Preparation of Crosslinked Carboxymethylcellulose (CMC) by ^{60}Co γ-ray Irradiation and Its Biodegradable Properties

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Abstract − Biodegradable and biocompatible carboxymethylcellulose (CMC) hydrogels for personal care products such as infants diapers and feminine hygiene products were prepared by a γ-irradiation crosslinking technique. Hydrogels were prepared as a function of the CMC concentration, total dose, and degree of substitution (DS), and their physical properties such as the gel fraction, swelling ratio, pH-responsibility and biodegradability were investigated. The irradiation of an aqueous CMC solution led to a gelation in an aqueous solution of more than 10 w%, and the gel percent increased as the CMC concentration, total dose and DS increased. The equilibrium swelling behaviors of the hydrogels prepared in various conditions were examined in an aqueous solution, and the pH-response at a pH of 1.2 and 6.8 was investigated. CMC hydrogels showed a high gelation at a high CMC concentration with DS 1.2. Lastly, the effects of the crosslinking degree of the CMC on the hydrolysis reaction were examined by cellulase from *Trichoderma reseei*. It was found that the degradable reaction depended on the degree of the crosslinking of the CMC. Intermolecular crosslinking reactions were confirmed by the ESR spectra.

Key words : Carboxylmethylcellulose, Crosslinking, Hydrogel, Biodegradability

INTRODUCTION

Cellulose is a renewable resource in nature, most of their derivatives being biodegradable and functional, so it is interesting and important to use them as feed stocks in the synthesis of an environmentally benign functional polymer. Sodium carboxymethyl cellulose is widely used in oral and topical pharmaceutical formulations because of its viscosity-increasing properties (Wade and Weller 1994). Viscous aqueous solutions are used to suspend powders intended for either topical oral or parenteral applications (Hussain *et al*. 1991).

It is an anionic linear polymer in which the original H atoms of the cellulose hydroxyl groups are replaced by a carboxymethyl substituent, -CH_2COO- (Fig. 1) (Baar *et al*. 1994). Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Peppas and Merril 1976a, b). To function as an absorbent for aqueous fluids, a polymer must have certain properties. It must be hydrophilic. The polymer must swell in aqueous fluids but must not dissolve. Although it is not a strict requirement, absorbents should have some ionic character, since a charge repulsion is an important factor in promoting polymer swelling in aqueous fluids. These polymers can be applied extensively as

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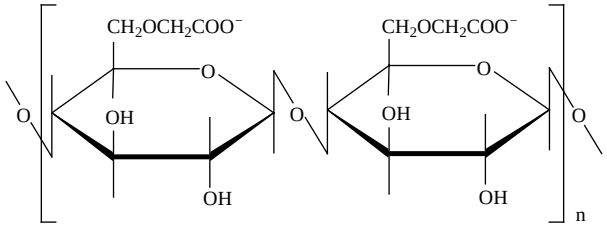

Fig. 1. Structure of the carboxymethylcellulose.

absorbents in personal care products such as infants diapers, feminine hygiene products, and incontinence products (Kinney and Scranton 1993). Currently, synthetic polymers such as polyacrylamide, polyacrylic acid and polymethacrylic acid have been used for the above purposes. But these polymers are not easily degraded in soil, and have a weak point which induces an allergy or stimulates the skin.

Therefore, many researchers have made every effort to prepare hydrogels by a natural polymer. Hydrogels can be fabricated by several ways (Rosiak and Ulanski 1999). Some attempts for a chemical crosslinking of CMC in order to obtain a hydrogel were conducted with divinylsulphone, glutaldehyde, and epichlorohydrin as a crosslinking agent (Anbergen and Oppermann 1990).

However, the remainder of the crosslinking agents have a harmful influence on the body. In this study, we investigated the radiation crosslinking of CMC as a function of the radiation dose, the CMC concentration and the degree of substitution. Additionally, the biodegradability of the CMC hydrogel by metabolite cellulase from *Trichoderma reseei* was examined as a function of the degree of the gel fraction. We studied the ESR spectra to investigate the concerned radicals in crosslinking reactions at 77 K and 243 K.

MATERIALS AND METHODS

1. Materials

Na-Carboxymethyl cellulose (DS value of CMC ranged from 0.7 to 1.2), obtained from the Aldrich Chem. Co. (MILWAUKEE WI, USA), was used without further purification. Molecular weight of the CMC was 2.5×10^5. Test cellulase (C-8546) for the biodegradability was supplied by the Sigma Co. (St. Louis, USA).

3, 5-Denitrosalicylic acid reagent (DNS reagent) was prepared in the laboratory to examine the reducing sugar (Miller 1959). Buffer solutions for the swelling experiment as a function of the pH were prepared by dissolving 2 g of sodium chloride and 7 ml of concentrated HCl in 1 L of distilled water (SGF, pH 1.2) and mixing 250 ml of 0.2 M KH_2PO_4 and 118 ml of 0.2 N NaOH (SIF, pH 6.8) (Somogyi 1952).

2. Methods

1) Preparation and swelling behavior of the CMC hydrogels

CMC hydrogels were prepared by radiation crosslinking in distilled water at room temperature (25°C). 10～40% CMC paste-like solutions were prepared by mixing 0.5～1 g of CMC in distilled water mechanically for 1 day. CMC paste-like solutions were packaged in polyethylene bags and N_2 was bubbled into the bags to eliminate the influence of O_2 on the crosslinking of the CMC. The samples were exposed to a gamma Co^{60} source (dose rate of 1.5 kGy h^{-1}) with various doses from 10 to 100 kGy.

The gel fraction of the crosslinked CMC was estimated by an extraction in the distilled water for 3days and being dried in a vacuum at 70°C for 48 h. The gel fraction was calculated by the following equation (a):

$$\text{Gel fraction}\,(\%) = W_d / W_i \times 100 \qquad \text{(a)}$$

where W_d is the weight of the dry gel after extraction and W_i is the initial weight of the polymer.

The measurement of the equilibrium swelling of the hydrogels was performed for 3days at room temperature to ensure an equilibrium.

Degree of swelling was calculated by the following equation (b):

$$\text{Degree of swelling}\,(\%) = (W_t - W_i) / W_i \times 100 \qquad \text{(b)}$$

where W_t is the weight of the swelling hydrogel at an equilibrium state and W_i is the initial weight of the dry gel.

2) Degree of the swelling ratio in aqueous solutions of various pHs

In order to estimate the pH-dependency of the CMC hydrogel, the CMC hydrogel was classified as the highest degree of swelling in distilled water. Swelling ratios were estimated at different times in SGF, and SIF buffers. Simi-

larly, the degree of swelling was calculated by (b).

3) Measurement of the biodegradability of the CMC hydrogels

CMC hydrogels were classified according to the degree of the gel fraction. A cellulase enzyme solution was prepared by adding a cellulase enzyme (2 mg) to 10 ml of a 0.1 M citrate buffer, at pH 4.8. An enzyme solution was divided into 2 ml in the 12 wells. 20 mg CMC hydrogels (CMC 30%, DS 0.9, gel fraction 63, 61, 52, 42%) were inserted into the 12 wells.

The mixture was incubated to 37°C in a shaker bath and stirred at 120 rpm for 2 to 3 days. Total reducing sugar was examined by the DNS method (Miller 1959). The reducing sugar was checked at λ_{max} 540 nm using a UV-VIS spectrophotometer (S-1100, SCINCO Co.).

4) ESR measurements

20% CMC paste-like solutions were prepared by dissolving the CMC in D_2O at room temperature for the trapped free radical measurement. These specimens were packed in ESR quartz tubes and N_2 was bubbled into the ESR tubes. The irradiation was conducted under a vacuum. The specimens were transferred into liquid nitrogen immediately after the irradiation was finished.

An X-band ESR spectrometer (EPR EMX, Bruker), operating at 9.648 GHz microwave and 100 kHz magnetic field modulation frequencies, was used at the liquid nitrogen temperature and above

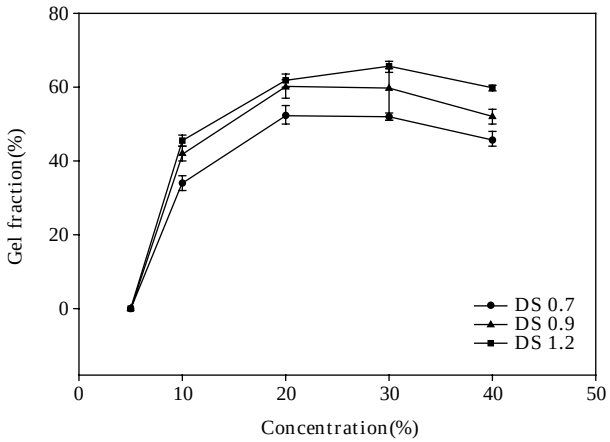

Fig. 2. Effect of the substitution degree of the CMC on the gel fraction of the CMC; Degree of the substitution of CMC, 0.7, 0.9, 1.2; irradiation in N_2, 30 kGy.

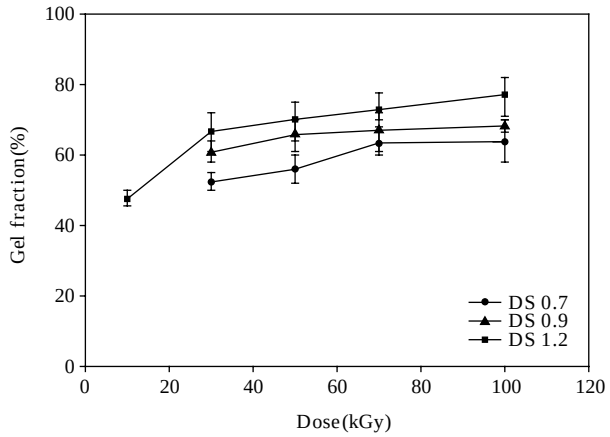

Fig. 3. Effect of the irradiation dose and the degree of the substitution on the gel fraction of the CMC; The concentration of the CMC, 20%; irradiation in N_2.

RESULTS AND DISCUSSION

1. Studies of the gel fraction and swelling ratio

The effect of the CMC concentration and the DS on the gel fraction is shown in Fig. 2. The irradiation led to the crosslinking of the CMC in an aqueous CMC solution of more than 10%, and the crosslinking was increased as the CMC concentration and DS increased. Maximum gel fraction was achieved at concentrations from 20% to 30%, while a high concentration, such as 40%, gave a low gel fraction. Also a low concentration, such as 5%, did not induce the gelation. The low gel fraction in the 40% high concentration of the CMC can be attributable to the fact that the water is not homogeneously dispersed into the CMC. Even though the 5% CMC is homogeneously dissolved in the water, the gelation is low because of the low combination between the CMC radicals. Water may contribute to the crosslinking of CMC in two ways. It enhances the mobility of the rigid molecules of the CMC, allowing an easy recombination of the CMC radicals. It also induces an increase of radical concentration of CMC by water radiolysis. By water radiolysis, hydrogen atoms and hydroxyl radicals are the most reactive species.

A higher DS is effective for the crosslinking of this polymer. Among the different types of CMC that are substituted, crosslinking took place most effectively for the one with the highest DS (1.2).

Fig. 3 shows the influence of the total dose on the cross-

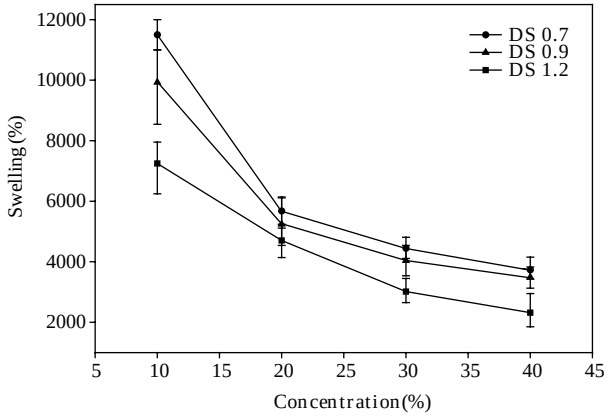

Fig. 4. Effect of the concentration and the substitution degree of the CMC on the swelling of the CMC; Degree of the substitution of the CMC 0.7, 0.9, 1.2; irradiation 30 kGy in N_2.

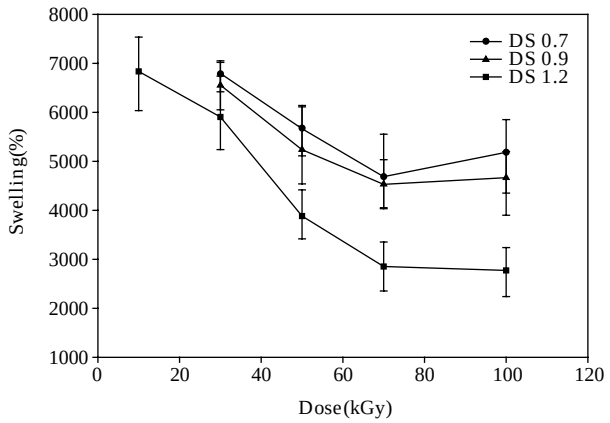

Fig. 5. Effect of the irradiation dose and the substitution degree on the swelling of the CMC; The concentration of the CMC 20%; irradiation in N_2.

linking of the CMC in a 20% aqueous solution. The gel fraction increased slightly with an increasing irradiation dose. This slow crosslinking rate as a function of the irradiation dose can be attributable to the fact that the CMC macromolecules are degraded and crosslinked by a gamma-ray at the same time.

The basic property of the hydrogel is its ability to absorb and hold huge amounts of solvent in its network structure. Figs. 4 and 5 show the swelling behaviors of the CMC hydrogels in deionized water. As shown in Fig. 4, the swelling decreases with an increasing concentration of the CMC and the total dose. It can be seen that the degree of swelling is controlled by changing the crosslinking density

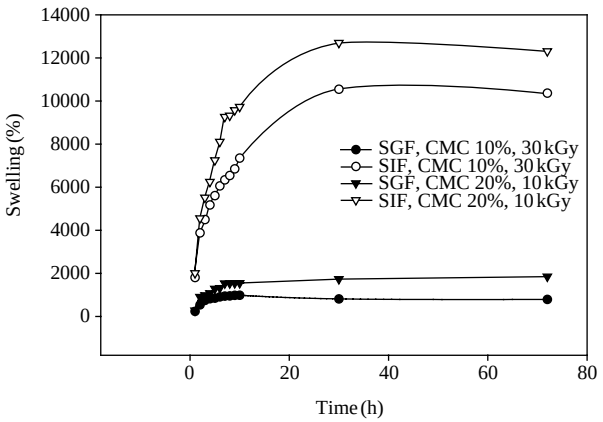

Fig. 6. The curve of the swelling test in SGF (pH 1.2), SIF (pH 6.8); CMC 10% (30 kGy), DS=0.7, CMC 20% (10 kGy), DS=1.2.

in the network. The equilibrium swelling of the hydrogels is a result of the balance of the osmotic forces determined by the affinity to the solvent and the network elasticity.

2. Degree of the swelling ratio in aqueous solutions of various pHs

It is known that a swelling or deswelling in response to changes of the pH is a typical phenomena of the polyelectrolyte gels like CMC. In this study, a pH buffer solution (SGF, SIF) was prepared. The effect of pH on the swelling of the CMC hydrogels is shown in Fig. 6. The dissociation degree is shifted to the right or left side of the Eq. accroding to the pH value of the solution.

$$R-CH_2-COOH \leftrightarrow R-CH_2-COO^- + H^+$$

At a neutral pH, the ionized form occurs more likely since the pK_a of the carboxylic groups in the polysaccharides is about 3.2 ~ 3.4. At a pH above the pK_a value, an electrostatic repulsion determines the interaction between the macromolecules (Hoogendam *et al*. 1998). In the case of SGF (pH < 3.2), the dominant one in the hydrogel is not the dissociated carboxyl group. The gel structure is devoid of a charge, and collapsing is observed as a result of the hydrogen bonding formation. In the case of SIF (pH > 6.0), the dominant charges in the gels are the unprotonated carboxyl group. Ionization allows for the hydration of the charged oxygen atoms, which also provokes a water uptake and the formation of the hydrogen bonds between polymer and water, and the interactions of the van der Waals type.

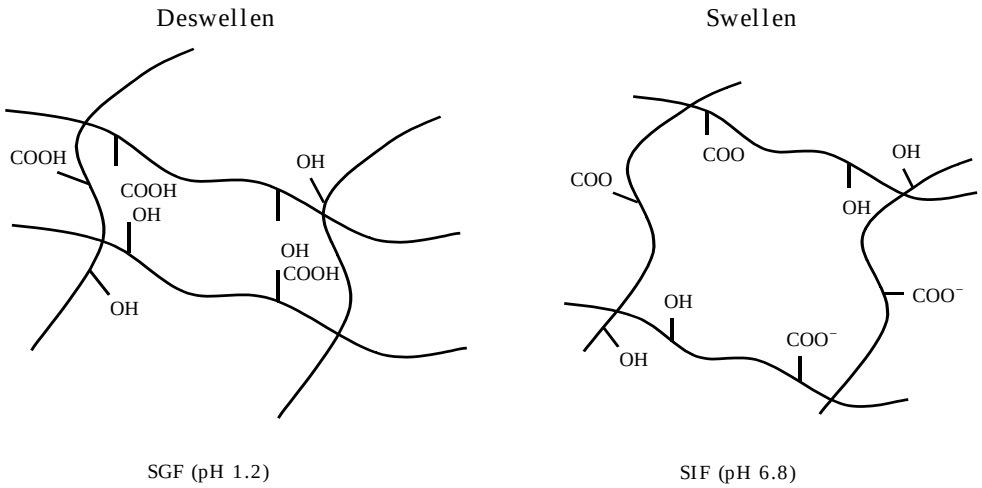

Fig. 7. Hypothetical sketch of the swelling and deswelling; SGF (pH 1.2), SIF (pH 6.8).

Fig. 7 shows the hypothetical sketch of the swelling and deswelling in the SGF and SIF buffers.

3. Measurement of the biodegradability of the CMC hydrogels

Cellulose-degrading microorganisms contain cellulase systems consisting of different enzymes which effectively hydrolyze cellulose by acting sequentially or in concert. Three categories of cellulolytic enzymes can be distinguished. Endoglucanases (EG) cleaves the cellulose chains internally. Exoglucanase degrades the cellulose starting from the free chain ends, producing cellobiose. The third enzyme, β-glycosidase, hydrolyses short cello-oligosaccharides to glucose. *Trichoderma reseei* is one of the most powerful producers of the cellulolytic enzymes and many commercial cellulose preparations are produced by this fungus. *Trichoderma reseei* produces at least two cellobiohydrolases and five endoglucanases of which EG I and EG II are the most abundant (Hoogendam *et al*. 1998).

In this study, the effect of cellulase on the degradability of the CMC hydrogel was kinetically examined by checking the quantity of the sugar product.

Fig. 8. shows the enzymatic hydrolysis of the CMC hydrogel. The hydrolysis reaction began immediately after an addition of the enzyme solution, and then the glucose production was leveled off. From the results, it was found that the degradable reaction depended on the degree of crosslinking of the CMC hydrogel.

An affinity of the enzyme was also investigated by using

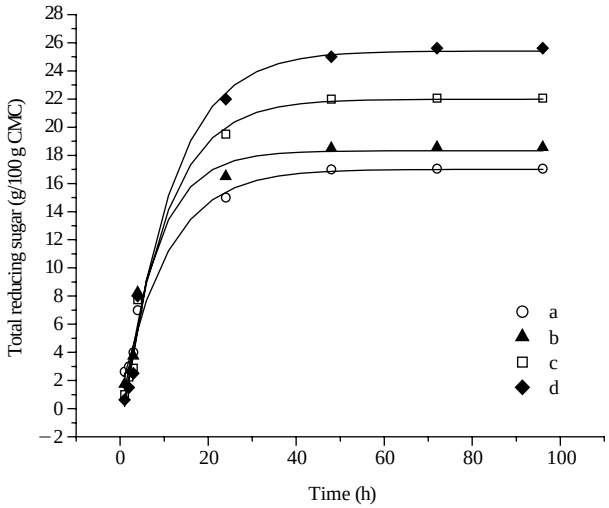

Fig. 8. The curve of the biodegradation with the gel fraction of the CMC; by Cellulase from Trichoderma reesei in a citrate buffer (30°C, pH 5.0); (a: DS=0.9, gel fraction 63%, b: DS=0.9, gel fraction 61%, c: DS=0.9, gel fraction 52%, d: DS=0.9, gel fraction 42%).

the correlation between V and K_m as a function of the gel fraction. Kinetic parameters of the enzymatic reaction can be estimated by the data of the initial hydrolysis rates by a direct linear method of the Lineweaver-Burk plot (Lineweaver and Burk 1934).

$$\frac{1}{V}=\frac{K_m}{V_{\max}}\frac{1}{[S]}+\frac{1}{V_{\max}} \qquad \text{(c)}$$

where V and $V_{\max}$ are the initial hydrolysis rate and maximum hydrolysis rate, K_m is the Michaelis constant, and [S]

is the CMC hydrogel concentration. Thus a plot of the reciprocal of the initial rate versus the reciprocal of the CMC hydrogel concentration for the experiments at a fixed enzyme concentration should give a straight line. Furthermore, the intercept with the ordinate gives $1/V_{max}$ and the slope is K_m/V_{max}, from which V_{max} and K_m can be calculated.

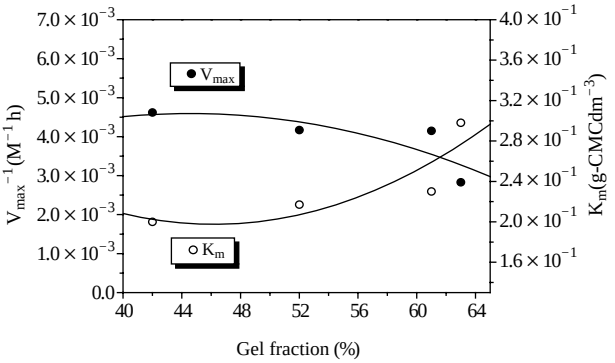

Fig. 9. Changes in the kinetic parameters with the gel fraction of the CMC hydrogels in a cellulase system.

In Fig. 9, V_{max} decreased with an increase of the gel fraction, after which it leveled off. On the other hands the K_m value increased with an increase of the gel fraction. As shown by the K_m value of Fig. 9, a high gel fraction lowers the affinity of the enzyme to the CMC.

4. ESR measurements

Fig. 10 shows the ESR figures of the unirradiated CMC (a) and the irradiated CMC hydrogels (b) kept in liquid nitrogen after being irradiated at a dose of 10 to 100 kGy at room temperature. The unirradiated CMC did not show any ESR signal, indicating the absence of the free radicals before irradiation. The ESR spectra of the gamma-irradiated CMC showed complex features, indicating the presence of multiple radical sites. They are ascribed to the radicals

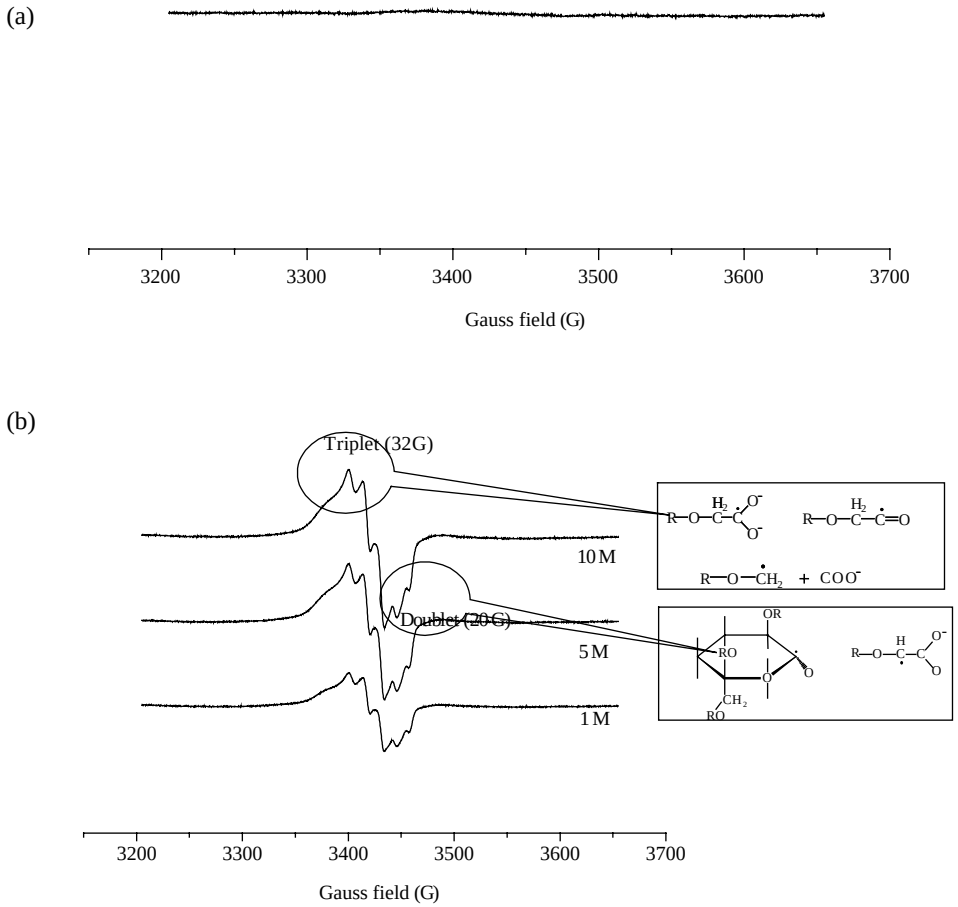

Fig. 10. ESR figures of the unirradiated CMC (a) and the irradiated CMC hydrogels (b) kept in liquid nitrogen after being irradiated at a dose of 10 to 100 kGy at room temperature.

which originated from the dehydrogenation. Fig. 10(b) shows that the radical intensity increases gradually until 100 kGy.

CONCLUSION

The objective of the study was to crosslink the biocompatible and biodegradable CMC by a radiation processing. A CMC hydrogel induced by irradiation is achievable only at certain conditions, i.e. at a high concentration of the polymer in an aqueous solution and a high DS. The swelling of CMC hydrogels decreased with an increasing concentration of the CMC and the total dose. The swelling measurements in the SIF and SGF buffer solution clearly showed the pH-dependency nature of the hydrogels. Biodegradability depended on the degree of the crosslinking of the CMC hydrogel. Thus, the CMC hydrogels are expected to be useful for infants diapers, feminine hygiene products, and incontinence products.

ACKNOWLEDGEMENTS

This work was carried out under the Nnclear R&D program by the Ministry of Science and Technology (MOST) of Korea.

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Manuscript Received: May 18, 2007
Revision Accepted: June 14 2007