



13 Radiation Processing of Biodegradable Polymer Hydrogel from Cellulose Derivatives

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Abstract

The effects of high-energy radiation on ethers of cellulose: carboxymethyl-, hydroxypropyl- and hydroxyethylcellulose have been investigated. Polymers were irradiated in solid state and aqueous solution at various concentrations. Degree of substitution (DS), the concentration in the solution and irradiation conditions had a significant impact on the obtained products. Irradiation of polymers in solid and in diluted solution resulted in their degradation. A novel hydrogels of such natural polymers were synthesized, without using any additives, by irradiation at high concentration. It was found that high DS of CMC promoted crosslinking and, for all of the ethers, the gel formation occurred easier for more concentrated solutions. Paste-like form of the initial material, when water plasticized the bulk of polymer mass, along with the high dose rate and preventing oxygen accessibility to the sample during irradiation were favorable for hydrogel preparation. Up to 95% of gel fraction was obtained from 50 and 60% CMC solutions irradiated by gamma rays or by a beam of accelerated electrons (EB). The other polymers were more sensitive to the dose rate and formed gels with higher gel fraction while processed by EB. Moreover, polymers (except CMC) treated by gamma rays were susceptible to degradation after application of a dose over 50 – 100 kGy. The presence of oxygen in the system during irradiation limited a gel content and was prone to easier degradation of already formed gel. Produced hydrogels swelled markedly by absorption when paced in the solvent. Crosslinked polymers showed susceptibility to degradation by cellulase enzyme and by the action of microorganisms in compost or under natural conditions in soil thus could be included into the group of biodegradable materials.

Keywords: Hydrogel, Radiation Crosslinking, Biodegradation, CMC, Cellulose Ethers

1. Introduction

Polysaccharides such as cellulose and its derivatives, exposed to ionizing radiation, had been recognized as degraded type polymers since long [1]. The ethers of cellulose suffer the reduction of molecular weight while exposed on the influence of gamma rays or high-energy electrons from accelerators. For such modified natural polymers the main occurring reactions, initialized by radicals placed on macromolecules, lead to random cleavage of glycoside bonds in the main chain. Furthermore, irradiation of polymers of this type, like i.e. carboxymethylcellulose [2] resulted in their faster degradation when processed in diluted aqueous solution. Presence of water in a system causes an acceleration of radio-chemical reactions by the action of intermediate products of water radiolysis. The decomposition of water molecules results in creation of unstable species, like hydroxyl radicals, hydrogen atoms and hydrated electrons. Out of them hydroxyl radicals are the mains responsible for the transfer of reactivity from water to the polymer chains. They abstract hydrogen atoms from macromolecules and consequently macroradicals are formed. But an irradiation of some cellulose derivatives in an aqueous solution upon certain conditions resulted in formation of three-dimensional network of crosslinked gel. The first successful attempts have been done by Leavitt [3, 4]. The author suggested that the crosslinking reaction involved macroradicals created through an indirect effect of radiation by water radiolysis products.

Hydrogels are polymeric networks, which may absorb and retain a large quantity of solvent increasing their volume but still maintain the initial shape and poses some mechanical resistant. Physical gels are held together by secondary forces including ionic, H-bonding or hydrophobic interaction and/or molecular entanglements. All of these interactions are reversible and can be disrupted by changes in physical conditions or stress. Chemical gels, on the contrary, are insoluble in water and their crosslinked network can be damaged only irreversibly by rupture of covalent bonds [5]. Usually the network of hydrogel consist of hydrophilic polymers, bond together by chemical crosslinkers or, which is more convenient, by reactions between these polymers initiated by an ionizing radiation. The hydrophilicity of these materials is due to the presence of

hydrophilic groups along the polymer chains, such as $-\text{OH}$, $-\text{COOH}$, $-\text{CONH}$, $-\text{CONH}_2$ and others [6, 7].

Hydrogels possessing abilities to absorption of solvents of various ionic strength or pH have found applications in a wide range of industries as super-absorbents, water reservoirs (i.e. agriculture, forestry). Also biomaterials in medicine and pharmacy are among one of the basic utilization of hydrogels, i.e. wound care coverings, controlled drug delivery systems, dental materials, implants, ophthalmic applications and others [8]. Particularly, natural polymers or hydrogels, which consists of natural macromolecules or their composites with synthetic polymers, formed by irradiation technique have been under an attention of researchers due to their biodegradability and availability at low costs [9, 10]. Ethers of cellulose, due to their novel feature of gel formation ability and easy biodegradation seem to be an excellent material. In the present research selected cellulose ethers were examined under ionizing radiation. It was found that carboxymethylcellulose (CMC), hydroxypropylcellulose (HPC) and hydroxyethylcellulose (HEC) form biodegradable hydrogels when irradiated in moderately concentrated solutions.

2. Experimental

2.1. Materials

Four ethers of cellulose were used in our investigations: carboxymethylcellulose (CMC) obtained from Daicel Co. Ltd., Japan, hydroxypropylcellulose (HPC) from Nippon Soda Co. Ltd. Japan and hydroxyethylcellulose (HEC) from Sumitomo Seika Co. Ltd. Japan. Characteristic of these polymers is summarized in Table 1. Average molecular weights were determined by measuring the intrinsic viscosity. Cellulase C-0901 enzyme, from *penicillium funiculosum* was obtained from Sigma Chemical Co., USA. All chemicals were analytical grade.

2.2. Sample preparation and irradiation

Deionized water was added to the polymer and mixed. For higher concentrations, above 5%, due to its high viscosity, the polymer was kneaded well with water. Then, material was kept for few days at room temperature to ensure complete dissolution and uniform distribution of polymer chains. Higher concentrated solutions had a form of very thick, thixotropic, paste-like gels. In the presence of air, irradiation of the mixture was carried

out in polyethylene bag; for air-free irradiation, the mixture was heat-sealed in poly(vinylidene chloride) bag to avoid the penetration of oxygen, after the removal of air by a vacuum machine.

Table 1. Characterization of cellulose ethers samples.

Sample	Degree of substitution (DS)	Intrinsic viscosity dL./g	Weight-average molecular weight
CMC 2.2	2.2	5.59	5.22×10^5
CMC 1.29	1.29	7.38	5.39×10^5
CMC 0.86	0.86	6.79	4.22×10^5
HPC1	3.0	4.49	6.60×10^5
HPC2	3.0	7.98	12.5×10^5
HEC7	2.0	3.72	2.02×10^5
HEC8	2.0	5.39	3.09×10^5
HEC9	2.0	9.35	5.82×10^5

Irradiation of samples was conducted with gamma rays generated from a ^{60}Co source at a dose rate of 10 or 1 kGy/h at inert temperature. For irradiation by high-energy electrons, the 2MeV accelerator was used at the irradiation parameters: current 1 mA, voltage 1 MeV and the dose per pass 10 kGy.

2.3. Viscosity and molecular weight of polymers

Initial weight-average molecular weights of the polymers were determined from an intrinsic viscosity on the basis of the Mark-Houwink equation $[\eta] = K \times \text{DP}^a$. K and a are constants, DP is the weight-average degree of polymerization and M_w is the weight-average molecular weight. To calculate a molecular weight obtained DP was multiplied by the average mass of the substituted anhydroglucose unit. The intrinsic viscosity was measured by an Ubbelohde viscometer in water or 0.1M NaCl solution, in the case of CMC at 25°C. The intrinsic viscosity were found by plotting obtained reduced viscosity η_{sp}/c and $\ln(\eta/\eta_{sp})/c$ against concentration [in g dl⁻¹] and extrapolating to zero concentration. For these polymers constant K is equal to 1.8×10^{-2} , 7.2×10^{-3} , 3.16×10^{-3} and a to 0.97, 0.915, 0.55 for CMC [11], HPC [12] and HEC [13], respectively.

Viscosity of degraded samples was examined with a rotary viscometer made by Tokyo Keiki Co. Ltd., Japan, after dilution to the concentration of 1%.

2.4. Gel content and swelling of hydrogel

The gel content was estimated gravimetrically by measuring its insoluble part after extraction of sol. Thus, hydrogel was kept in deionized water for 7 days at room temperature, occasionally shaken. The residue was made up of the crosslinked gel only.

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, G_d is the weight of insoluble part after extraction with water. The swelling of crosslinked hydrogel was estimated according to Japan Industrial Standard (JIS) K8150. The dry gel was immersed in deionized water for 24h at room temperature. Swelling, in grams of absorbed solvent per gram of dried gel, was calculated as follows

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

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

2.5. Biodegradation methods

Enzymatic degradation was carried out using a Cellulase C-0901 enzyme, from *penicillium funiculosum* in an acetic acid - NaOH buffer of pH 5.0 at 37°C with shaking. After an extraction of the soluble part, samples of gel of about 10mg were incubated in an enzyme solution at different times. Concentration of the enzyme in buffer was 0.1 mg mL⁻¹. Washing with an excess of distilled water and drying in vacuum at 35°C to a constant weight followed the incubation. The result of degradation is expressed as a percentage of the weight loss

$$\text{Weight loss \%} = (W_0 - W_1) / W_0 \times 100\% \quad (3)$$

where W_0 and W_1 are, respectively, the weights of gel before and after enzymatic treatment.

The microbial degradability of polymer under the controlled soil was evaluated by the production of CO₂. Specially designed apparatus – MODA: Microbial Oxidative Degradation Analyzer [14], comprised of 4 independent lines of columns, was used. 10 grams of the sample along with rinsed sea sand - 450 g, and compost - 130 g, after mixing, was placed in a heated reaction column. Inside the column monitored temperature of the test was 35°C and the flow of the carbon dioxide-free but moisturized air was 30 ml min⁻¹. After flowing by the sample, the air, caring formed due to polymer decay CO₂ was passing through a series of columns filled in turn with silica gel, calcium

chloride, soda lime and calcium chloride. Ammonia, also formed from the sample, was trapped in sulfuric acid solution, and water vapor was absorbed into first two columns (silica gel and calcium chloride). The CO_2 was collected quantitatively by soda lime and producing during the reaction water was caught in the last CaCl_2 column. Thus mass of produced carbon dioxide was calculated as a difference in the weight of two last columns (containing soda lime and calcium chloride) 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.

3. Results and discussion

3.1. Degradation

The all examined polymers exposed on γ -rays in solid state and in diluted aqueous solution in the atmosphere of air as well as under vacuum undergo mainly degradation. At Figure 1 there is presented the degradation of HPC2 as the ratio of the viscosity: V_{irr}/V_0 (viscosity after irradiation / initial viscosity of the polymer). Solutions were examined after dilution to the concentration of 1%. Samples lessen their viscosity the most significant at the early stages of irradiation, at low doses. Then, the rate of decreasing becomes not so high, reaching the level of pure solvent at high doses. The rate of viscosity reduction is lower for polymer irradiated in solid than irradiated in solution. It proves that presence of water accelerates radiation-induced reactions by indirect effect, through intermediate products of water radiolysis, here – breaking of the main chain. The reduction of the solution viscosity is directly related to molecular weight with emphasis

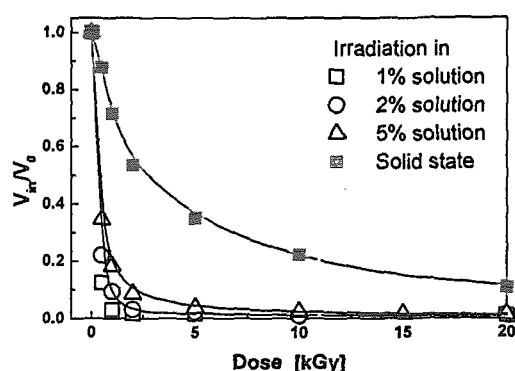


Fig. 1. Degradation of HPC2 irradiated in solid and diluted aqueous solution. Viscosity after dilution to 1% concentration: V_{irr} – after irradiation, V_0 – initial.

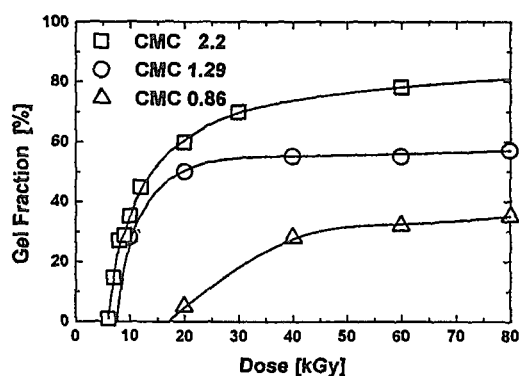


Fig. 2. Crosslinking of CMC of different degree of substitution.

on the presence of longer chains. Polymers decompose to the form of low molecular compounds, like oligo- or mono- saccharide. The cleavage of bonds in normal chain occurs randomly; thus the longest chains suffer from the most frequent breaks. It was postulated that cellulose and its derivatives degrade by the rupture of glycosidic bonds, which are the weakest points in such macromolecules. [15, 16]. HPC1, the lower molecular fraction of the same polymer, CMC and HEC, when irradiated in solid state or in dilution, degrade at the same manner under gamma rays.

3.2. Hydrogel of carboxymethylcellulose

3.2.1. Crosslinking of CMC and swelling

Crosslinking successfully competes with glycoside bond cleavage as the concentration of polymer in a solution oversteps the critical value, then insoluble gel appears. Gelation occurrence depends on the molecular weight, the degree of substitution (DS) of the cellulose unit and irradiation conditions. CMC with higher degree of substitution build gel easier and more efficiently than that of lower DS as presented at Figure 2 for irradiation of 20% solutions. CMC of DS 2.2 gives the highest gel content among the three samples, about 80%, moreover, CMC of DS 1.29 and 0.86 produce ca. 60 and 35% of gel, respectively. Hence, it can be concluded that intermolecular linkages are formed as a result of the ether function. At Figure 3 the gel fraction against delivered dose is showed for CMC 2.2 irradiated at high concentrations after degassing by EB, which does not differ from the results got for EB processed samples. Irradiation of polymer in concentration of 50 to 60% by gamma rays and by a beam of accelerated electrons resulted in obtaining high gel fraction ca. 90 and 95%, respectively.

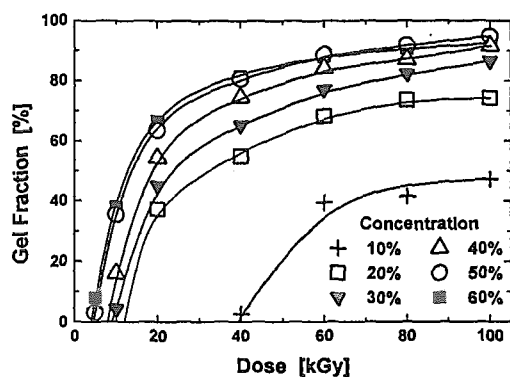


Fig. 3. Crosslinking of CMC 2.2 irradiated in aqueous solution by EB without an access of air.

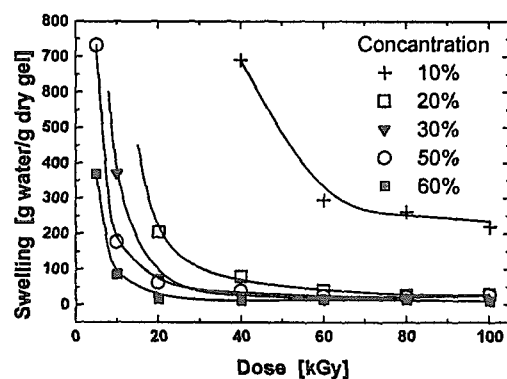


Fig. 4. Swelling of CMC 2.2 hydrogels formed by EB in water.

Medium concentrated solutions form not as high gel fraction but the maximum water uptake greatly enhances. In Figure 4 swelling of hydrogels is presented. CMC 2.2 formed by EB exhibit various swelling properties related to initial concentration of polymer and applied dose. Swelling has the maximum value for hydrogels at low doses of irradiation and drastically reduces with increasing the dose. After the dose oversteps so called 'gelation dose' the gel is formed, statistically one crosslink per chain is necessary [17]. The network is weak and susceptible for breaking but, because of a relatively low number of intermolecular bonds, is able to expand by holding a solvent into its voids. With subsequent increasing the density of crosslinks due to the further irradiation the absorption ability of the gel decreases. The hydrogel structure becomes more tightly connected and rigid. The swelling remains at the level of ca. 20, even for hydrogels irradiated at the dose as high as 100 kGy. An exception is the hydrogel formed from 10% CMC solution at 100 kGy, which can absorb over 220 grams of water per one gram of dried gel.

3.2.2. Enzymatic degradation of CMC

Crosslinked hydrogels were degraded by cellulase enzyme in acetic acid – NaOH buffer pH 5.0, The results shown in Figure 5, for an example of CMC 2.2, demonstrates high degradability of the investigated material. Hydrogels were prepared from 50% CMC aqueous solutions by gamma irradiation at various doses. Hydrogels of less applied dose degrade faster than those crosslinked more tightly at higher dose due to the increasing

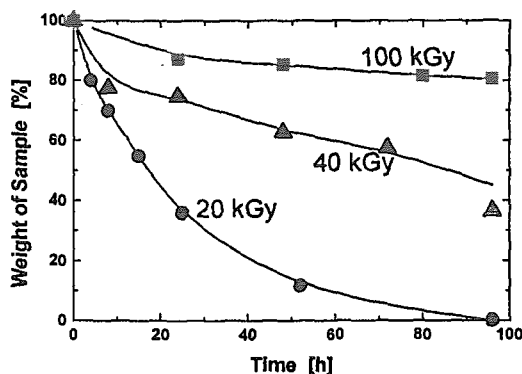


Fig. 5. Enzymatic degradation of CMC 2.2 hydrogel formed from 50% aqueous solution by γ -rays in with no access of air, cellulase enzyme in acetic acid – NaOH buffer, pH 5.0 at 37°C.

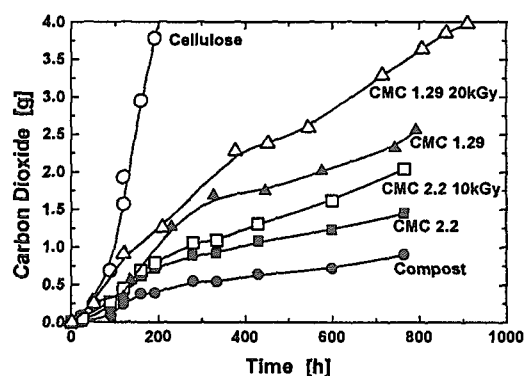


Fig. 6. Microbial oxidative degradation of CMC 2.2 and 1.29 unirradiated and irradiated in the compost soil at 35°C.

number of intermolecular bonds. Samples formed by the energy of 20 kGy disintegrate completely after 95 hours, while those formed by 10 kGy disappear before 8 hours of enzymatic treatment – not showed at the picture – despite, they possess relatively high gel fraction, over 40%. For gels irradiated at dose 40 kGy and above the degree of degradation do not exceed 60% after 96 hours of incubation. The material characterized by a huge swelling ability – the most desired from the point of view of further applications – irradiated at low doses, undergo the fastest biodegradation by the action of the enzyme.

3.2.3. Microbial degradation of CMC

Microbial degradability was evaluated by measuring of carbon dioxide produced from decomposed polymeric material. Samples of carboxymethylcellulose of DS 1.29 and 2.2, also cellulose as a reference material, were used in the experiment. The results are presented in Figure 6. Cellulose undergoes the fastest degradation, which reaches c.a. 80% after 1 month (after subtraction of the carbon dioxide produced in blank test – only compost). CMC decompose much slower but the rate of producing of CO₂ is still significant. After 1 month CMC 2.2 degrades in 3.8% - unirradiated and 8.4% - irradiated and CMC 1.29 degrades in 12.3% and 18.7%, respectively. The higher substituted samples disintegrate slower. Side chains prevents from cleavage of backbone by making the access of microorganisms to the vicinity of glycosidic bond difficult due to a spatial atomic configuration. Radiation processing of the polymer improves its susceptibility for bacterial digestion. Polymers irradiated in 20% aqueous solution at low dose, 10 and 20 kGy for DS 2.2 and 1.29 respectively, yield faster decomposition, in spite of some crosslinks were introduced. The gel fraction of examined hydrogels was 15% for CMC 2.2 and 47% for CMC 1.29. It can be explained in that way, that even in crosslinked hydrogels, polymer chains are shorter than in initial material. During irradiation of polysaccharides scission is the predominant reaction, but with increasing the degree of etherification crosslinking enhances its share. Random scission causes in diminishing average molecular weight and consequently, degraded as well as not degraded chains can be crosslinked. Hence, after irradiation unbonded macromolecules exist beside the network of gel and it is apparent that they undergo the fastest biodegradation. Crosslinked material also experience decomposition process. Hydrogel consist of chains, which are mainly cut for smaller fractions but bonded each to another. The existence of a

few intermolecular crosslinks per initial chain should not inhibit greatly the total degradation. Thus, it is postulated that on the increasing of biodegradation rate of irradiated hydrogel, apart the radiation degraded chains, has also crosslinked fraction.

3.3. Hydrogel of hydroxypropyl and hydroxyethylcellulose

Opposite to CMC 2.2, crosslinking of HPC and HEC differs considerably due to the type of irradiation. The gel fraction is significantly higher when polymer is irradiated with high dose rate as presented in Figure 7 for an example of HPC2 in 20% aqueous solution. EB irradiation results in the content of gel as high as 85%. Gamma processing with the dose rate 10 and 1 kGy/h gives maximum part of gel less than 60 and 30, respectively. The concentration of free radicals in the system, determined by the dose rate, is the important factor influencing the gel formation. To the intermolecular recombination it is required the existence of two such species on neighboring chains at the same time. The probability of such a coupling is higher as higher is a dose rate. Under the present conditions of irradiation the concentration of radicals in the system should have differed 500 (or 5000 for 1 kGy/h) times. It is due to the fact that EB gives more than 500 times higher dose rate comparing to 10 kGy/h gamma irradiation.

The presence of air causes degradation, by increasing the yield of scission. For γ -irradiated HPC2 glycosidic bond cleavage finally prevails over crosslinking and the created network of the gel once more becomes soluble. During irradiation the oxygen present in the system diminishes the maximum of gel fraction for the all investigated polymers. It is due to the reaction of oxygen with macroradicals, which transform into

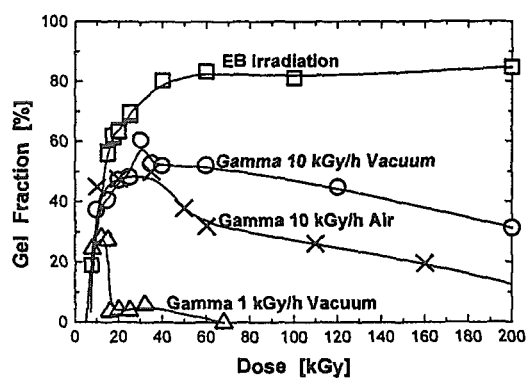


Fig. 7. Crosslinking of HPC2 irradiated at 20% aqueous solution at different dose rate.

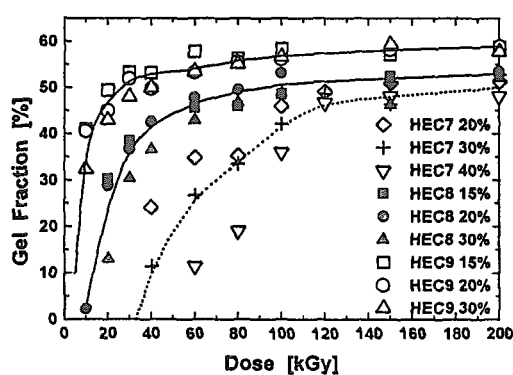


Fig. 8. EB irradiation of various molecular weight samples of HEC in aqueous solution without an access of oxygen.

peroxy-macroradicals, and subsequently decompose by rupture of main chain bond of the macromolecule [18]. Also HEC and CMC undergo degradation while irradiated in air.

The length of a polymer chain affects the results of irradiation as depicted in Figure 8 for an example of HEC; the degree of molecular substitution was 2.0 for all fractions. Higher initial average molecular weight of the polymer enhances a share of gel. The maximum gel fraction reaches the level of 50, 53 and nearly 60% for increasing molecular weight of samples. Moreover, growing of the insoluble part at initial stages of gelation also occurs earlier, lower gelation dose, and faster for larger macromolecules. Thus, the energy utilized to produce 50% of gel of HEC9 corresponds to 25 kGy, while for HEC8 and HEC7 is as much as 80 and 200 kGy, respectively. Comparable results, with a strong dependence on the initial molecular weight and irradiation conditions were obtained for the other investigated polymers, except the effect of dose rate on CMC.

Conclusion

Hydrogels from four kinds of cellulose ethers were synthesized by ionizing radiation without any additives. It was found that high concentration in aqueous solution and more complete substitution by a side chains in glucopyranose ring of a polymer were favorable for crosslinking. Considerable higher gel fraction was obtained for solutions irradiated by EB than that processed by gamma rays, except for CMC for which gel formation was only slightly dependent on the dose rate. Access of oxygen to the sample during irradiation causes diminishing of the maximum gel content. Formed hydrogels was able to swell significantly by water absorption, and the swelling varied due to the concentration in which the polymer was irradiated and to applied dose. Test carried out by using a cellulase enzyme and in activated compost, confirmed that obtained hydrogels are biodegradable.

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