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Synthesis of biodegradable hydroxyethylcellulose cryogels by UV irradiation

Petar Petrov [a,*], Elissaveta Petrova [a], Bozidar Tchorbanov [b], Christo B. Tsvetanov [a]

[a] *Institute of Polymers, Bulgarian Academy of Sciences, ''Akad. G. Bonchev'' str. 103A, 1113 Sofia, Bulgaria*
[b] *Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

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Abstract

Biodegradable macroporous hydroxyethylcellulose (HEC) cryogels of good quality and high gel fraction yield (95%) were synthesized via a facile method. The latter involved a relatively fast preparation of homogeneous semidilute solution of polymer and photoinitiator, (4-benzoylbenzyl)trimethylammonium chloride, followed by freezing at a defined negative temperature, an extremely short UV irradiation and subsequent thawing. HEC cryogels were successfully prepared also by using H_2O_2 as a photoinitiator. The effects of the temperature of freezing, the HEC molecular weight and the concentration of HEC solution on the cross-linking efficiency, the swelling ratio and the enzymatic degradation of HEC cryogels were investigated. Due to the cryoconcentration phenomenon, cryogels are formed at substantially low initial concentrations of the studied polymers. The highest values of gel fraction yield are achieved in the 1–2 wt.% concentration range at −20 °C. As a rule, the higher the molecular weight, the greater the gel fraction yield of the resulting cryogels. Scanning electron microscopy (SEM) analysis reveals that the interior structure of HEC cryogels is completely different from the conventional HEC hydrogels. HEC cryogels undergo decomposition by the action of cellulase enzyme, however, due to their specific morphology, the rate of degradation is slower compared to the conventional HEC hydrogel of similar gel fraction yield.

Keywords: Cryogels; UV irradiation; Hydroxyethylcellulose

1. Introduction

Hydrogels based on both natural and synthetic polymers have found various applications in medicine and pharmacy as drug and cell carriers, tissue engineering matrices, membranes for biosensors, contact lenses, etc. [1–3]. Due to environmental issues, there is a growing interest in developing natural polymer-based hydrogels that guarantee biodegradability. In particular, the cellulose derivatives have received considerable attention because of their water solubility, easy biodegradation and low costs. Hydrogels of cellulose derivatives can be obtained either by reaction with chemical reagents [4–6] or by ionizing radiation [7–11]. Generally, two competing processes, degradation and cross-linking, take place at high-energy irradiation of cellulose derivatives with either electron beam or gamma rays [7,8,10]. It has been found that the examined polymers undergo degradation when exposed to ionizing radiation at ambient temperature in solid state and in aqueous solutions of low concentration (less that 10 wt.%), while the best results of cross-linking have been obtained at paste-like conditions (25–40 wt.%, depending on the polymer). Noteworthy, in the case of highly concentrated polymer solutions several days are required for complete dissolution of the cellulose derivative in water [7].

Nowadays, there is an increasing interest on macroporous polymer gels prepared by cryotropic gelation [3,12–18]. The cryogels obtained are macroporous materials with an open porous structure, which significantly increases their equilibrium

* Corresponding author. Tel.: +35929792281; fax: +35928700309.
E-mail address: ppetrov@polymer.bas.bg (P. Petrov).

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sorption properties due to capillary effects. The process of cryogel formation involves a moderate freezing of the system, a reaction of cross-linking and a subsequent thawing. In this process, most of the water is frozen and forms ice crystals while the non-freezable water and the soluble substances are accumulated in a non-frozen liquid microphase (NFLMP). The gel formation occurs in this liquid microphase and the ice crystals perform as porogens. Due to the so-called cryoconcentration effect and some other factors (for example, increased dielectric constant of the medium upon cooling), an acceleration of chemical reactions is observed within a defined range of negative temperatures [13].

In the last decade, in our laboratory, numerous hydrogels based on high molecular weight poly(ethylene oxide) have been prepared by employing the UV-irradiation technique [19–24]. The advantages of the UV irradiation are the very low capital outlay and the extremely short time for efficient gel formation. Very recently, Petrov et al. [25] reported on the first effective cross-linking of various cellulose derivatives via UV irradiation of moderately frozen systems on the basis of semidilute aqueous solutions of the polymers. It was suggested that due to the cryoconcentration effect, the polymer concentration in the non-frozen liquid microphase is very high and the reaction conditions resemble the conditions in the paste-like state. Therefore, during UV irradiation the reactions of cross-linking prevail over the chain scission reactions and cryogels of cellulose derivatives can be formed.

In this paper, the effect of different synthesis conditions, including polymer concentration, molecular weight and temperature of freezing, on the preparation of hydroxyethylcellulose cryogels is reported. The interior morphology and the enzymatic degradation of these new materials were investigated and compared to a conventional hydroxyethylcellulose hydrogels.

2. Experimental part

2.1. Materials

Hydroxyethylcelluloses (Table 1) were donated by Hercules Inc. (Aqualon Division) and used as received. (4-Benzoylbenzyl)trimethylammonium chloride (BBTMAC), H_2O_2 (30 vol.% water solution) and divinylsulphone (DVS) were purchased from Aldrich and used without purification. Cellulase enzyme [EC 3.2.1.4] or 1,4-β-D-glucan 4-glucano-hydrolase from *Basidiomyces* sp. was purchased from Merck.

Table 1
Characteristics of HECs as specified by the supplier

Hydroxyethylcellulose type	Approximate molecular weight (g/mol)	Degree of substitution	Molar degree of substitution
Natrosol 250HHR	1,300,000	1.5	2.5
Natrosol 250GR	300,000	1.5	2.5
Natrosol 250LR	90,000	1.5	2.5

2.2. Cryogels preparation

An appropriate amount of each polymer, dissolved in 13 mL of distilled water to obtain semidilute solution (0.3–8 wt.%), was heated at 40 °C for 20 min and then the sample was kept for 16 h at room temperature to ensure complete dissolution and homogeneity. Given amount of photoinitiator, BBTMAC (2 wt.% with respect to the polymer) dissolved in 2 mL water was added under stirring at room temperature. The resulting homogeneous solution was poured into eight Teflon dishes (20 mm diameter) forming a 2.5 mm thick layer, which was then kept in a freezer at defined temperature for 2 h. The dishes were then quickly placed in a thermostated open chamber connected with a ''Julabo'' cryostat apparatus. The frozen system was irradiated with full spectrum UV–vis light at given negative temperature with a ''Dymax 5000-EC'' UV curing equipment with 400 W metal halide flood lamp for 2 min (dose = 11.4 J/cm^2; input power = 93 mW/cm^2).

In control experiments, hydrogels of hydroxyethylcellulose were obtained by reaction with a cross-linking agent, DVS. First, 0.15 g of polymer was dissolved in 13 mL aqueous solution of NaOH (pH = 12.5), heated at 40 °C for 20 min and then the sample was kept for 16 h at room temperature to ensure complete dissolution and homogeneity. DVS (0.0087 mL, 0.0217 mL, 0,0433 mL and 0.0867 mL) dissolved in 2 mL distilled water was added to the polymer solution under stirring and the reaction was carried out for 15 h at 30 °C under air.

2.3. Measurements of gel fraction yield and equilibrium degree of swelling

Gel fraction (GF) yield and equilibrium degree of swelling (ES) of the cryogels and hydrogels were determined gravimetrically. The GF content in the dried sample was estimated by weighing the insoluble part after extraction in distilled water for 7 days at room temperature. GF yield [%] = (weight of dried sample/initial weight of polymer) × 100.

The ES was determined by applying two different approaches for drying the samples: (i) freeze drying and (ii) air-drying at 30 °C overnight and subsequent drying under vacuum. Disks of dried cryogel were weighted and then immersed in distilled water at room temperature and an equilibrium water uptake was reached (at least 72 h). The surface of the cryogel was blotted by filter paper prior to weighing. ES = weight of swollen sample/weight of dried sample. ES before drying of the samples was determined by first weighing the swollen cryogels extracted for 7 days in water and then by drying to obtain the weight of the dried samples.

The experimental errors of the GF yields and the ES calculations are ±3%. In all figures, the size of the symbols corresponds to the experimental error.

2.4. Scanning electron microscopy studies

The extracted gels were quickly frozen in liquid nitrogen, fractured and freeze dried in an ''Alpha 1-2 Freeze Drier''

(Martin Christ) at −55 °C and 0.02 mbar for 24 h. The interior morphology of the gels was studied by using a JEOL JSM-5510 scanning electron microscope operating at 10 kV. Before observations, the gel specimens were fixed on a glass substrate and coated with gold for 60 s.

2.5. Enzymatic degradation

Enzymatic degradation was carried out in an acetic acid–sodium hydroxide buffer at pH 5.0 by cellulase enzyme. About 10 mg of a freeze dried gel was immersed in 10 mL of the enzyme solution for a given time. The enzyme concentration was 0.2 mg/mL or 2 units/mL. The cellulose activity was assayed using 0.6% solution of sodium carboxymethylcellulose as substrate at 30 °C and pH 5.0. One unit of cellulase activity is defined as the amount of enzyme liberating 1 μmol reducing sugars in 1 h. Tests were carried out at the most appropriate temperature for the enzyme activity, 30 °C, without shaking. After incubation, the samples were immersed in an excess of distilled water and extracted for 24 h to wash away the degraded polymer. Finally, the gels were dried at 30 °C under vacuum. The result from enzymatic degradation (D) is expressed as a percentage of the initial sample weight: D [%] = (weight of dried sample after enzymatic degradation/initial weight of dried gel) × 100.

3. Results and discussion

3.1. Synthesis of hydroxyethylcellulose cryogels

Hydroxyethylcellulose cryogels were prepared by UV irradiation of moderately frozen systems for 2 min using a water soluble photoinitiator, (4-benzoylbenzyl)trimethylammonium chloride (2 wt.% with respect to the polymer), and subsequent thawing. The cryogels obtained are opaque materials and a significant part of water (capillary-bound water) can be easily separated by compression. The influence of the irradiation dose and the amount of BBTMAC on the cross-linking efficiency of moderately frozen systems based on cellulose derivatives were described elsewhere [25]. It is established that 2 min irradiation at an irradiation dose rate of 5.7 J/cm^2 min is adequate for cross-linking of semidilute polymer solutions containing at least 2 wt.% BBTMAC. In this work, we have studied in detail the effect of the temperature of freezing, the polymer concentration and the polymer molecular weight on the cross-linking efficiency and the cryogels properties.

The important features in the process of cryogel preparation are: (i) to freeze the solvent before the beginning of cross-linking reactions and (ii) to find a range of negative temperatures within which a fraction of non-frozen water remains in the system (moderately frozen system) [13]. Due to the non-freezable solvent molecules, the non-frozen microphase retains liquid and thus the solutes accumulated into NFLMP possess sufficient molecular or, in the case of polymers, segmental mobility, which is the critical point for the successful preparation of cryogels of cellulose derivatives by UV irradiation. In order to ensure regular cryostructuration, the semidilute hydroxyethylcellulose solutions were at first kept at a given negative temperature for 2 h and then irradiated with UV light. The influence of the temperature of freezing on the cross-linking efficiency within the temperature range from −10 °C to −30 °C was investigated. Fig. 1 shows the gel fraction yield of cryogels obtained by freezing followed by irradiation with UV light of 1 wt.% hydroxyethylcellulose (Natrosol 250HHR; molecular weight *ca*. 1,300,000) solutions in water at various negative temperatures. Cryogels of good quality and high GF yield were prepared in the range between −15 °C and −30 °C, however, the maximum value of GF yield (95 ± 3%) was reached at −20 °C. In a control experiment, 1 wt.% HEC solution was frozen in liquid nitrogen (−196 °C) for 30 min and then immediately irradiated with UV light. The resulting gel was torn during the extraction with water and the determined GF yield was as low as 40%. This experiment shows the important role of the conditions used to prepare the specimens. It seems that the fast freezing in liquid nitrogen hinders the process of regular cryostructuration, possibly decreases the amount of non-frozen water which results in lower yield of gel fraction.

Fig. 2 shows the dependence of the GF yield of HEC cryogels prepared at −20 °C on the concentration of HEC solutions. Cryogels are formed at substantially low initial concentrations of HEC solutions in the studied range of molecular weights. Undoubtedly, this phenomenon is due to the cryoconcentration of polymer and photoinitiator in the NFLMP. The GF yield increases with increasing concentration and reaches a maximum at polymer concentration of 1 wt.% for HECs with molecular weight 1,300,000 wt.% and 300,000 wt.%, and 2 wt.% for HEC with molecular weight 90,000 and decreases at higher concentrations. Obviously, the best conditions for cryostructuration and cross-linking of the studied HECs systems are achieved in the 1–2 wt.% concentration range. Noteworthy, the higher the molecular weight of the

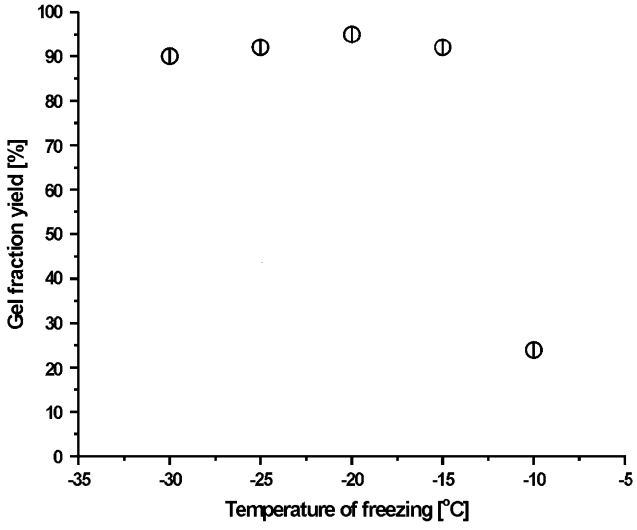

Fig. 1. Gel fraction yields of 1 wt.% HEC (Natrosol 250HHR) systems frozen and irradiated with UV light at various negative temperatures (2 wt.% BBTMAC; irradiation time: 2 min).

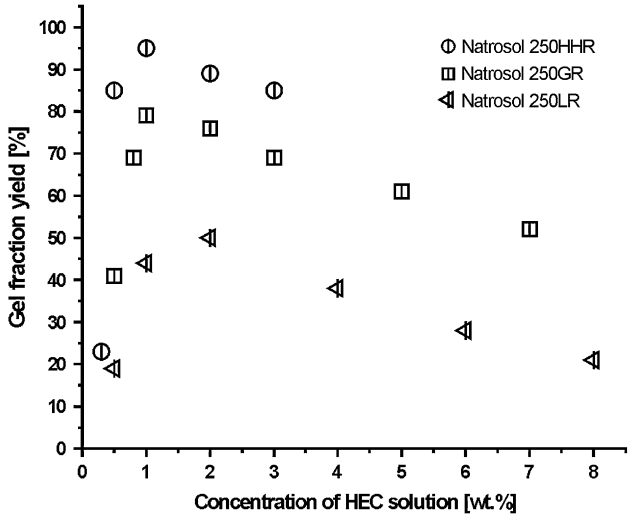

Fig. 2. Gel fraction yields of HEC cryogels prepared from HECs of different molecular weights at different polymer concentrations (temperature of freezing: −20 °C; 2 wt.% BBTMAC; irradiation time: 2 min).

polymers used in this study the higher the GF yield of cryogels.

The main advantages of our method are the relatively fast preparation of a homogeneous semidilute solution of the polymer and BBTMAC and the extremely short irradiation time required for obtaining high quality cryogels. Moreover, in preliminary experiments, HEC cryogels were successfully prepared by using H_2O_2 as a photoinitiator. Thus, these HEC cryogels could be considered as ''green'' materials that can be degraded to natural products. The GF yield of HEC cryogels obtained with 2 wt.% H_2O_2 (30 vol.%) is in the 70–80% range, however, more detailed studies are in progress.

3.2. Interior morphology

Although the drying process can affect the gels morphology, we believe that the structure of the freeze dried specimens closely resembles the morphology of hydrated gels. The interior morphologies of a HEC cryogel (Natrosol 250HHR; molecular weight *ca.* 1,300,000) and a conventional HEC hydrogel (prepared by chemical cross-linking of the same HEC with DVS) are shown in Fig. 3. The HEC cryogel has a macroporous structure with large interconnected pores (50–200 μm) surrounded by dense thin walls (*ca.* 1 μm). This is a typical example of a sponge-like morphology that imparts opacity of the cryogels. The conventional HEC hydrogel is a transparent material and exhibits a distinctly different interior structure consisting of nearly round shaped and closed pores surrounded by thick walls.

As already mentioned above, in a moderately frozen system, two phases exist: solvent crystals and non-frozen liquid microphase. After thawing the frozen HEC systems, pores are formed in the spaces originally occupied by ice. Since these ice crystals act as the pore-forming agent, the size and the number of the pores will depend on the size and the

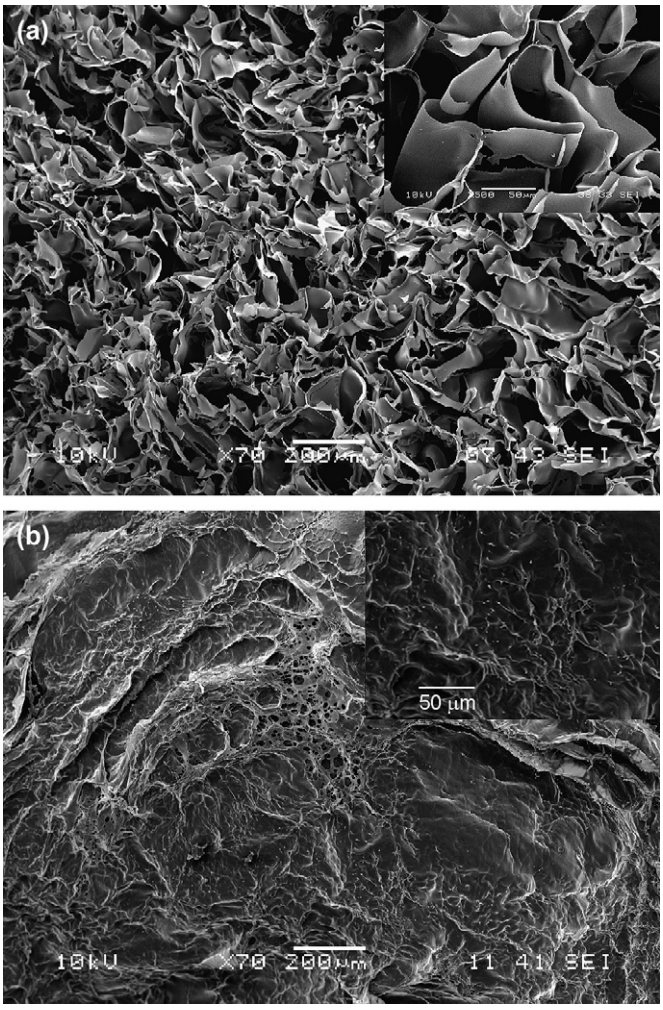

Fig. 3. SEM micrographs of (a) HEC cryogel (1 wt.% Natrosol 250HHR frozen at −20 °C; 2 wt.% BBTMAC; irradiation time: 2 min) and (b) conventional HEC hydrogel (1 wt.% Natrosol 250HHR cross-linked with DVS).

number of crystals formed in the process of cryostructuration, which depends mainly on the freezing procedure and the relative concentration of the solution [3,12,13].

3.3. Equilibrium degree of swelling

We found that the drying methods used to determine ES affect the swelling behavior of the HEC cryogels in water. When air-dried at 30 °C, the material shrinks and loses its macroporosity. Such dried material undergoes only partial recovery of porosity on rewetting. As shown in Fig. 4, the air-dried HEC hydrogels prepared at different experimental conditions exhibit almost the same very low ES. This could be explained by the fact that during air-drying the strong surface tension of the evaporating water causes cohesion of the cellulose macromolecules resulting in a tight mass [26], which increases the hydrophobicity of the gel. In contrast, the freeze dried HEC cryogels show a much better swelling in water and the ES almost reaches the estimated ES of HEC cryogels before drying. Evidently, in the case of freeze dried specimens,

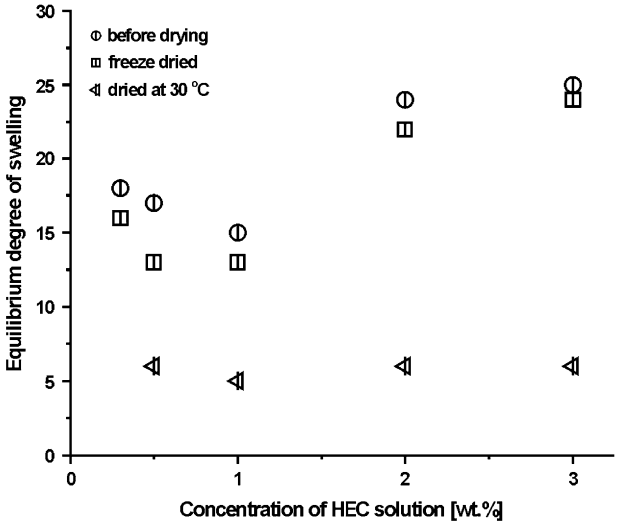

Fig. 4. Equilibrium swelling of HEC cryogels (Natrosol 250HHR frozen at −20 °C; 2 wt.% BBTMAC; irradiation time: 2 min) at different polymer concentrations dried in different modes prior to swelling measurements.

the materials preserve their original morphology and the dependence of the swelling ratios on the experimental conditions like polymer concentration and molecular weight is well manifested (Figs. 4 and 5). It is found that ES strongly depends on the GF yield and increases proportionally (except the first two concentrations of each HEC) with the decrease of the GF yield. Here, one can speculate that in the HEC cryogels with lower GF yield the cross-linking density is lower which results in higher ES, however, the HEC cryogels are heterogeneous materials and more complex systems compared to the conventional HEC hydrogels. In the cryogels different types of water exist in the polymer network, water bound to the polymer via hydrogen bonds which characterizes the cross-linking efficiency and capillary-bound water that fills the space of macropores. We found that the capillary-bound water is the major fraction of our systems since more that 65% of water can be easily separated by compression. Therefore we assume that the calculated ES values are only apparent values giving information about the overall amount of water in the cryogels and cannot be applied for exact characterization of the cross-linking density of heterogeneous macroporous cryogels.

From a practical point of view, HEC cryogels with different swelling properties can be prepared either by varying the experimental conditions or by applying different methods of drying. Thus, the HEC cryogels could meet some specific requirements for the uptake and release of compounds.

3.4. Enzymatic degradation

Degradation of hydrogels is a desired process when they are utilized in medicine, agriculture, etc. It is known that HEC hydrogels undergo decomposition by cleavage of the glycosidic linkages by the action of cellulase enzyme or microorganisms from compost [10]. Here we report, as far as we know, the first test of enzymatic degradation of HEC cryogels by cellulase enzyme. Fig. 6 shows the weight loss of HEC cryogels with different GF yield as a function of the degradation time. A blank test in buffer solution without the enzyme showed a mass decrease of each gel by less than 3%. The degradation of the cryogels proceeded at a higher rate during the first 12 h and then slowed down. The results are strongly linked with the GF yield and the cross-linking density, respectively. The HEC cryogel of the lowest GF yield (50%) is completely decomposed after 12 h, while the cryogel of the highest GF yield (95%) exhibits the strongest resistance to the enzymatic degradation. In other words, the cryogel of lower cross-linking density is affected more severely by the enzyme because of

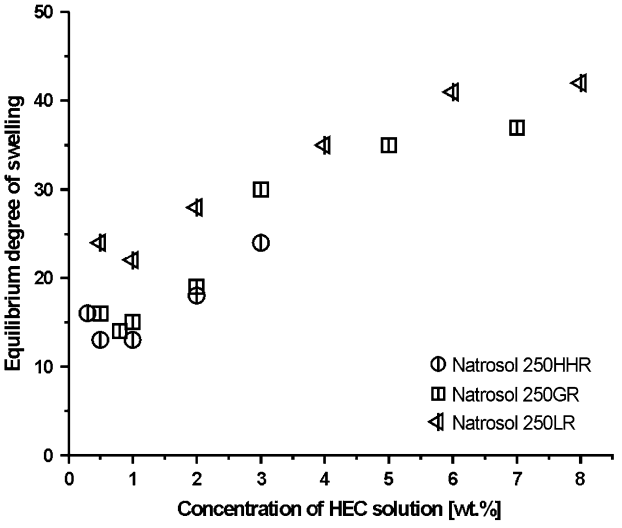

Fig. 5. Equilibrium swelling of HEC cryogels prepared from HECs of different molecular weights at different polymer concentrations (temperature of freezing: −20 °C; 2 wt.% BBTMAC; irradiation time: 2 min; freeze dried samples).

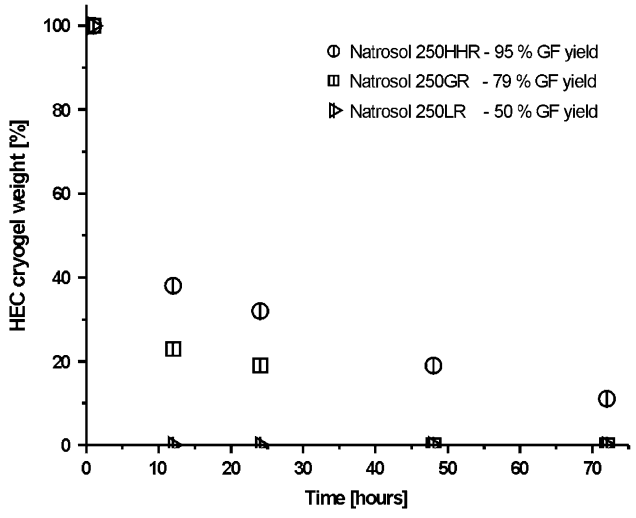

Fig. 6. Enzymatic degradation of HEC hydrogels by the cellulase enzyme in an acetic acid–sodium hydroxide buffer (pH 5.0) at 30 °C. Cryogels were prepared by freezing of 1 wt.% Natrosol 250HHR and Natrosol 250GR solutions and 2 wt.% Natrosol 250LR solution containing 2 wt.% BBTMAC (with respect to the polymer) at −20 °C and irradiation with UV light for 2 min.

the smaller number of intermolecular bonds and therefore degrades faster.

In a control experiment, conventional HEC hydrogels were prepared by chemical cross-linking with divinylsulphone and tested at the same conditions of enzymatic degradation. Series of HEC hydrogels of high GF yield (82–98%) and different cross-linking densities were prepared by varying the amount of the cross-linking agent. As expected, the higher the cross-linking density the lower becomes the rate of degradation, however, the HEC hydrogels degrade more rapidly in comparison with the HEC cryogels of similar GF yield. All conventional HEC hydrogels were completely decomposed in the interval of 12–48 h of action of the cellulase enzyme, while the HEC cryogel with GF yield of 95% still retained 20% of its weight after 48 h of enzymatic degradation. It seems that the specific morphology of the cryogels and in particular their dense walls are responsible for the slower degradation rate compared to the hydrogels.

Fig. 7 shows the interior of a HEC cryogel (Natrosol 250HHR; molecular weight *ca*. 1,300,000) without and after 12 h of enzymatic degradation. SEM analysis illustrates that the enzyme molecules attack the HEC network not only at the gel surface, but they also penetrate into the macropores and digest the whole polymer structure. Evidently, the cryogel walls appear thinner and partially destroyed by the cellulase enzyme after 12 h, which is in good agreement with the weight loss of 62% estimated for this sample (Fig. 6).

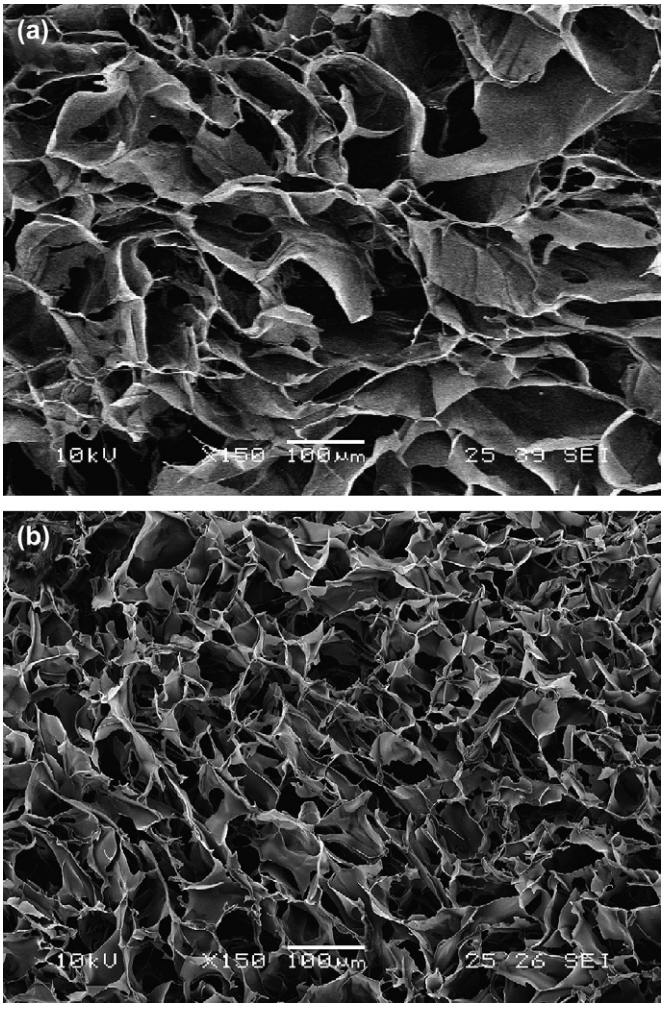

Fig. 7. SEM micrographs of HEC cryogel (1 wt.% Natrosol 250HHR frozen at −20 °C; 2 wt.% BBTMAC; irradiation time: 2 min): (a) without and (b) after 12 h action of the cellulase enzyme.

The general trend observed for both the HEC cryogels and the HEC hydrogels is that the rate of enzymatic degradation depends on the gels morphology.

4. Conclusion

Hydroxyethylcellulose hydrogels of good quality and high gel fraction yield were prepared by UV irradiation of moderately frozen HEC systems for only 2 min at an irradiation dose rate of 5.7 J/cm^2 min. All HEC cryogels possess macroporous structure with large interconnected pores surrounded by dense thin walls, which impart opacity to the material. The gel fraction yield and the swelling of HEC cryogels can be controlled by varying the synthesis conditions like temperature of freezing, polymer molecular weight and concentration of HEC solution. Importantly, the morphology and the swelling behavior of HEC cryogels are dependent on the drying methods applied. The enzymatic degradation tests showed that the degradation of the HEC cryogel network occurs at a slower rate than that of the conventional HEC hydrogel of similar gel fraction yield.

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