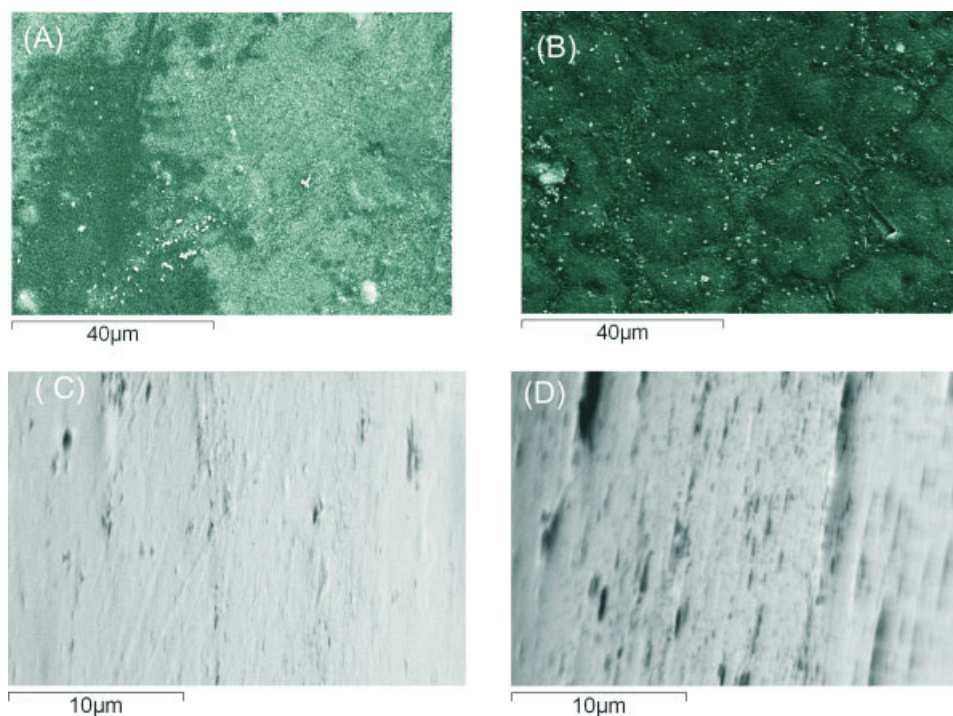
$$H = P_{max}/A \qquad (3)$$

Figure 4 SEM images of (A) chitosan (surface view), (B) crosslinked chitosan (surface view), (C) chitosan (cross-sectional view), and (D) crosslinked chitosan (cross-sectional view). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
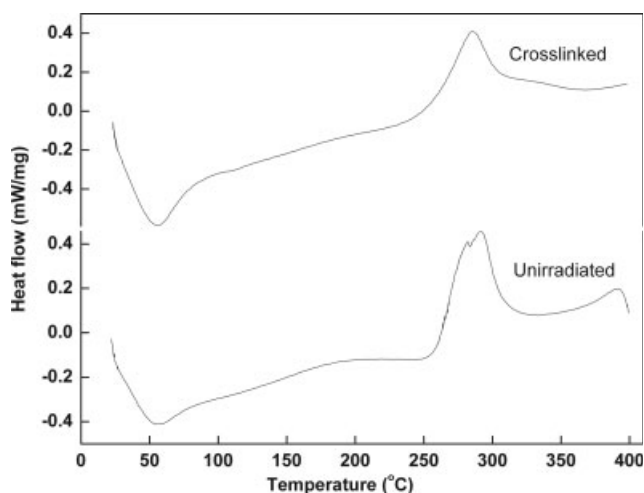
Figure 5 DSC thermograms of chitosan and crosslinked chitosan.

where P_{max} is the maximum load and A is the contact area, which is a function of displacement and is determined according to the following formula

$$A(h) = 24.5 \, h^2 \tag{4}$$

A constant value of 24.5 is used because it is assumed that a Berkovich indenter has a perfect tip.[11] The elastic modulus is also calculated from the unloading portion of the curves and it is given by

$$S = dP/dA \tag{5}$$

Calculated values of modulus and hardness is presented in the Table III. From the table it is clear that as the irradiation dose increases hardness and modulus of the polymer increases, which may be due to increase in the crosslinking fraction. But modulus and hardness values of gamma irradiated sample is less compared to electron beam irradiated sample, which may be due to the difference in the dose rate as explained earlier in the text.

Role of CCl_4 in the crosslinking of chitosan

When chitosan is irradiated with electron beam in the presence of mixed solvent 30 : 35 : 5 parts by weight of water, methanol, and CCl_4, respectively, energy is absorbed by both chitosan and solvent. The free radicals are produced on the back bone of chitosan, particularly at the radical sites on the 1 and 4 carbon atoms, which are responsible for scission of the 1,4 glycosidic bond, but in the presence of CCl_4, chitosan will react with $^\bullet CCl_3$ instead of undergoing chain scission, resulting in the attachment of carbon atom of $^\bullet CCl_3$ to the chitosan backbone. Irradiation effect of CCl_4 is well established in literature.[12] Radiolysis of CCl_4 results in the formation of $^\bullet CCl_3$ radicals by the following reaction:
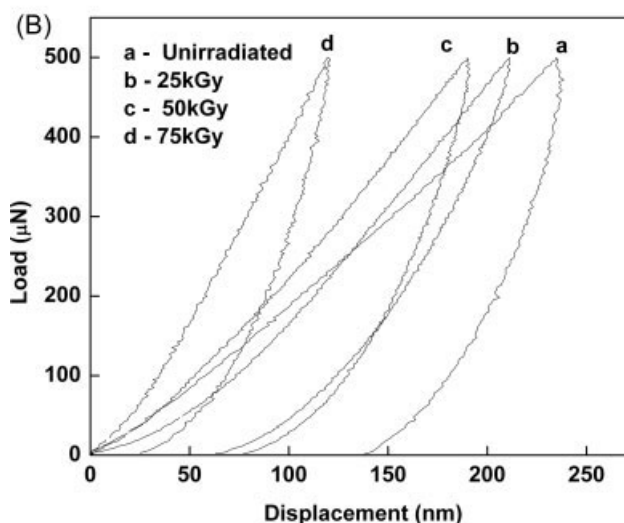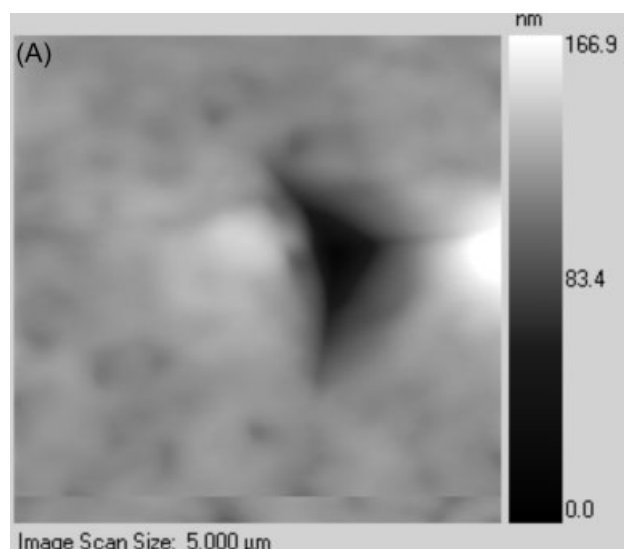

Figure 6 (A) AFM image after indentation (tip Berkovich). (B) Load versus displacement cures for different doses.

$$CCl_4 \xrightarrow{\text{Electron Beam}} {}^\bullet CCl_3 + Cl^\bullet \tag{6}$$

The C—Cl bond has lower dissociation energy than the C—H bond,[12] so CCl_4 forms more radicals upon irradiation than chitosan. The radical $^\bullet CCl_3$ formed may either react with the radicals formed on the chitosan backbone, resulting in the attachment of

TABLE III
Nanoindentation Experimental Results

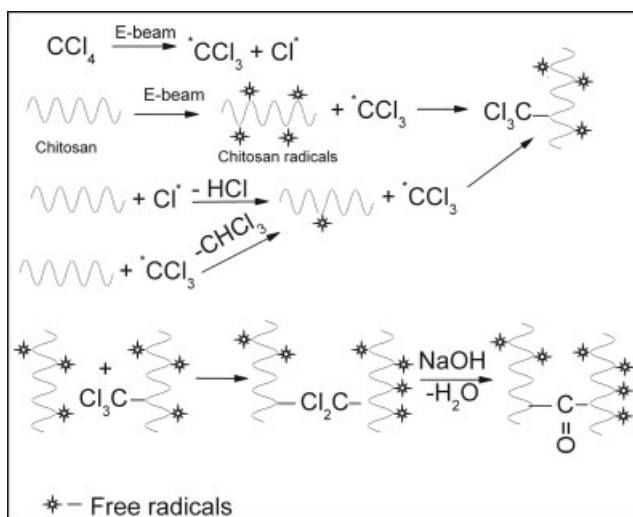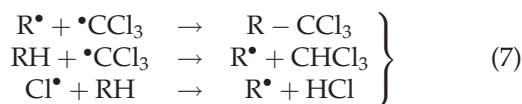| Dose (kGy)	Modulus (GPa)		Hardness (GPa)	
	EB	γ-rays	EB	γ-rays
0	4.82	4.82	0.19	0.19
25	4.93	4.86	0.28	0.22
50	5.36	5.17	0.34	0.27
70	7.85	6.68	0.37	0.31

Figure 7 Suggested crosslinking mechanism.

the $^\bullet CCl_3$ group to the chitosan chain, or it may react with chitosan and result in the formation of radical sites in the chitosan backbone. Also $Cl^\bullet$ radical reacts with chitosan to produce radicals in the chitosan backbone [See (7)].

$$\left. \begin{array}{rcl} R^\bullet + {}^\bullet CCl_3 & \rightarrow & R-CCl_3 \\ RH + {}^\bullet CCl_3 & \rightarrow & R^\bullet + CHCl_3 \\ Cl^\bullet + RH & \rightarrow & R^\bullet + HCl \end{array} \right\} \quad (7)$$

This $^\bullet CCl_3$ attached chitosan chain will react with adjacent chains having radicals, resulting in the formation of crosslinked chitosan structure [See (8)].

$$R-CCl_3 + R^\bullet \rightarrow R-CCl_2-R+Cl^\bullet \quad (8)$$

When crosslinked chitosan is treated with NaOH two Cl atoms of $-CCl_2-$ bridge is replaced by $-OH$ groups and two OH group on same carbon atom will be unstable and undergoes dehydration resulting in loss of water molecule, with formation of carbonyl group ($>C=O$). Hence the crosslinked structure is retained even after treatment with NaOH. All the above results are depicted in Figure 7.

CONCLUSIONS

Chitosan which normally undergoes degradation when exposed to EB irradiation can be crosslinked when irradiated in the presence of CCl_4. Ionic capacity measurements confirms the presence of NH_2 group, which is further supported by FTIR results. From the dissolution study it is seen that electron beam crosslinked chitosan has better crosslinked fraction compared to gamma ray crosslinked chitosan. From nanoindentation study it is found that mechanical properties of crosslinked chitosan are increased with irradiation dose due to the increase in crosslinking fraction. Electron beam irradiated sample shows better mechanical properties compared to gamma irradiated samples. Hence it can be concluded that electron beam is more efficient in crosslinking chitosan and has got better mechanical properties compared to gamma ray crosslinked chitosan.

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