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Radiation synthesis and characteristic of the hydrogels based on carboxymethylated chitin derivatives

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Abstract

Carboxymethylchitin (CM-chitin) and its deacetylated product carboxymethylchitosan (CM-chitosan) were irradiated in aqueous solutions at various irradiation dose by high-energy radiation electronic accelerator in vacuum. Carboxymethylated chitin derivatives irradiated at paste-like conditions were found to introduce crosslinking structure. When the dosage of electron beam irradiation was 20 kGy or more, transparent hydrogels could be produced. In the case of CM-chitosan, high degree of deacetylation was found to negatively correlate to crosslinking even if it has a high degree of substitution. The hydrogels created from carboxymethylated chitin derivatives, exhibited excellent mechanical properties and good swelling in water. The further study on the swelling properties at various conditions indicated that CM-chitin and CM-chitosan hydrogels displayed characteristically pH-sensitive character in their swelling behavior. The gel of CM-chitosan with a high DS (0.91) swelled in acid ($pH < 3.5$) and alkaline ($pH > 6$) conditions and deswelled between pH 3.5 and 6.0 due to the ionic composition changes of the gel network. In addition, the antibacterial activity against *E. coli* was also found in CM-chitosan hydrogel. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Carboxymethylchitin; Carboxymethylchitosan; Irradiation; Hydrogels; Crosslinking; pH-sensitive; Antibacterial activity

1. Introduction

Chitin with its deacetylated product (chitosan) is one of the most abundant natural amino polysaccharides. Chitin and chitosan have a variety of applications in industry pharmacy, and biotechnology (Majeti & Kumar, 2000). Recent years have seen extensive investigations on the utilization of chitin and its derivatives for developing drug carriers for transmucosal administration (Saiki, Watanabe, Tokura, & Azuma, 1992; Senel et al., 2000; Tokura et al., 1990). Chitin and its derivatives also find applications as wound dressings that have been proven to be effective in promoting wound healing (Winter, 1965). However, the poor solubility seriously limits the application of chitin and chitosan. Thus, water-soluble chitin and chitosan derivatives which are soluble in both acid and basic physiologic circumstances, might be good candidates for application. In their utilization, many shapes and material properties are required, such as hydrogels which no single type of chitin or chitin derivatives can provide satisfactorily.

Chemical modifications of chitin and chitosan have been studied widely (Blair, Guther, Law, & Turkington, 1987), but up to recent years only a few researches on their irradiation modification have been reported (Liu, Guan, Yang, Li, & Yao, 2001b; Liu, Zhai, & Wu, 2001b; Singh & Ray, 1998). No report has been found in the literature on radiation crosslinking of chitin derivatives. In previous work, we have applied irradiation to prepare hydrogels from cellulose, starch water-soluble derivatives at high concentrated paste-like condition (Fei, Wach, Mitomo, Yoshii, & Kume, 2000; Kume, Nagasawa, & Yoshii, 2002; Wach, Mitomo, Yoshii, & Kume, 2001). In this paper, novel hydrogels consisting of pure carboxymethylchitin (CM-chitin), carboxymethylchitosan (CM-chitosan) prepared by electron beam (EB) irradiation is described, and the characteristics of the prepared hydrogels are studied in detail.

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Table 1
Characterization of CM-chitin and CM-chitosan used in this study

Sample	DS	DDA (%)	Intrinsic viscosity (dl/g)	M_w
CM-chitin	0.83	31.4	2.32	3.06×10^{4a}
CM-chitosan	0.91	84.0	2.42	2.93×10^4

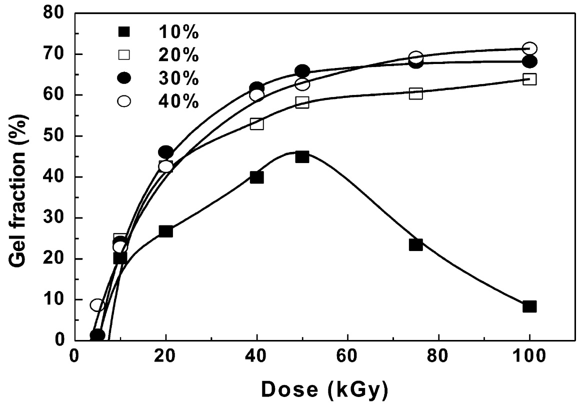
[a] Weight-average molecular weight of CM-chitosan was determined the same as the CM-chitin.

2. Experimental

2.1. Materials

Carboxymethylchitin (CM-chitin) and carboxymethylchitosan (CM-chitosan) used in this study were obtained from Koyou Chemical Industrial Co. Ltd, Japan. The degree of substitution (DS) of the CM-chitin, CM-chitosan was 0.83 and 0.91, respectively. The average molecular weight of the sample was estimated from intrinsic viscosity. Characteristic of these polymers is summarized in Table 1.

(a) CM-chitin



(b) CM-chitosan

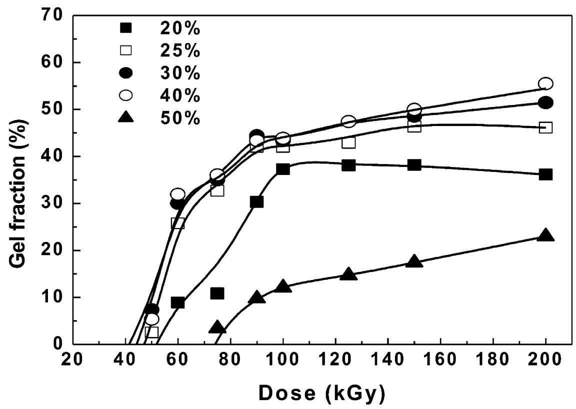


Fig. 1. (a) Effect of concentration on crosslinking of CM-chitin (irradiation in vacuum); (b) effect of concentration on crosslinking of CM-chitosan (irradiation in vacuum).

2.2. Sample preparation and irradiation

Deionized water was added to the polymer, and then the material was mixed until homogenous. For high concentrations, i.e. above 10%, due to its high viscosity, the polymer was kneaded well with water by a glass bar. To prevent penetration of air to the sample during irradiation the mixture was heat-sealed in a poly (vinylidene chloride) bag, after removal of the air by a vacuum machine. Irradiation of samples was done with EB generated from the accelerator that was used at the following irradiation parameters: current = 1 mA, voltage = 2 MeV, and dose per pass = 1 kGy.

2.3. Gel fraction and swelling of hydrogel

After irradiation, the CM-chitin, CM-chitosan mixed with water was dried by lyophilization. Then the gel content in the dried crosslinked sample was estimated by measuring its insoluble part after extraction in distilled water for 48 h at room temperature. The gel fraction was calculated according to the following equation

$$\text{Gel fraction}(\%) = (G_d/G_i)100 \qquad (1)$$

where G_i is the initial weight of dried sample after lyophilization and G_d is the weight of the insoluble part after extraction with water.

The swelling of crosslinked CM-chitin and CM-chitosan was estimated according to Japan Industrial Standard (JIS) K8150. The dry gel was immersed in distilled water for 48 h at room temperature. After swelling, the hydrogel was filtered by a stainless-sheet net of 30 mesh. Swelling was calculated as follows

$$\text{Swelling} = (G_s - G_i)/G_i \qquad (2)$$

where G_s is the weight of hydrogel in swollen state.

Effect of some factors such as acid, base, as well as inorganic salt on swelling behavior of the hydrogels also were performed by determining the swelling ratio of CM-chitin, CM-chitosan hydrogels at above condition for 48 h.

2.4. Mechanical properties

In order to estimate the mechanical properties of crosslinked material, hydrogels were examined in relaxed state. The hydrogels were cut into dumbbell (ASTM D-1822-L), and then tensile strength and elongation at break were measured by Strograph-R1 Material Tester (Toyoseiki Co. Ltd, Japan) with a crosshead speed of 50 mm/min. At least five measurements for each sample were recorded and a mean value was calculated.

2.5. Antibacterial assessment

Antibacterial activity of the CM-chitosan hydrogels against *E. coli* B/r were evaluated by using the optical

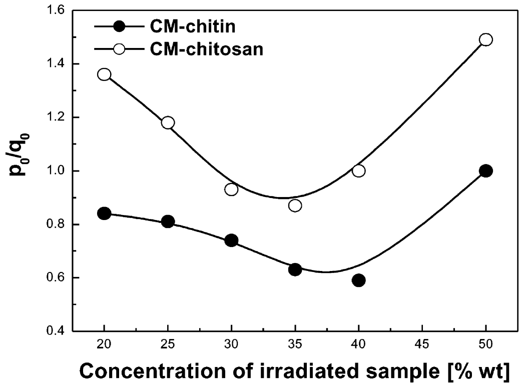
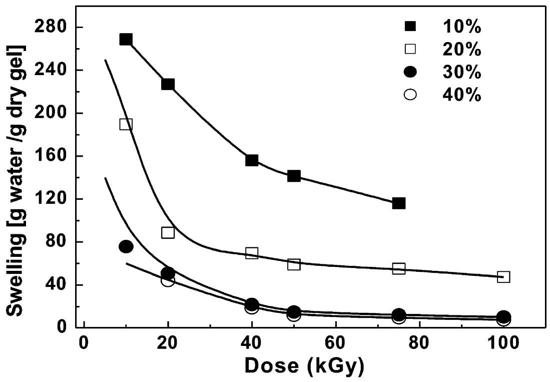


Fig. 2. p_0/q_0 ratio of CM-chitin and CM-chitosan after irradiation.

density (OD) method. 0.02 g dry CM-chitosan hydrogel film was added into 5 ml medium (Nutrient broth from Difico, Michigan, USA) including two percent *E. coli* B/r culture where *E. coli* B/r grew in a logarithmic growth phase and then incubated at 37 °C for 48 h. During the incubation, the turbidity of the medium was measured at 650 nm every 2 h. All of the operation was done in aseptic condition or by aseptic technique.

(a) CM-chitin



(b). CM-chitosan

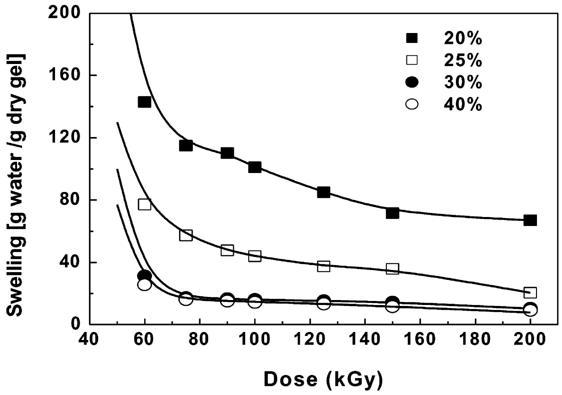


Fig. 3. Swelling of hydrogels of CM-chitin and CM-chitosan in distilled water. (a) The swelling of hydrogel formed from CM-chitin; (b) the swelling of hydrogel formed from CM-chitosan.

3. Results and discussion

3.1. Synthesis of hydrogel

Crosslinking successfully competes with glycoside bone cleavage as the concentration of polymer in a solution oversteps some critical values. CM-chitin and CM-chitosan undergo degradation at solid state and diluted solution (below 10%). Crosslinking is observed only for irradiation at paste-like state of high concentration.

The effects of the concentration of CM-chitin, CM-chitosan in aqueous solution on the gel fraction are shown in Fig. 1. Plots representing both samples indicate that the gel usually degrade in solid phase or in a diluted aqueous solution by irradiation. Gel fraction rises sharply just after exceeding the gelation point and levels off later. A high concentration in an aqueous solution is favorable for crosslinking. Fig. 1(a) shows the gel fraction of CM-chitin against delivered dose at high concentration (10–40%). The gel fraction rises till 60–70%. The gel fraction obtained in 10% aqueous solution is lower than those of higher concentrations, and when irradiation dose exceeds 75 kGy, the gel fraction decreases due to the degradation. Concentration of 30–40% is preferable to form gels of CM-chitin. A similar situation is observed for CM-chitosan, as shown in Fig. 1(b). In the case of CM-chitosan, the gelation occurred in the condition that is above 10% in concentration and at least 50 kGy dose. The gel fraction rises till 50–60%, and maximum gel fraction was achieved at concentration from 25 to 35%. In a high concentration, such as 50%, low gel fraction was obtained due to the heterogeneous state with water.

It is known that the presence of water enhances the mobility of the rigid molecules of polymer allowing macroradicals to recombine each other, and moreover the products of water radiolysis can induce more macroradicals. Hence the presence of water enhances the yield of macroradicals. So from practical point of view, the polymer should be homogeneously mixed with water to prepare homogeneous sample. However, for a higher concentration (i.e. 50%), it is difficult to obtain homogenously dispersed polymer in the whole volume of sample.

It can be seen that crosslinking of CM-chitosan was more difficult to occur than CM-chitin in the same condition. This was confirmed by calculating the value of p_0/q_0 according to Charlesky–Rosiak equation (Olejniczak, Rosiak, & Charlesby, 1991). Fig. 2 shows the data concerning the ratio of degradation to crosslinking density (p_0/q_0) versus the concentration of CM-chitin and CM-chitosan. p_0/q_0 is the parameter that allows the determination of the final results of irradiation and is equal to half of the scission yield/crosslinking yield ratio ($0.5 \times G_{(S)}/G_{(x)}$). If p_0/q_0 is lower, crosslinking occurs more efficiently. It was found in the case of CM-chitosan that higher degree of deacetylation (DDA) negatively correlates to the ability to crosslinking. Chemically, CM-chitosan with a high DDA, above 50% is

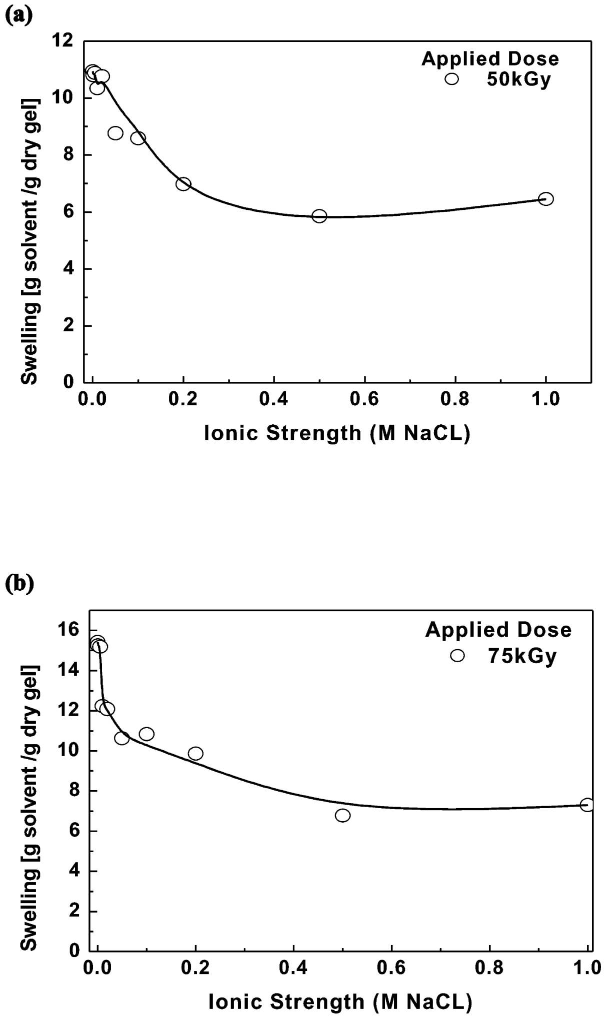


Fig. 4. (a) Swelling of a CM-chitin hydrogel (prepared from 30% aqueous solution at 50 kGy) in NaCl aqueous solutions of various ionic strengths; (b) swelling of a CM-chitosan hydrogel (prepared from 30% aqueous solution at 75 kGy) in NaCl aqueous solutions of various ionic strengths.

similar to CM-chitin, containing a strong ionic interaction due to its polyamphoteric property. We can assume that acid groups interact with the amino groups both intra- and inter-molecularly through ionic interaction. And this ionic interaction may limit the mobility of polymer chain to get close to each other for recombining, like rigid molecules. Unlike carboxymethylcellulose, CM-chitin and CM-chitosan containing two different functional groups give a more complicated effect on crosslinking by irradiation.

On the other hand, the two different functional groups also add more characteristics such as antibacterial activity for application. Therefore the effect of amino group and carboxymethyl group on the crosslinking behavior is worthy of further investigation.

3.2. Swelling of hydrogel

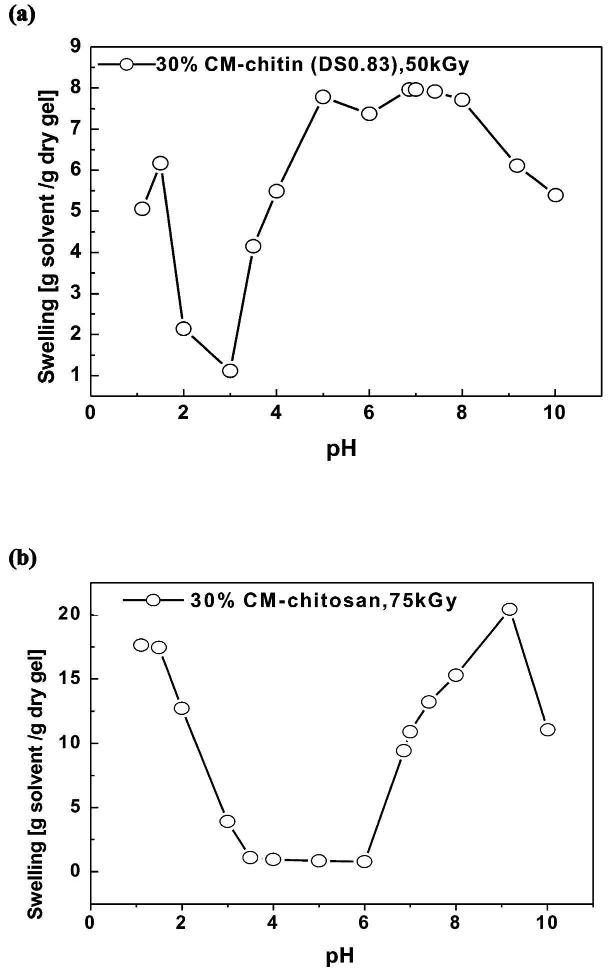


Fig. 5. (a) Swelling of a CM-chitin hydrogel (prepared from 30% aqueous solution at 50 kGy) in aqueous solutions of several pH values; (b) swelling of a CM-chitosan hydrogel (prepared from 30% aqueous solution at 75 kGy) in aqueous solutions of several pH values.

The equilibrium swelling of hydrogels is a result of the balance of osmotic forces determined by the affinity to the solvent and the network elasticity. Fig. 3 shows the swelling of the hydrogels of CM-chitin and CM-chitosan. It can be seen that the degree of swelling is controlled by changing the crosslinking density in the network. The degree of swelling decreases with an increasing dose in each concentrations, and the gel made from low concentration aqueous solution shows higher swelling ability in water than that made from high concentration solution. This is because of the maximum crosslinking density of the hydrogels prepared from 30 to 40% aqueous solution (Fig. 2). As usually swelling is highest just after the gelation dose and decreases with increasing absorbed energy at the early stages of gel formation; then the decreasing becomes slower but still observable. The swelling corresponds well to the crosslinking behavior. In this study, the hydrogel made from the CM-chitin shows the maximum swelling ratio of 270, and the gel from CM-chitosan shows the maximum swelling ratio of 134, respectively.

The effects of inorganic salt on the swelling of CM-chitin (conc. 30%, 50 kGy) and CM-chitosan hydrogel (conc. 30%, 75 kGy) are shown in Fig. 4. Because CM-chitin and

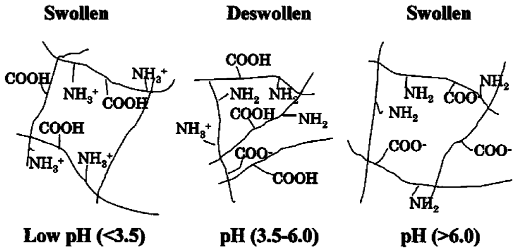


Fig. 6. Hypothetical sketch of swelling–deswelling molecular structure of CM-chitosan hydrogel in aqueous solutions at <3.5, 3.5–6.0, and >6.0 pH region.

CM-chitosan were polyelectrolyte polymer, swelling of the gels showed a typical behavior of polyelectrolyte under different ionic strength of solution. The increase of ionic strength in the solution led to an increase of the osmotic pressure of the solution ions and a decrease of the excess osmotic pressure of the network ions resulted in deswelling of the gel.

It is well known that swelling or deswelling in response to changes of pH is a typical phenomena of polyelectrolyte gels. In this study, a series pH buffer solution was prepared with a total ionic strength varied from 0.05 to 0.1 M because no obvious ionic strength effect on the swelling was observed in this range (Fig. 4). The effect of pH value on the swelling of CM-chitin (conc. 30%, 50 kGy) and CM-chitosan hydrogel (conc. 30%, 75 kGy) are shown in Fig. 5. As shown in Fig. 5(b), the CM-chitosan hydrogels displayed characteristically pH-sensitive character in their swelling behavior. The CM-chitosan hydrogel swelled at low pH (<3.5) and also in the pH range 6.0 < pH < 11.0; however, deswelling occurred in the range of pH 3.5–6.0. Because CM-chitosan gel contains both carboxyl and amino groups, and thus forms a network with oppositely charged structures which could change the charge state of the ionic groups varying with pH. In the case of low pH (<3.5) the dominant charges in the gel are the protonated amino group; In the case of high pH (>6.0) the dominant charges in the gel are the unprotonated carboxyl group. In these pH regions, the CM-chitosan gel is swelled due to the increase in ionic swelling pressure (ionic repulsion > elastic force). If we can assume the gel having an isoelectric point (pI) like protein molecule, the pI should be in the pH region 3.5–6.0. Because at pH 3.5–6.0, most of the ionic groups are absent due to protonation of the carboxyl group and deprotonation of the amino group, and the gel thus deswelled in this region (repulsion < elastic force). The explanation can be schematically depicted in Fig. 6. Choi et al. reported the pH-sensitive hydrogels prepared from poly(glutamic acid) (PGA) and poly(lysine) (PL) by γ irradiation (Choi, Yang, & Kunioka, 1995). The swelling behavior of PGA/PL gel is similar to CM-chitosan gel due to the similar structure containing both carboxyl and amino groups. While the swelling behavior of CM-chitin hydrogel (Fig. 5(b)) containing more carboxyl group in composition is similar to the PGA/PL gel with high PGA ratio in mixture. These data are basically similar, and the only difference is that in the case of CM-chitosan and CM-chitin the two kinds of ionic group are in a pure system. The difference can offer a possibility for easily controlling the swelling properties in application. Comparing the swelling data of CM-chitin and CM-chitosan, we found that as the amino groups in composition were increased, a broad transition for curve of equilibrium swelling as a function of pH was observed. The pH sensitivity with a broad transition in CM-chitosan gels is believed to play an important role in biomedical application.

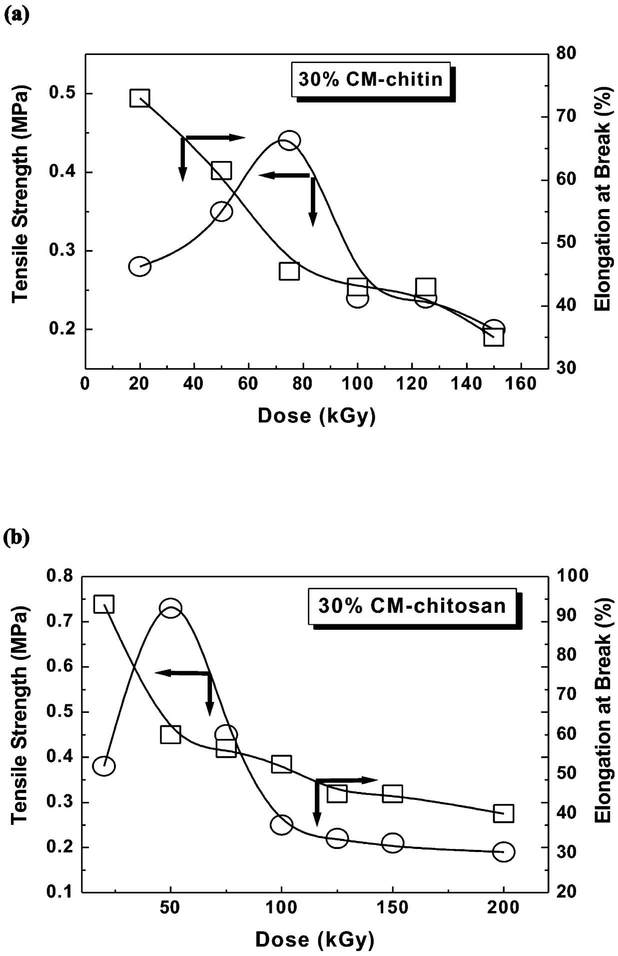


Fig. 7. (a) Tensile strength and elongation at break of CM-chitin hydrogel film (prepared from 30% aqueous solution at various doses) in relaxed conditions; (b) tensile strength and elongation at break of CM-chitosan hydrogel film (prepared from 30% aqueous solution at various doses) in relaxed conditions.

3.3. Mechanical properties of hydrogel

Wach et al. have already studied the mechanical properties of some pure polysaccharide derivatives hydrogels prepared by irradiation, such as hydroxypropylcellulose (HPC), carboxymethylcellulose (CMC) (Wach, 2002; Wach, Mitomo, Yoshii, & Kume, 2002) The relatively low mechanical strength was found in CMC gels due to polyelectrolyte characteristic comparing with HPC gels. It is

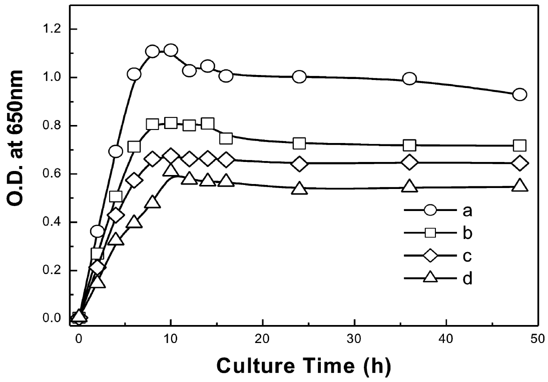


Fig. 8. OD versus culture time of medium (a), CM-chitosan gel without sol part (b), CM-chitosan gel (c), and CM-chitosan raw material against *E. coli* (CM-chitosan hydrogel was prepared from 40% aqueous solution at 75 kGy).

well known that CM-chitin and CM-chitosan are typical polyelectrolyte polymer. Therefore, from practical point of view, it is necessary to investigate the mechanical properties of CM-chitin and CM-chitosan hydrogels.

The effect of irradiation dose on mechanical properties of CM-chitin and CM-chitosan irradiated by EB in 30% concentration was discussed. Tensile strength and elongation at break of hydrogels samples in relaxed state (after irradiation with the original water content) are shown in Fig. 7. The tensile strength of both polymers was increased with absorbed dose at early stage, after reaching a maximum and then decreased again. This effect is due to the increase of crosslinking density when at early stage, and after reaching a maximum, higher absorbed doses led to degradation and destroy the network structure. Tensile strength of 0.45 MPa at a dose of 75 kGy was obtained in CM-chitin hydrogels, and 0.73 MPa tensile strength at a dose of 50 kGy was obtained in CM-chitosan hydrogels, respectively. Elongation at break of both polymers was decreased with increasing of absorbed dose. This is due to the irradiation-induced brittleness of hydrogel film. We noted that the highest elongation value was obtained in unirradiated state, and before irradiation the polymer samples still have certain tensile strength. It can be explained that CM-chitin and CM-chitosan have a strong ionic interaction in their network structures. This ionic interaction can enhance the mechanical properties in spite of the irradiation (Zhao, Kato, Fukumoto, & Nakamae, 2001). Comparing these data to the results obtained for HPC and CMC in same conditions, we can infer that carboxymethylated chitin derivatives are able to form hydrogels with good mechanical properties due to the ionic interaction in network structure. It seems that polyampholytic polymer is favorable for producing hydrogels with good mechanical properties.

3.4. Antibacterial activity of hydrogel

Hydrogels have been widely used for cosmetic and biomedical applications such as burns wound dressing, contact lens, artificial skin and in drug delivery system (Rosiak & Yoshii, 1999). However, hydrogels prepared for biomedical application, have to be sterilized or prevented from many microorganisms. Antibacterial activity will be the most valuable properties in the filed of biomedical application. Liu et al. reported antibacterial action of chitosan and CM-chitosan against *E. coli* (Liu et al., 2001a). It was found that the antibacterial activity is influenced by molecular weight. They noticed that chitosan and its carboxymethylated derivatives with molecular weight ranging from 5×10^3–9.16×10^4 would be helpful in restraining the growth of bacterial.

In this study, the antibacterial activity of CM-chitosan hydrogel with the huge network structure was checked by using OD method. Fig. 8 shows the OD versus the culture time for the CM-chitosan material, CM-chitosan gel with or without sol part against *E. coli*, respectively. All samples exhibit significant antibacterial activity. Antibacterial activity was found to increase in the order of the gel without sol part, the gel with sol part, raw materials. Form the result of Fig. 8, it can be seen that the gel prepared from CM-chitosan could provide satisfying antibacterial activity, even when the gel have only the gel part. The most interesting phenomenon in this study is that a huge crosslinking structure was found to exhibit antibacterial activity in spite of the limitation of molecular weight.

4. Conclusion

A series novel polyampholytic hydrogels of carboxymethylated chitin derivatives were synthesized by irradiation without any additives. High concentrated paste-like condition was favorable for crosslinking. The hydrogels produced from CM-chitin and CM-chitosan can provide satisfying properties such as pH-sensitivity in swelling behavior, good mechanical properties, etc. for application. Moreover, CM-chitosan hydrogel can provide satisfying antibacterial activity against *E. coli*. Thus, the CM-chitin and CM-chitosan hydrogels are expected to be useful as biomedical, agricultural, and cosmetic materials components.

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