Radiation Crosslinking of Biodegradable Carboxymethylchitin and Carboxymethylchitosan

J. M. Wasikiewicz,[1] H. Mitomo,[1] N. Nagasawa,[2] T. Yagi,[2] M. Tamada,[2] F. Yoshii[2]

[1]*Department of Biological and Chemical Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan*
[2]*Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Agency, Takasaki, Gunma 370 – 1292, Japan*

Received 29 November 2005; accepted 5 February 2006
DOI 10.1002/app.24203
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Radiation induced crosslinking of carboxymethylchitin (CMCht) and carboxymethylchitosan (CMChts) has been investigated both by electron beam (EB) and γ-irradiations. The highest crosslinking efficiency, measured as gel content of obtained hydrogels, by EB irradiation was 72 and 50% for CMCht and CMChts, respectively, at polymer concentration of 40% at room temperature. For irradiation crosslinking at different temperatures by γ-irradiation, the best ratio of crosslinking to degradation processes was obtained at low temperature for low concentrated polymers and at high temperature for high concentrated ones. Both hydrogels revealed significant swelling properties in deionized water, with maximum water uptake 241.2 and 63 g water/g dry gel for CMCht and CMChts, respectively. Biodegradation tests, including enzymatic controlled microorganisms degradations and soil burial, have indicated that both hydrogels undergo spontaneous biodegradation with satisfactory results. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 758–767, 2006

Key words: irradiation; biodegradable; hydrogels; carboxymethylchitin; carboxymethylchitosan

INTRODUCTION

Carboxymethylchitin (CMCht) and carboxymethylchitosan (CMChts) are the most popular among the derivatives of chitin and chitosan. Structurally, they are molecules in which some of the hydrogen atoms of hydroxyl groups are replaced with carboxymethyl substituents. In comparison to pure chitin and chitosan, their carboxymethylated derivatives possess very useful features such as dissolution in acidic as well as neutral and basic solutions.[1] This property significantly widens the applications of these derivatives in the industry[2,3] and in medicine/pharmacy.[4,5]

Some further modifications of these polymers may result in their even more advanced practical applications. Crosslinking, for example, leads to a creation of hydrogels that have excellent solvent sorption properties.[6] Chemical crosslinking has been reported[7] as one of the crosslinking methods. However, in this method, addition of glutaraldehyde is necessary to initiate the process. Glutaraldehyde reacts as a crosslinker, and during the reaction, it is incorporated inside the polymer matrix, and so the final product is a network mixture of a polymer and the crosslinker. Another option is an ultraviolet crosslinking, but in this case, addition of a photoinitiator is required, which also causes further problems with purification of the final product.

A new method of crosslinking has been proposed, using ionizing radiation. Its main advantages, compared to other methods, are the lack of any additives to start the process, hence the final product contains only polymer in its structure. It is definitely a great improvement due to the fact that the final product does not require further purification. Moreover, ionizing radiation usually allows the combination of the synthesis and sterilization of polymeric materials in a single technological step,[8,9] thus reducing costs and production time. Therefore, ionizing radiation method is an excellent tool in fabrication of materials for biomedical applications, such as wound dressing, drug delivery systems, stimuli-responsive systems, implants, etc.

Public concern for environmental protection forced governments in many countries to observe strict regulations on materials production and waste management. It refers, especially, to synthetic polymeric material because of their common utilization and a longer degradation period. Natural polymers, due to their renewable sources and spontaneous degradation by naturally occurring microorganisms, have been suggested as suitable materials to overcome this problem. Such polymers are not harmful to natural environment, since they do not produce any toxic products as degradation proceeds. CMCht and CMChts due to the

Correspondence to: J. M. Wasikiewicz (wasikiewicz@yahoo.com).

natural origin of their parental chitin undergo biodegradation, which was confirmed in this work.

In recent years, research on polymers had been focused on their possible utilization in biomedical and pharmaceutical applications. A promising group of polymers for these purposes is polysaccharides, due to their unique structure, exceptional properties, and biocompability. However, insolubility in aqueous solution and particular shapes are required in these applications. It can be achieved by radiation induced crosslinking, which results in creation of water-insoluble hydrogels of chosen shapes.

We have applied ionizing radiation to induce crosslinking reaction in water-soluble derivatives of cellulose,[10] starch,[11] and chitin/chitosan.[12] It was found that radiation induced crosslinking of their carboxymethyl derivatives occur in high concentrated aqueous solution (higher than 10%), and as result water insoluble hydrogels are formed.

Although radiation synthesis of CMCht and CMChts has been reported,[12] detailed radiation parameters, irradiation at various temperatures, and biodegradability have not been revealed yet; thus, this article completed research in these areas.

EXPERIMENTAL

Materials

Carboxymethylchitin (CMCht) and carboxymethylchitosan (CMChts) used in all experiments were purchased from Koyo Chemicals, Japan. The degree of substitution, which indicates the number of original H atoms of hydroxyl groups being replaced by carboxymethyl substituent in molecule of CMCht and CMChts, were 0.81 and 0.91, respectively. The average molecular weights estimated by gel permeation chromatography (GPC) measurements were 2.5×10^5 for CMCht and 6.0×10^4 for CMChts. The degree of deacetylation values (DDA), the ratio of glucosamine to glucosacetylamide groups per monomer unit, was 24.6 and 84.0% for CMCht and CMChts, respectively. All chemicals were of analytical grade.

Samples preparation and irradiation

Polymer, in the appropriate amount, was added to deionized water and mixed by Keyence HM-500 hybrid mixer. The prepared materials were kept for 2 days to ensure complete dissolution and homogenous distribution of polymer chains in all volume. After that, samples were cold pressed (200 kPa) for about 45 min to obtain thin film shape (1 mm) and then sealed in polyethylene bags to ensure air-free conditions after degassing using vacuum apparatus.

Irradiation was performed by electron beam (EB) accelerator at the following parameters: current = 1 mA, voltage = 2 MeV, and dose per pass = 1 kGy. A wide range of doses has been applied, from 10 up to 200 kGy to prepare hydrogels.

Alternatively, irradiation at different temperatures was carried out by γ-rays generated from ^{60}Co source at the dose rate of 10 kGy/h. At temperatures of 70 °C and 50°C, samples were placed in a heater with controlled temperature, whereas at a temperature of 0°C, samples were placed in polystyrene box filled out with mixture of ice and water. After irradiation, crosslinked samples were dried.

Gel content and swelling of hydrogels

The gel content of radiation crosslinked hydrogels was determined gravimetrically by measuring the insoluble part after extraction of the sol part. Thus, hydrogel was kept in deionized water for 5 days at room temperature and was occasionally shaken. This period is sufficient for the extraction of sol fraction from hydrogel. The gel fraction was calculated as follows:

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

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

Swelling was conducted by immersing a dried gel sample in deionized water at room temperature. After an equilibrium water uptake was reached, i.e., when the mass of the gel at two consequent weighing did not differ by more than 0.5%, the hydrogel was filtered by a stainless steel net of 30 mesh and lightly blotted out by filter paper to remove surface water, prior to weighting. Swelling, in grams of absorbed solvent per gram of dried gel, was calculated as follows:

$$\text{Swelling} = (G_s - G_d)/G_d \quad (2)$$

where G_s is the weight of hydrogel in a swollen state.

Morphology of hydrogels

The scanning electron microscope (SEM) pictures of hydrogels at equilibrium state were taken by Hitachi Integrated System Type N SEM-EDX spectrometer running at an accelerating voltage of 15 kV, at a magnification of 1000 times. Samples were prepared from swollen hydrogel films dipped into liquid nitrogen, which preserves the hydrogel structure, and next they were broken to view their cross-section. Frozen water was removed from samples during vacuum sublimation at a temperature of 28°C. After drying and before examination, samples were coated by graphite thin layer to enhance their conductivity.

Biodegradation of hydrogels

Enzymatic degradation

Enzymatic degradation of CMCht and CMChts hydrogels was carried out in a phosphate buffer solution at pH 8.0, containing chitinase and at pH 5.6 containing chitosanase enzymes, respectively. After removing the sol part, 1-mm thin, dry film gel samples with weight of about 10 mg were dipped into enzyme solution for a given time, temperature, and enzyme concentration. After incubation, the samples were washed and kept in an excess of deionized water to rinse out the degraded polymer and finally dried at 35°C. Result of the enzymatic degradation is expressed as a decrease in sample weight:

$$\text{Degradation } (\%) = G_e/G_d \times 100\% \qquad (3)$$

where G_e and G_d denote the weights of films after and before enzymatic tests, respectively.

Soil burial test

Soil burial test was performed in a plastic trough, using the soil from Takasaki, JAEA area. The conditions were completely natural, without any improvements or additives. The test was carried out for 10 weeks from October to December. Samples were placed in direct contact with the soil completely buried at the depth of 8 cm under the surface. Because of the difficulties emerging from the ability of samples to swell water and adhere to the soil, the degradation was observed as a change in their appearance. The thin films, with thickness of 1 mm and size of 20 × 30 mm^2, of dry hydrogels prepared by EB irradiation of CMCht and CMChts were used in this experiment.

Biodegradation under controlled conditions in composed soil

The microbial degradability of polymer in composted soil was evaluated by measurements of produced CO_2 gas. Specially designed apparatus, microbial oxidative degradation analyzer (MODA)[13] comprising four independent lines of columns, was used. Polymer sample was mixed with rinsed sea sand (450 g) and compost of moisture content 52% (120 g) and was then placed in a heated reactor. Inside the column, the monitored temperature was 35°C and the flow of the carbon dioxide-free but moisturized air was 30 mL/min. After flowing through the sample reactor, the air, carrying CO_2 formed due to polymer decay, was passed through a series of columns filled with silica gel, calcium chloride, soda lime, and calcium chloride. Ammonia, which could be formed from the decomposing sample, was trapped in sulfuric acid solution, and water vapor was absorbed into the first two columns (silica gel and calcium chloride). The CO_2 was collected quantitatively by soda lime and water, produced during the reaction, and it was trapped in the last $CaCl_2$ column. Thus, mass of produced carbon dioxide was calculated as a difference in the weight of the two last columns at the beginning and the end of the test. Pure compost mixed with sea sand was used as a blank and cellulose as a reference sample. Samples designated for examination by this method were initial CMCht and CMChts and their irradiated hydrogels in the amount of 10 g.

Unirradiated polymer was in the form of dry powder, whereas hydrogel in relaxed state was cut into small pieces and swelled in water for 1 h before mixing with sand and compost.

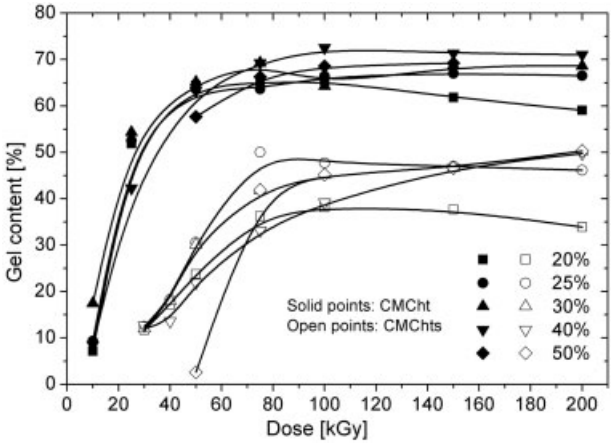

Figure 1 Effect of radiation dose and polymer concentration in aqueous solution on crosslinking (gel content) of carboxymethylchitin (solid points) and carboxymethylchitosan (open points). Irradiation of degassed solutions by EB, at the dose rate 1 kGy/pass.

RESULTS AND DISCUSSION

Crosslinking of CMCht and CMChts by EB irradiation

Degradation and crosslinking of the polymer, under ionizing radiation, occur simultaneously, and the one which predominates determines the final reaction products. However, in highly concentrated aqueous solutions of CMCht and CMChts (~15% w/w) exposed to ionizing radiation, crosslinking prevails upon the degradation reaction, resulting in the formation of hydrogel matrix.[12]

The effect of polymer concentration on the gel fraction of radiation crosslinked hydrogels is shown in Figure 1. The highest gel contents for CMCht and CMChts were obtained for a concentration of 40% w/w, exceeding 72% and 50%, respectively. The gel content increases with increasing of the solution concentration. When polymer concentration is higher, the

distance between the neighboring radicals sited on macromolecules becomes shorter. Moreover, as reaction proceeds in an aqueous medium, macroradical mobility is not sustained, what with reduced proximity indeed facilitates their recombination reaction, leading to formation of a network of covalent bonds—hydrogel. Finally, water itself makes a significant contribution to the crosslinking process, which is induced by reactions of water radiolysis products. Among them, the most reactive species are hydrogen atom and hydroxyl radical.[14] These radicals can create macroradicals through abstracting hydrogen atom from the polymer chain. Thus, the presence of water may enhance the number of macroradicals, because they are created both directly on polymer chain and during indirect reaction of water radiolysis products with macromolecules.

However, for very high polymer concentration of about 50%, gel content of radiation induced hydrogel is lower than that at 40% concentration. The explanation is that at such high concentration, the polymer is not homogenously distributed in entire volume. As a result, the crosslinking reaction occurs only at certain, preferred region of solution, therefore the total reaction efficiency, observed as gel content is decreased. The other factor is that with increase of polymer concentration, radicals mobility is being restricted, what inhibits their recombination reaction and enhances degradation reaction probability.

For samples at all concentrations, the gel fraction dramatically increases, just after exceeding the gelation point, with increasing radiation dose and then it levels off at the dose of 75 kGy for CMCht and 100 kGy for CMChts.

It is also worth mentioning that the dose required to crosslink CMChts is higher than that of CMCht. CM-chitosan chemical structure differs from CM-chitin in the content of amine groups. Amine group of chitosan derivatives can interact with its acidic groups both inter- and intramolecularly, through ionic interactions. Such created species may create rigid structure, which would shield access for other radicals and thus significantly reduce possibility of their recombination. This effect is compensated by applying a higher dose, which ensures a larger number of created radicals.

Crosslinking of CMCht and CMChts by γ-irradiation at different temperatures

Temperature is one of the factors that significantly influences most of the physicochemical reactions. Therefore, irradiation of carboxymethyl-chitin, -chitosan at various temperatures has been investigated. The results are shown in Figure 2 and radiation crosslinking parameters are summarized in Table II. As can be seen, for both polymers crosslinking reaction was the most efficient (the highest gel content) at

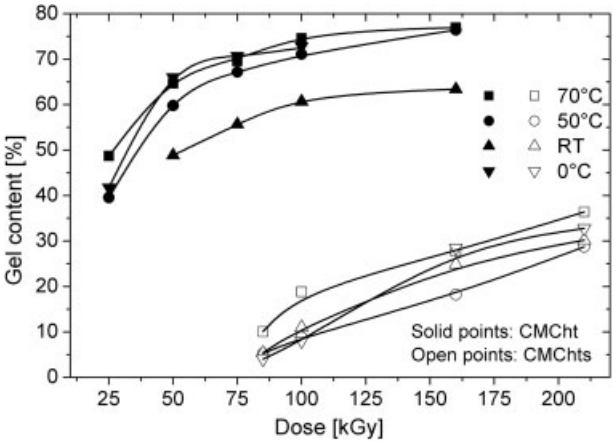

Figure 2 Effect of radiation dose and temperature on the gel fraction of carboxymethylchitin(solid points) and carboxymethylchitosan(open points). Irradiation of degassed 40% aqueous solutions by γ-irradiation, at the dose rate 10 kGy/h.

temperatures 70°C and 0°C. During irradiation next to crosslinking, chain scission reaction also occurs. At high temperatures, chain scission is concluded to be the main reaction, but at lower temperatures, crosslinking should prevail,[15] which is consistent with the presented results for temperature 0°C.

The effect of temperature on the crosslinking reaction of CMCht and CMChts is closely related to the radical mobility and polymer conformation. At low temperature, mobility of the radicals is restricted, and thus the mutual recombination of each of the two neighboring ones is facilitated, leading to the creation of crosslink bond. As a result, higher gel content is observed at lower temperature. On the other hand, at high temperature, radical mobility increases, and this favors chain-scission reaction. However, increasing temperature also causes decrease in polymer chains entanglement and this conducts to crosslinking reaction. It seems that in elevated temperature the latter effect predominates. It is easier for macroradicals located on the polymer side-chains to recombine with each other in untangled form, because access to them is unshielded and this facilitates creation of intermolecular crosslinks. As it was observed, the straight dependence of temperature on the radiation crosslinking of CMCht and CMChts is difficult to determine. Temperature influences on few parameters and the final result depend on which one will predominate.

A comparison of irradiation behavior of CM-chitin by γ- and EB-sources at ambient temperature shows that there is no big difference in the obtained results (Figs. 1 and 2). However, in case of CM-chitosan, gel fraction of hydrogels formed by γ-radiation is lower than that by EB radiation. The main reason is the energy density of both sources, and it is explained in detail in the next section.

Radiation parameters of gelation

The gelation dose (D_g), minimum required energy to initiate gelation process, the ratio of scission to crosslinking densities (p_0/q_0) together with yields of crosslinking and degradation are the most important factors for determining efficiency and properties of crosslinking reactions.

D_g and p_0/q_0 values were calculated using a specially designed computer program (Gelsol), which works based on the following Charlesby-Rosiak equation:[16]

$$s + \sqrt{s} = \frac{p_0}{q_0} + \left(2 - \frac{p_0}{q_0}\right)\frac{D_v + D_g}{D_v + D} \tag{4}$$

Yields of crosslinking and degradation were calculated using equations:

$$G(x) = \frac{c}{M_{w0} D_g d\left(2 - \frac{p_0}{q_0}\right)} \tag{5}$$

$$\frac{G(s)}{G(x)} = 2 \times \frac{p_0}{q_0} \tag{6}$$

where s is sol fraction, p_0 is degradation density–average number of main chain scission per monomer unit and per unit dose; q_0 is crosslinking density-proportion of monomer units crosslinked per unit dose; D is absorbed dose; D_v is the virtual dose—a dose required to change the distribution of molecular weight of the certain polymer in such way that relation between weight-average and number-average molecular weight would be equal to two; D_g is the gelation dose—minimum required energy to start gelation process; M_{w0} is initial weight-average molecular weight of polymer; c is polymer concentration; d is solution density and $G(x)$ and $G(s)$ are yields of crosslinking and scission, respectively.

The data summarized in Tables I and II show that crosslinking of CM-chitin, both by EB and by γ-rays irradiation, is much easier than crosslinking of CM-chitosan, with lower gelation dose D_g. It is because of the fact that the molecular weight of CMCht is about four times higher that that of CMChts; this means its chains are longer and statistical probability of the creation of multiple radicals on a single chain and their recombination with radicals located on other chains significantly increases leading to the formation of gel matrix. Thus, CMCht required lower radiation dose than CMChts to start the gelation process.

Another reason of such behavior was described earlier that because of the presence of amino group, strong ionic interaction in water solution appears, and as a result, two radicals recombination reaction leading to crosslinking is impeded.

TABLE I
Comparison of D_g, $G(x)$, and $G(s)$ Values of CMCht and CMChts Irradiated at Various Solution Concentration by EB at the Dose Rate 1 kGy/pass

Concentration of polymer (%)	D_g	p_0/q_0	$G(x)$ (10^7mol/J)	$G(s)$ (10^7mol/J)
CM-chitin				
20	9.63	0.93	0.78	1.44
25	9.22	0.86	0.95	1.64
30	8.37	0.80	1.19	1.91
40	8.65	0.68	1.40	1.91
50	27.79	0.74	0.57	0.85
CM-chitosan				
20	47.79	1.41	1.18	3.33
25	48.89	1.23	1.11	2.72
30	34.09	1.15	1.73	3.97
40	27.00	0.99	2.44	4.84
50	49.68	1.19	2.07	4.93

In Table I, we can notice that the lowest p_0/q_0 ratio is for polymer concentration of 40% for CM-chitin as well as for CM-chitosan, which means that this concentration is the optimum for crosslinking reaction.

If we compare values of D_g and p_0/q_0 for both polymers, for example, at concentration of 40%, irradiated in ambient temperature by EB 8.65 kGy, 0.68 for CMCht and 27.0 kGy, 0.99 for CMChts with γ-irradiated ones 17.78 kGy 0.84 for CMCht and 74.39 kGy 1.19 for CMChts, it becomes obvious that gelation parameter are much better for EB irradiation. These two kinds of radiation cause similar effect on matter; however, the energy which they are carrying for time unit is different. To obtain the same total radiation dose by EB it takes minutes, whereas γ-irradiation requires hours. As a result, in the same time during EB radiation about two orders of magnitude radical are created in comparison with γ-source. When the number of radicals increases, the possibility of recombination raises significantly, which results in enhanced efficiency of a gel creation.

Swelling of CMCht and CMChts hydrogels in deionized water

The ability of hydrogels to swell and hold significant amounts of solvent inside their network structure is one of their most important features. This property makes hydrogel the perfect material as a solvent absorber. Usually swelling is expressed as the amount of adsorbed solvent in grams, to 1 gram of dried gel.

Swelling measurements of CMCht and CMChts hydrogels created by EB and γ-irradiations are shown in Figures 3 and 4. All curves present the same tendency; swelling is the highest just after the dose crosses the gelation point and radically decreases with increasing

TABLE II
Comparison of D_g, $G(x)$, and $G(s)$ Values of CMCht and CMChts Irradiated at Various Solution Concentration and at Different Temperatures by γ Irradiation at the Dose Rate 10 kGy/h

Temperature	Concentration of polymer (%)	CM-chitin				CM-chitosan			
		D_g (kGy)	p_0/q_0	$G(x)$ (10^7 mol/J)	$G(s)$ (10^7mol/J)	D_g (kGy)	p_0/q_0	$G(x)$ (10^7mol/J)	$G(s)$ (10^7mol/J)
70°C	20	12.28	1.09	0.72	1.56				
	25	27.79	0.99	0.36	0.71	44.30	1.25	1.25	3.14
	30	16.25	0.78	0.61	0.94	69.19	1.04	0.75	1.57
	40	6.88	0.57	1.63	1.85	63.10	1.09	1.16	2.53
50°C	25	15.14	0.90	0.60	1.08	45.06	1.08	1.01	2.17
	30	26.85	0.74	0.36	0.53	72.91	1.22	0.88	2.15
	40	9.58	0.54	1.14	1.24	62.08	0.89	0.97	1.72
RT	25	16.71	0.88	0.12	0.14	33.58	1.09	1.14	2.93
	30	14.06	0.74	0.53	0.94	54.69	0.84	1.36	2.97
	40	17.78	0.84	0.68	1.00	74.39	1.19	0.79	1.32
0°C	20	10.98	0.81	0.78	1.30	40.70	1.10	1.11	2.63
	25	17.89	0.75	0.61	0.99	28.71	0.64	0.91	2.00
	30	13.90	0.54	0.45	0.67	76.01	1.19	1.07	1.37
	40	16.55	0.65	0.59	0.64	79.88	1.04	0.81	1.93

of absorbed energy at the early stages of gel formation. Then, the decreasing becomes slower but is still observable. At the beginning of the gelation process, the hydrogel is very weak and fragile, but because of a relatively low number of network bonds, it is able to expand by absorbing and holding large amounts of solvent in its voids. With a subsequent increase in dose, the gel content increases, thus the crosslink density grows, and the hydrogel becomes more tightly packed and firm. Hence, the water permeability through hydrogel and its sorption abilities lessen.

At this point, a significant problem arises, because of two linked factors of an opposite tendency. When applied dose is low, hydrogel absorbs enormous amount of solvent, but hydrogel possesses poor mechanical properties, which are vulnerable to crushing and breaking. On the other hand, at high radiation dose, hydrogel has a good mechanical characteristic but low solvent sorption values. Thus, the compromise has to be made between good swelling and mechanical properties for practical applications.

Swelling of hydrogels obtained by EB irradiation is lower than that obtained by γ-irradiation. This dependence is opposite to the gel content value for these hydrogel, which is in good accordance with the aforementioned assumption that EB creates hydrogel with denser crosslinking bonds, so hydrogel is stiffer and less vulnerable for solvent penetration.

The same tendency has been revealed for hydrogels produced by γ-radiation at different temperatures that solvent swelling has an opposite dependence to the

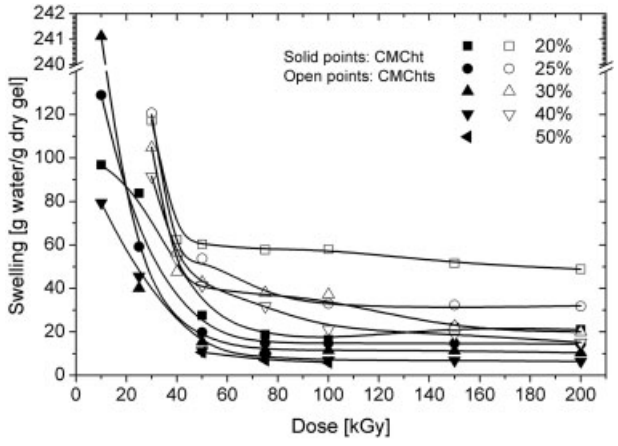

Figure 3 Swelling of CM-chitin (solid points) and CM-chitosan (open points) hydrogels obtained by EB irradiation at different radiation doses and at various solution concentrations. Irradiation of degassed solutions at the dose rate 1 kGy/pass.

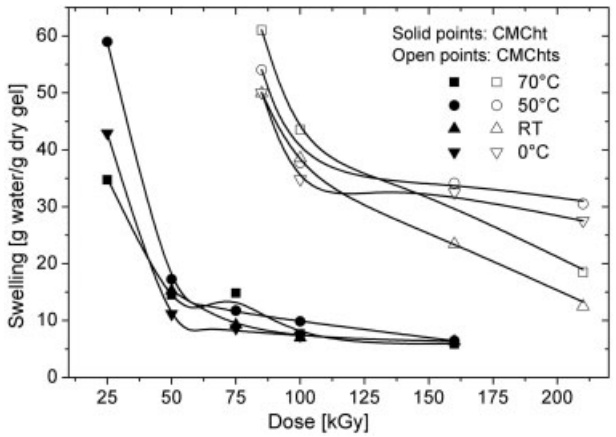

Figure 4 Swelling of CM-chitin (solid points) and CM-chitosan (open points) hydrogels obtained by γ-irradiation at different radiation doses and at various temperatures. Irradiation of degassed solutions, at the dose rate 10 kGy/h.

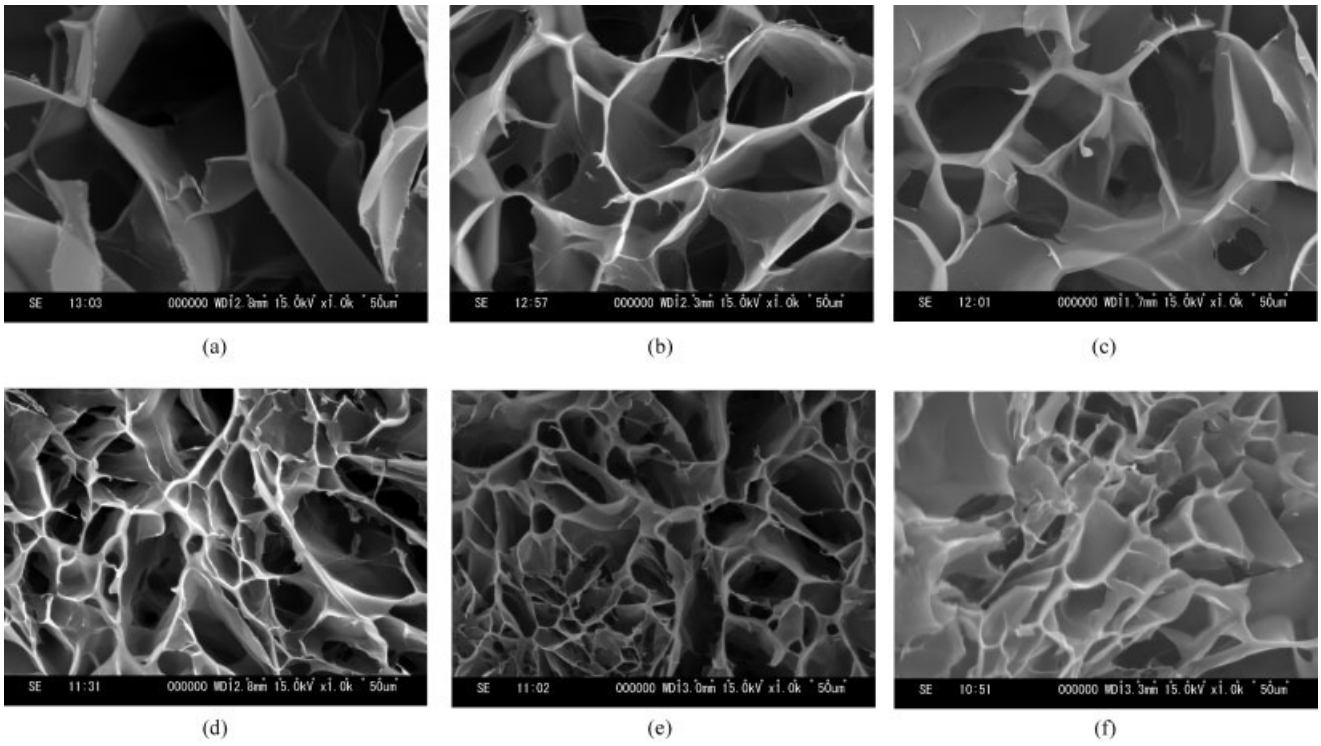

Figure 5 About 1000 times zoomed SEM photographs of CMCht hydrogels cross section obtained at different irradiation dose: (a) 25 kGy; (b) 50 kGy; (c) 75 kGy; (d) 100 kGy; (e) 150 kGy; (f) 200 kGy. Irradiation of degassed 40% solutions by EB.

gel content. The higher the gel content, the lower the water uptake by these gels.

Morphology of hydrogels

During radiation induced crosslinking reaction, microporous gels are created because of the formation of new inter- and intramolecular crosslinks. Moreover, at the size level of microns, gel also forms a macroporous structure, which can be observed by SEM technique.

Photographs of a cross section structure of swelled CMCht and CMChts hydrogels are shown in Figures 5 and 6, respectively. SEM images have been taken at 1000 times magnification for hydrogels obtained by irradiation by EB beams at different doses.

Pictures of both gels show that with increasing of the radiation dose, the pore size of hydrogels become smaller in diameter and a denser, stiffer, and ordered structure is obtained. Comparison of pore size for CM-chitin irradiate at doses 10 and 200 kGy gives back the result of 42 and 8 μm, respectively, that is a difference of about 34 μm in total. Similarly, the pore size of irradiated CM-chitosan at 75 and 200 kGy are 85 and 38 μm, respectively, that is about 47 μm in difference. A diminishing of the pore size of these hydrogels is connected with increasing of the radiation dose that is in good consistence with the results of swelling studies discussed earlier, lower water uptake was obtained for higher doses.

Biodegradation of CMCht and CMChts hydrogels

Enzymatic degradation

Enzymes are biological catalysts, usually proteins, which help most chemical reactions in living systems to occur. Enzymes are true catalysts, which mean that they initiate the reaction or increase its rate but do not participate in it. Thus, like all catalysts, enzymes return to their original form at the end of the reaction cycle.[17] Enzymatic rate acceleration may range from 10^3 to 10^{16} times the rate of uncatalyzed reaction. The degradation by microorganisms might be the result of multistep reactions catalyzed by enzymes and/or reactions not involving enzyme catalysts.

It has been reported that chitinase enzyme catalyzes chitin degradation[18] and adequately chitosanase acts in the same way for chitosan.[19] It was assumed that these enzymes also enhance degradation yields of carboxymethylated chitin and chitosan derivatives.

The relationship between enzyme concentration and hydrogel weight loss is shown in Figure 7. Increasing the enzyme concentration leads to increase in the reaction rate. If we assumed that at the given incubation time the degradation process is not fully completed than it did in solutions with higher enzyme content, degradation proceeds faster and exceeds higher polymer weight loss at the same time unit. For further experiments concentration of 0.2 mg/mL has been selected.

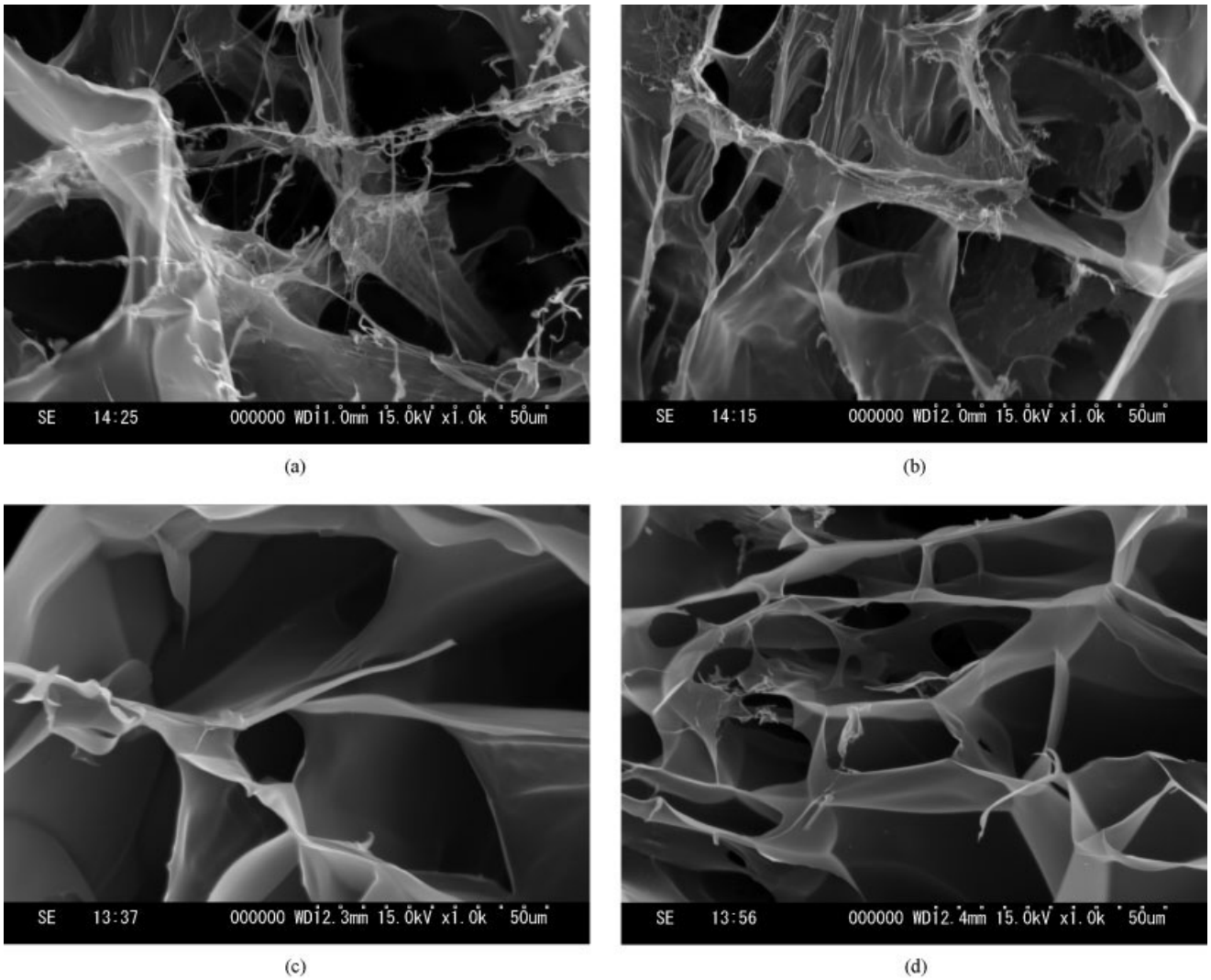

Figure 6 About 1000 times zoomed SEM photographs of CMChts hydrogels cross-section obtained at different irradiation dose: (a) 75 kGy; (b) 100 kGy; (c) 150 kGy; (d) 200 kGy. Irradiation of degassed 40% solutions by EB.

Temperature dependence on enzymatic degradation of CM-chitin, -chitosan and hydrolysis without enzymes as their weight loss results are presented in Figure 8. In both cases, maximum degradation peak occurred. For CMCht degradation with chitinase peak appears at temperature of 60°C as a result of 42% weight loss. Maximum efficiency of chitosanase for CMChts degradation is at temperature of 55°C with 35% of initial polymer weight loss. Before reaching maximum of the peak, polymers degradation increases with increasing temperature, because of enhanced enzyme reactivity. However, after reaching peak point, degradation decreases gradually due to the fact that enzyme is being deactivated in denaturation process of its proteins.

Kinetic of CMCht and CMCht enzymatic degradation by chitinase and chitosanase are presented in Figure 9. CMCht hydrogel loses its weight sharply until the time of 48 h and then it levels off. Although

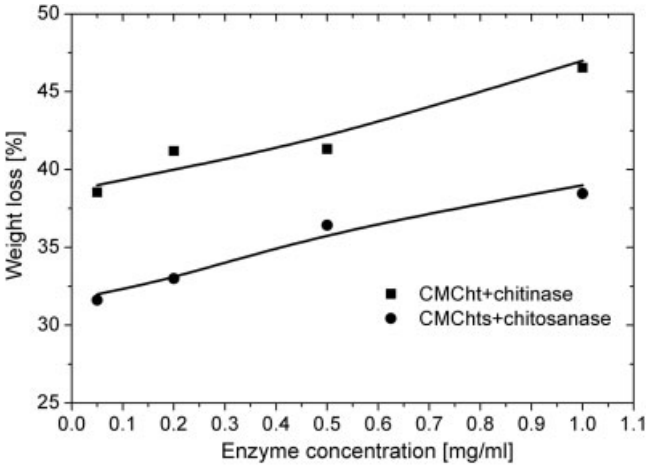

Figure 7 Enzyme concentration dependence on the degradation of EB crosslinked CMCht and CMChts hydrogels at the dose of 75 and 100 kGy, respectively. Enzyme incubation time, 48 h; temperature, 55 and 60°C for CMChts and CMCht, respectively.

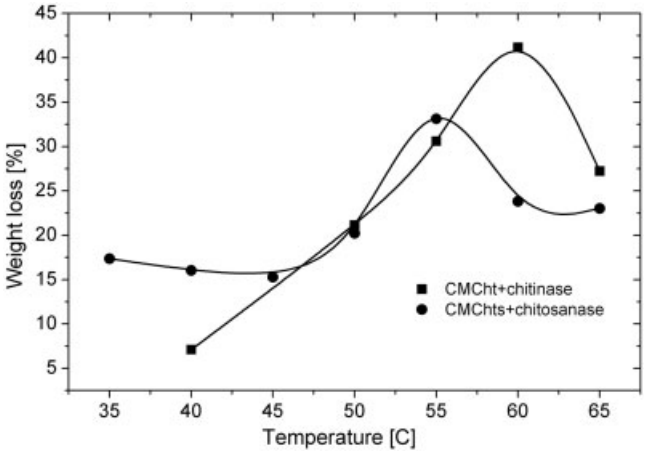

Figure 8 Effect of reaction temperature on the weight loss of CMCht and CMChts radiation crosslinked hydrogels. Radiation conditions as in Figure 7. Enzyme concentration 0.2 mg/mL, incubation time 48 h.

CMChts weight decrease more gradually, it stabilizes after the same time period.

Hydrolysis of subjected hydrogel (control samples) reveals lower degradation effect than enzymatic treatment. However, we should expect much bigger difference between these two methods as it was observed for unmodified chitin and chitosan.[20] These enzymes are efficient for chitin and chitosan but for their carboxymethylated derivatives it is very probable that carboxymethyl groups stand as a structural hindrance for enzyme to fit in chitin, chitosan molecules.

Soil burial test

Biodegradation in a natural environment of hydrogels is difficult for quantitative evaluation. The gel swells

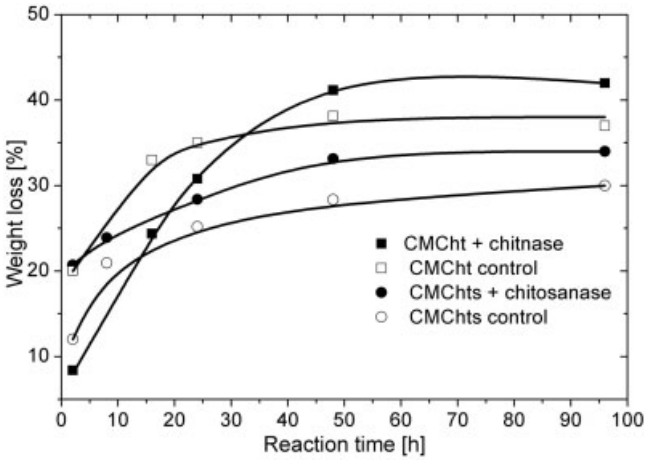

Figure 9 Reaction time influence on weight loss of CMCht and CMChts radiation crosslinked hydrogel. Radiation conditions as in Figure 7. Enzyme concentration 0.2 mg/mL, temperature 55 and 60°C for CMChts and CMCht, respectively.

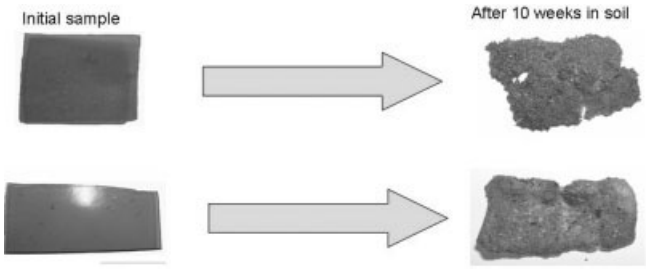

Figure 10 Pictures of (a) CM-chitosan and (b) CM-chitin kept in soil for 10-weeks time period.

during rain and collapses while drying under dry condition of atmospheric surroundings. Initial polymers for hydrogel fabrication are soluble in water and consequent crosslinked material is water swellable, which is in distinction with water-insoluble plastics. During the test, swelling/shrinking process of hydrogel can take place several times due to the fluctuations of weather conditions. Swelling occurs by imbibing natural water along with organic and inorganic particles such as sand. After the test duration, removal of those remains from the bulk of gel is impossible, and thus, evaluation of the degradation ratio becomes unrealizable.[21] The only estimation can be done by comparison of the shape and visual evaluation of samples.

Figure 10 shows photographs of CM-chitin and CM-chitosan hydrogel samples just before and after keeping in soil for 10 weeks. The size and thickness of samples kept in soil become smaller than their initial ones. In the case of CM-chitosan, even creation of some small wholes is noticeable. We can conclude that elongated storing of these samples in the ground would cause their complete diminishing as a result of bacterial activity.

Biodegradation under controlled conditions in composed soil

Microorganisms that are always present in compost interact with organic matter. They are involved in various processes such as production of enzymes, which speeds up the degradation process. According to ASTM standard,[22] the biodegradation efficiency was evaluated by measuring carbon dioxide secretion. Initial polymer samples as well as hydrogel synthesized by EB radiation have been keep in compost under controlled conditions for the period of 21 days and results are presented in Figure 11.

Amount of secreted CO_2 has been recalculated to a total degradation percentage and results are also shown in Figure 11. After 21 days, CM-chitin lost 8% and CM-chitosan 4% of their initial weight, whereas their unirradiated counterparts 6 and 2%, respectively. One can notice that degradation of both hydrogels is more effective than their initial polymers. An expla-

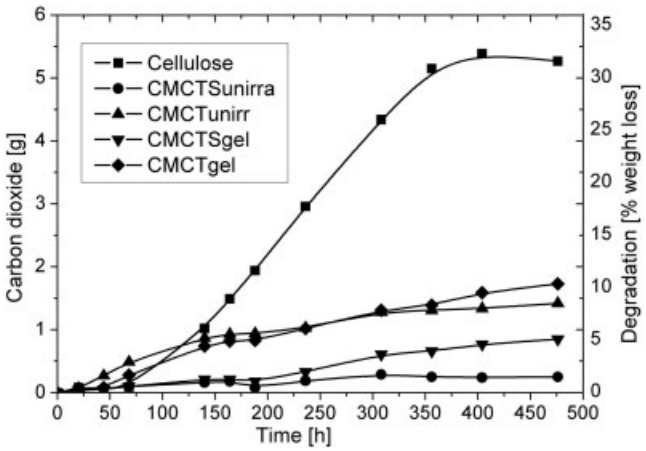

Figure 11 Microbial oxidative degradation analysis at 35°C of pure and irradiated by EB CMCht and CMChts samples.

nation for this phenomenon should be searched in a consideration of irradiation process itself. It was mentioned before that irradiation crosslinking reaction is always accompanied with chain scission reaction. During irradiation, about 70% of CM-chitin and 50% of CM-chitosan samples initial weights are changed in a form of gel; the rest remains soluble in a sol fraction. Crosslinking is accompanied by degradation of polymers residing in the sol part. It is apparent that these shorter chains undergo biodegradation more efficiently than the crosslinked hydrogels.

However, even introduction of a few crosslinking bonds do not restrain biodegradation process of hydrogel. For irradiated materials what we observed is in fact the sum of two degradations: partial degradation of unbonded polymer and crosslinking gels as a post radiation effect[23] of ionizing irradiation together with microbial biodegradation process.

CONCLUSIONS

CMCht and CMChts hydrogels have been synthesized without any additives, using ionizing radiation sources. The most efficient crosslinking process has been obtained for polymer concentration of 40% w/w for EB irradiation at ambient temperature and at 20–30% for γ-irradiation at low temperatures.

Hydrogels swelling was the highest just after exceeding gelation point and decreased with applied radiation dose. Biodegradation experiments revealed that both CMCht and CMChts undergo spontaneous degradation in soil and by enzymes, with satisfactory results.

Ease of radiation synthesis, outstanding swelling properties and biodegradability makes CMCht and CMChts excellent material for many future applications, especially in the biomedical field.

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