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# Stimuli sensitive super-macroporous cryogels based on photo-crosslinked 2-hydroxyethylcellulose and chitosan

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ABSTRACT

Original pH sensitive cryogels, based on two biodegradable natural polymers chitosan (CS) and 2-hydroxyethylcellulose (HEC), were obtained via cryogenic treatment of semi-dilute aqueous solutions and UV induced crosslinking in frozen state. $H_2O_2$ and *N,N′*-methylenebisacrylamide (BisAAm) were used as photoinitiator and crosslinking agent, respectively. BisAAm facilitated the formation of polymer co-network and increased both the gel fraction yield and mechanical strength of cryogels. The influence of chitosan content on the physico-mechanical properties of HEC–CS cryogels was investigated. In general, the increase of CS fraction in the polymer co-network increased the degree of swelling and enhanced significantly the storage modulus of materials. All HEC–CS cryogels obtained were opalescent sponge-like materials, which quickly release/uptake water due to their open porous structure. The incorporation of CS provided pH dependent swelling and good bioadhesive properties of cryogels. HEC–CS cryogels were further exploited as drug delivery systems of the highly water soluble drug metronidazole belonging to BCS Class I.



## 1. Introduction

Nowadays, the development of new products and materials based on renewable organic resources using innovative processes is of increasing interest and deserves the attention of both academic and industrial research (Marcì, Mele, Palmisano, Pulito, & Sannino, 2006). Thus, many natural polymers, especially polysaccharides, have been used to synthesize superabsorbent hydrogels (Chang & Zhang, 2011). These materials are promising for application in the biomedical field because of their unique advantages like non-toxicity, biocompatibility, biodegradability and some biological functions (Chang, Lue, & Zhang, 2008). Practically, cellulose is the most abundant polymer in nature and its derivatives are very popular due to their solubility in water, compatibility with tissues and blood, and low cost (Trombino et al., 2009). Hydrogels of cellulose derivatives can be obtained either by physical or chemical crosslinking (Chang & Zhang, 2011). In the case of physically crosslinked gels, there is no covalent bonding formation and the network is formed through ionic bonds, hydrogen bonds, or polymer–polymer interaction. The chemically crosslinked hydrogels of cellulose derivatives have been prepared by reaction with chemical reagents (Sannino & Nicolais, 2005; Sannino et al., 2005) or by ionizing radiation (Fei, Wach, Mitomo, Yoshii, & Kume, 2000; Wach, Mitomo, Yoshii, & Kume, 2002). However, most of chemicals used are toxic and the removal of non-reacted crosslinking reagents requires large amounts of water. On the other hand, it was demonstrated that cellulose derivatives undergo degradation when exposed to ionizing radiation at ambient temperature in solid state and in dilute aqueous solutions (less that 10 wt.%). Cross-linked gels have been obtained at paste-like conditions (25–40 mass%, depending on the polymer), but in this case several days are required for complete dissolution of the cellulose derivative in water.

Several years ago a facile method for preparation of hydrogels of cellulose derivatives based on cryogenic treatment and UV irradiation was reported by our team (Petrov, Petrova, Stamenova, Tsvetanov, & Riess, 2006; Petrov, Petrova, Tchorbanov, & Tsvetanov, 2007). Firstly, semi-dilute aqueous solutions of different cellulose derivatives, containing photoinitiator, were frozen at negative temperature (usually at minus 20 °C) and, then, the polymer network was formed by UV induced crosslinking. The cryogenic treatment resulted in formation of super-macroporous hydrogels, also referred as cryogels (Lozinsky, 2002). During the freezing process most of water forms ice crystals while the non-freezable water and the soluble substances are accumulated in a non-frozen liquid microphase (NFLMP). Ice crystals act as porogens and the formation of polymer network occurs only in the NFLMF. It was suggested that the polymer concentration in NFLMP is very high (cryo-concentration effect) and the reaction conditions closely resemble the conditions in the paste-like state, which is the main reason for

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domination of cross-linking over chain scission reactions during the irradiation with UV light. All cryogels of cellulose derivatives obtained by UV irradiation were opalescent macroporous materials with an open porous structure, which determined the very rapid water uptake by the freeze dried samples (2–3 s) due to capillary effects. Another advantages of this method are the very low capital outlay and the relatively short time for efficient gel formation. Furthermore, the usage of $H_2O_2$ as a photoinitiator allowed preparation of environment friendly materials which can be applied without any additional purification procedure.

Despite the many advantages, cryogels of cellulose derivatives posses relatively low mechanical strength. In our previous work we demonstrated that the elastic modulus of (hydroxypropyl)methylcellulose cryogel can be increased from ca. 150 Pa to 1000 Pa by incorporation of the crosslinking agent *N*,*N*′-methylenebisacrylamide into the polymer network (Petrov et al., 2006). Another useful strategy for improvement or modification of the physicochemical properties of cryogels is the combination of two polymers within a co-network of two interpenetrating networks (Chang et al., 2008; Dragan, Lazar, Dinu, & Doroftei, 2012). Preliminary studies showed that the formation of co-network from 2-hydroxyethylcellulose and acrylamide (2:1 w/w) by UV irradiation in frozen state increased three times the yield strength of the aerogels obtained (Petrov & Georgiev, 2012).

The present paper describes the preparation of original supermacroporous cryogels based on 2-hydroxyethylcellulose and chitosan via UV induced crosslinking in frozen semi-dilute aqueous solution of the two polymers. Chitosan, similar to HEC, is biodegradable and biocompatible natural polysaccharide with outstanding bioadhesion properties (Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012). In this study, chitosan was incorporated into the polymer co-network to increase the elasticity and adhesiveness of HEC cryogel. The effect of co-network composition on the swelling behavior, elastic modulus and adhesive properties of HEC–CS cryogels was investigated and compared to the cryogels of 2-hydroxyethylcellulose. HEC–CS cryogels were tested as matrices for loading and release of the hydrophilic drug metronidazole.

## 2. Experimental

### 2.1. Materials

2-Hydroxyethylcellulose (1.300 kg/mol; degree of substitution 1.5; molar degree of substitution 2.5) was donated by Hercules Inc. (Aqualon Division, USA). Chitosan (high molecular weight; 800–2000 cP (1 wt.% in 1% acetic acid, 25 °C, Brookfield); >75% deacetylated), *N*,*N*′-methylenebisacrylamide and acetic acid were purchased from Sigma–Aldrich. $H_2O_2$ (30 vol.% water solution) was purchased from Acros Organics.

### 2.2. Synthesis of cryogels

An appropriate amount of CS was dissolved in 13 ml of acidic water (pH 3) to obtain semi-dilute solution, then, given amount of HEC was added and the system was heated at 40 °C for 20 min and finally kept for 16 h at room temperature to ensure homogeneous solution (1–2 wt.%; HEC:CS wt. ratio from 1:0.1 to 1:1). Given amounts of crosslinking agent, BisAAm (15–35 wt.% to the polymers) dissolved in 2 ml water and $H_2O_2$ (5 wt.% to the polymers), were added under stirring at room temperature. The resulting homogeneous solution was poured into 8 Teflon dishes (20 mm diameter) forming a 2.5 mm thick layer, which was then kept in a freezer at minus 20 °C for 2 h. The frozen samples were irradiated with a full spectrum UV–vis light using a Dymax 5000-EC UV curing equipment with a 400 W metal halide flood lamp for 2 min (irradiation dose rate: 5.7 J/cm$^2$ min; input power: 93 mW/cm$^2$).

Cryogels containing metronidazole (10 wt.% to polymers) were prepared by the same procedure, except that the active substance was added to the polymer solution prior freezing and crosslinking.

### 2.3. Measurements of gel fraction yield

The gel fraction (GF) yield of the cryogels was determined gravimetrically. To calculate the GF content, the samples were extracted consequently in distilled water at pH 4 and pH 7 for 6 days at room temperature. After that, the samples were freeze-dried, weighed and the GF yield was calculated by the relationship GF (%) = (weight of freeze-dried sample/initial weight of polymers and crosslinking agent) × 100.

### 2.4. Measurements of degree of swelling

The degree of swelling (DS) was measured in water at pH 4 and pH 7. Disks of cryogel, were equilibrated at given pH, blotted with filter paper and weighed. Finally, the samples were freeze-dried. The degree of swelling was calculated as DS = (weight of wet sample/weight of freeze-dried sample). The experimental errors of the GF yield and the DS calculations were in the range of 2–5%.

### 2.5. Dynamic rheological measurements

Dynamic rheological measurements of the cryogels were performed on a Haake RheoStress 600 rheometer with a parallel plate sensor system (20 mm diameter) and Peltier temperature controller. Disks of the cryogels were first extracted for 6 days at pH 4 and the dynamic storage ($G'$) and loss ($G''$) moduli were measured in the 0.03–10 Hz frequency range at 25 °C in CD-mode at $\gamma = 0.005$, which is inside the linear viscoelastic regime. Three runs of each sample were conducted.

### 2.6. Drug release studies

Drug release profiles were evaluated using a dissolution test apparatus (Erweka DT 600, Heusenstamm, Germany). The USP paddle method wits slight modification was selected. Six freeze-dried samples were immersed in 500 ml acetate buffer (pH 4.5) and the test was carried out at a paddle rotation speed of 50 rpm, at temperature = 37 ± 0.5 °C. The quantity of metronidazole in the release medium was analyzed by UV absorbance at 320 nm using DU 800 spectrophotometer (Beckman Coulter). The cumulative percentage of drug release was calculated and the average of six determinations was used in the data analysis.

## 3. Results and discussion

Cryogels from high molecular weight HEC and CS linear precursors at initial weight ratio in the range 1:0.1–1:1 were obtained by UV irradiation of frozen aqueous systems in the presence of photoinitiator $H_2O_2$ and crosslinking agent BisAAm, and subsequent thawing. Then, cryogels were extracted successively in acidic (acetic acid, pH 4) and neutral water (pH 7) to wash the non-crosslinked polymers and acetic acid, and used for determination of the gel fraction yield, degree of swelling and storage modulus. The incorporation of given amount of BisAAm into the polymer co-network aimed to increase both the gel fraction yield and mechanical strength of cryogels. We found that the cryogenic treatment of semi-dilute polymer solutions afforded formation of cryogels of good quality which means that upon UV irradiation the reactions of cross-linking prevail over the chain scission reactions. The obtained cryogels maintained their original compactness when

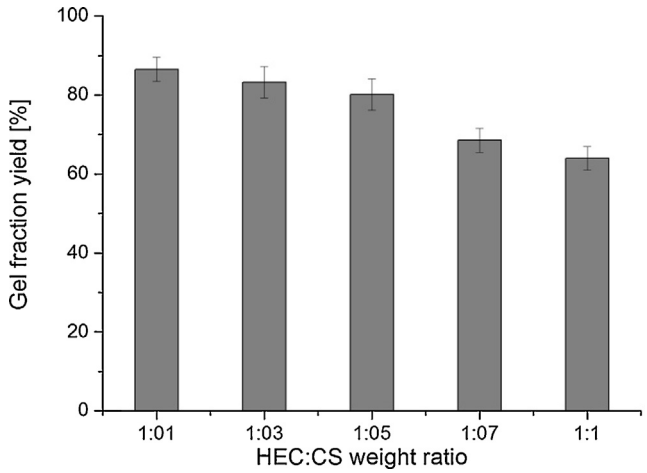


**Fig. 1.** Gel fraction yield of HEC–CS cryogels of different composition prepared at temperature of freezing – 20 °C, initial polymer concentration 1.5 wt.%, 30 wt.% BisAAm, irradiation time 2 min.

exposed to an excess of water. The calculated values of the gel fraction yield varied between 65% and 85%, depending on the cryogels composition (Fig. 1). In general, the increase of CS content in the initial polymer solution resulted in decreased GF yield. Thus, one can point out that the addition of CS hinders to some extend the process of crosslinking.

The proposed mechanism of photocrosslinking involves photohomolysis of $H_2O_2$ to hydroxyl radicals, generation of macroradicals by hydrogen atom abstraction from both HEC and CS chains and subsequent intermolecular recombination of macroradicals to form co-network. We suggest that the high polymer concentration in the liquid microphase favors the recombination of macroradicals. Since the GF yield of pure chitosan cryogels prepared via the same method did not exceed 30%, a general question is whether CS chains participate equivalently in the formation of polymer network. Obviously, for pure CS, at the reported experimental conditions, the reactions of crosslinking do not prevail over the chain scission. The first evidence that CS was successfully incorporated into the polymer co-network came out from the different degree of swelling of cryogels in acidic and neutral water (Fig. 2). Whatever the composition, DS of HEC–CS cryogels at pH4 was higher than DS at pH 7. Since HEC is a non-ionic polymer, its network has similar swelling

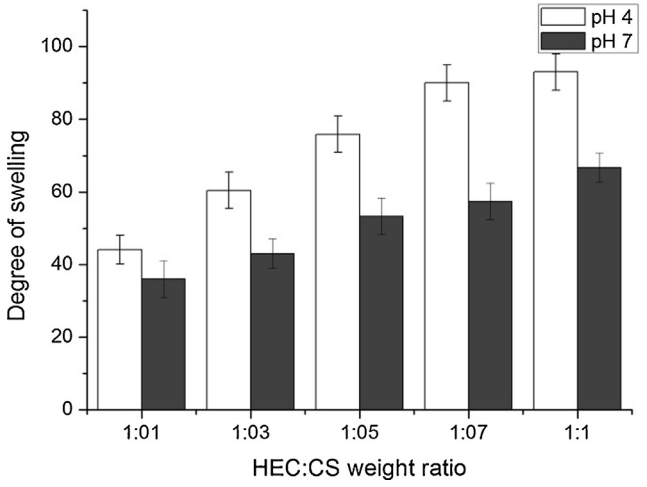


**Fig. 2.** Degree of swelling of HEC–CS cryogels of different composition in acidic and neutral water prepared at temperature of freezing – 20 °C, initial polymer concentration 1.5 wt.%, 30 wt.% BisAAm, irradiation time 2 min.

behavior at both pH 4 and 7. In contrast, in acidic conditions amino groups in chitosan are protonated and, therefore, chitosan segments swell more at pH4 than in neutral water. Thus, the different degree of swelling of cryogels at different pH can be attributed to the incorporation of chitosan into the polymer co-network. It was found that DS strongly depends on the co-network composition and increases proportionally with increasing CS content.

Information that the composition of polymer co-network closely resemble the composition of initial polymer mixtures was obtained by elemental analysis (Table 1). These results are direct proof that the three constituents HEC, CS and BisAAm were regularly incorporated into the formed co-network without any preference. Noteworthy, if CS molecules are not covalently attached to the network they will be removed from the cryogel matrix at the extraction procedure as demonstrated for semi-penetrating networks (Dragan et al., 2012). It seems that the presence of HEC suppress the chain scission of CS macromolecules upon UV irradiation and, on the other hand, not only extraction of CS contributes to the decreased GF yield (Fig. 1).

The concentration of the initial HEC/CS solution is one of the most important factors for obtaining cryogels of good quality and high GF yield. In this study, we found that cryogels of fairly high GF yield can be formed in the concentration range between 1 and 2 wt.%, however, the highest value was reached at initial concentration of 1.5 wt.%. Therefore, most of our experiments were conducted at this polymer concentration.

All HEC–CS cryogels obtained at the reported experimental conditions were opalescent sponge-like materials which easily release water by compression due to their open porous structure. The morphology of HEC–CS cryogels is identical with HEC cryogels and consist of smooth polymer walls surrounding interconnected pores (Petrov et al., 2007). This specific morphology also determines the very fast water uptake (2–3 s) observed for the freeze dried gels. One should mention that two types of water exist in the cryogels – water bound to the polymer network via hydrogen bonds and capillary-bound (free) water that fills the space of macropores (>60%) and that can be released. Therefore, we assume that the DS values are only apparent ones giving information about the overall amount of water in the cryogels and cannot be applied for exact calculation of the crosslinking density.

As mentioned above BisAAm was added to the reaction mixture to facilitate the formation of polymer network and, thus, to increase the gel fraction yield of the resulting cryogels. It was established that the maximum GF yield was reached at 30 wt% BisAAm with respect to the two polymers (Fig. 3).

Expectedly, the degree of swelling of HEC–CS cryogels decreased proportionally with the increase of the amount of crosslinking agent (Fig. 4). Here, one can speculate that the main purpose for this behavior is the increased crosslinking density of the co-network. However, due to the heterogeneous nature of HEC–CS cryogels and the existence of free water, the results obtained cannot be interpreted unambiguously.

It was described elsewhere that the incorporation of crosslinking agent remarkably increases the elastic modulus of polymer cryogels (Petrov et al., 2006; Petrov, Petrova, & Tsvetanov, 2009). This effect is attributed to both the increase of GF yield and crosslinking density of the network. In this study, we investigated the influence of *N*,*N*′methylenbisacrylamide content on the viscoelastic properties of HEC–CS cryogels. Dynamic rheological measurements (Fig. 5) revealed that storage modulus increases gradually with the amount of BisAAm incorporated into the polymer co-network. Remarkably, $G'$ of cryogels containing 30 wt.% BisAAm (with respect to polymers) was 20 times higher than $G'$ of the sample without crosslinking agent.

Dynamic rheological analysis were also used for investigating the influence of HEC to CS weight ratio on the viscoelastic

**Table 1**
Compositions of the initial HEC–CS mixtures containing 30 wt.% BisAAm and the corresponding extracted polymer cryogels.

| HEC:CS weight ratio | Before irradiation | | | After irradiation and extraction | | |
|---|---|---|---|---|---|---|
| | C (%) | H (%) | N (%) | C (%) | H (%) | N (%) |
| 1:0.7 | 45.45 | 7.58 | 8.56 | 45.43 | 7.73 | 8.71 |
| 1:0.3 | 46.80 | 7.80 | 7.39 | 46.23 | 7.87 | 6.84 |

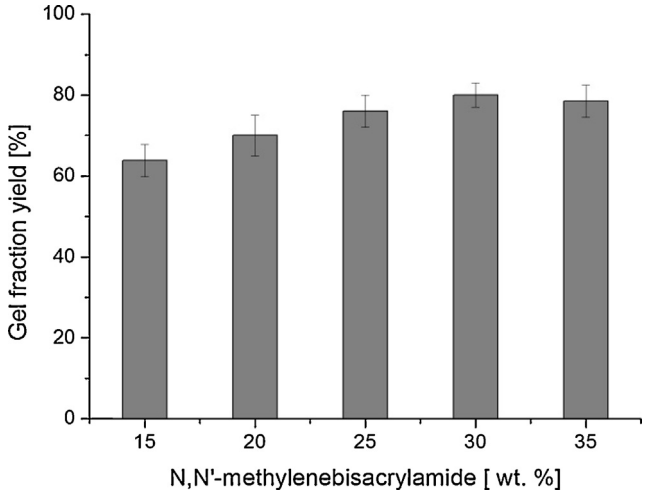


**Fig. 3.** Gel fraction yield of HEC–CS cryogels (1:0.5 w/w) containing different amount of BisAAm, prepared at temperature of freezing – 20 °C, initial polymer concentration 1.5 wt.%, irradiation time 2 min.

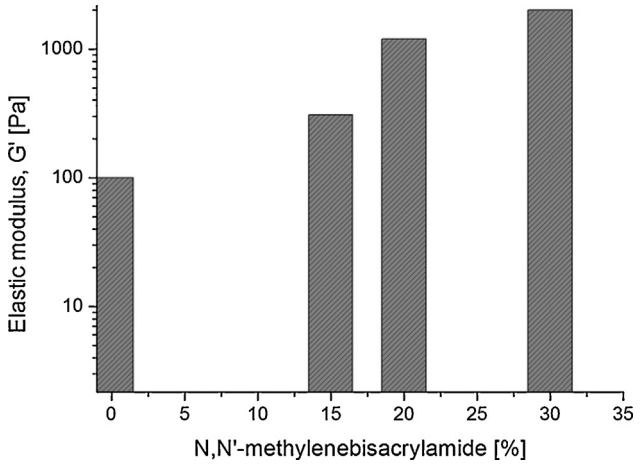


**Fig. 5.** Influence of the amount of crosslinking agent on the storage modulus of HEC–SC cryogels prepared at temperature of freezing – 20 °C, HEC:CS ratio 1:0.5 w/w, initial polymer concentration 1.5 wt.%, irradiation time 2 min.

properties of cryogels at constant content of crosslinking agent. All cryogels exhibited similar behavior typical for elastic gels, i.e. the storage modulus was frequency independent in the 0.03–10 Hz range and $G'$ was more than an order of magnitude higher than $G''$. Importantly, the incorporation of chitosan to the HEC network increased significantly the storage modulus of cryogels (Fig. 6). Furthermore, a strong dependence of $G'$ on the chitosan fraction was registered. Clearly, the higher the content of CS, the larger the storage modulus of the resulting cryogels. Based on rheological measurements we can undoubtedly conclude that the elastic properties of HEC cryogels were remarkably enhanced by formation of co-network containing CS segments and BisAAm.

Bioadhesion is one of the intriguing properties of chitosan that makes it attractive for application in biomedical field (Leithner & Bernkop-Schnurch, 2012). It is supposed that amino groups in CS interact with negatively charged surfaces and the electrostatic attraction is the major mechanism for chitosan bioadhesion. In addition, adhesion is also attributed to hydrogen bonding and hydrophobic effects (Sogias, Williams, & Khutoryanskiy, 2008). The adhesive properties of HEC–CS cryogels obtained via photo-crosslinking were tested in a simple experiment. Two samples, a pure HEC cryogel and a HEC–CS (1:0.5 w/w) cryogel, were placed on the two fingers (Fig. 7a) and, then, the hand was shaken ones. As shown in Fig. 7b, the pure HEC cryogel fell down, while HEC–CS cryogel remained attached to the finger. This experiment unambiguously demonstrates that the incorporation of chitosan into the polymer co-network provided good adhesive properties of cryogels.

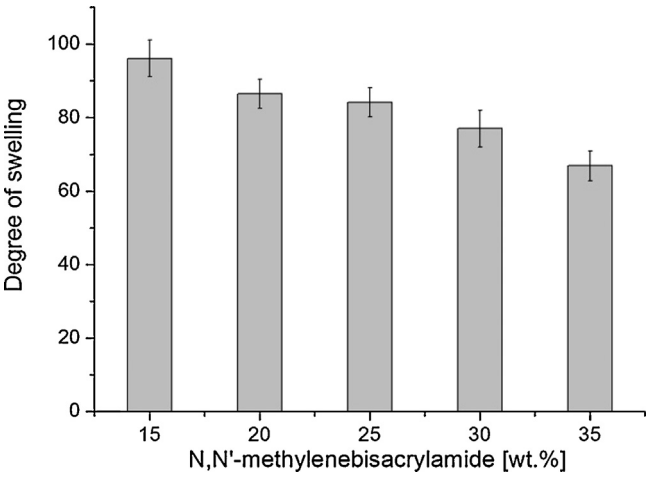


**Fig. 4.** Degree of swelling of HEC–CS cryogels (1:0.5 w/w) containing different amount of BisAAm at pH4, prepared at temperature of freezing – 20 °C, initial polymer concentration 1.5 wt.%, irradiation time 2 min.

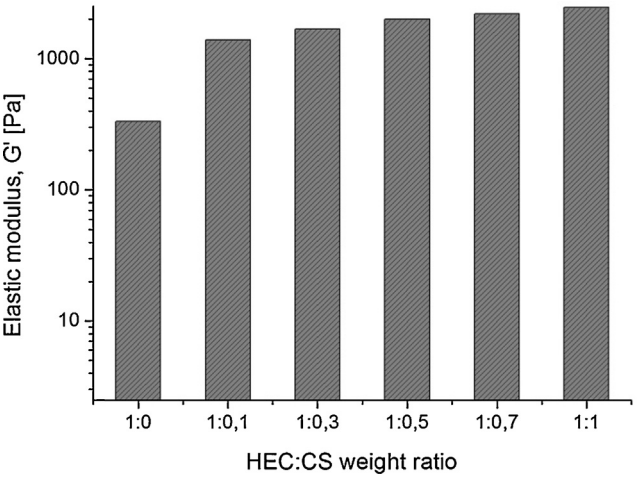


**Fig. 6.** Dependence of the storage modulus of cryogels on the HEC to CS weight ratio. Cryogels were prepared at temperature of freezing – 20 °C, 30 wt.% BisAAm, initial polymer concentration 1.5 wt.%, irradiation time 2 min.

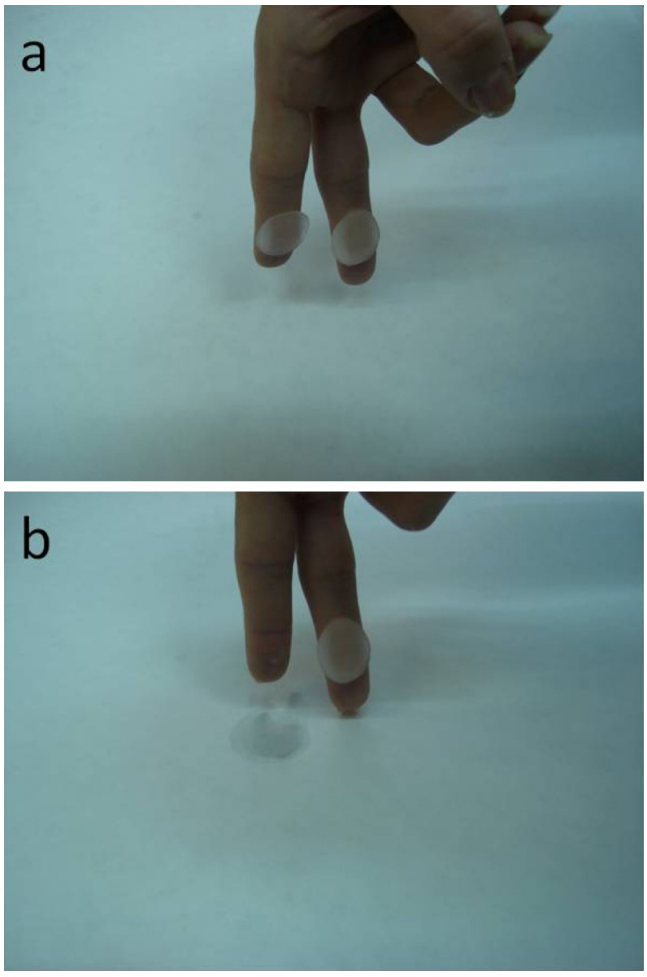


**Fig. 7.** Digital photographs of HEC (left) and HEC–CS (right) cryogels placed on the fingers before (a) and after (b) hand shaking.

Based on the fore mentioned adhesive properties of tested materials it was interesting to investigate whether these cryogels could be of potential use as excipients for preparation of drug delivery systems for vaginal application for which the mucoadhesion is considered an important prerequisite for increasing the therapeutic efficacy of the dosage forms. For that reason we sought to load a model drug metronidazole – a broad spectrum agent commonly use for treatment of vaginal infections caused by trichomonas vaginalis and anaerobic bacteria.

The release kinetic curve of metronidazole loaded into the HEC–CS (1:0.5 w/w) cryogels containing 30 wt.% BisAAm is shown in Fig. 8.

As is evident from the figure the release profile of metronidazole is characterized with initial burst release, approximately 40% of the drug is released at 30 min, followed by sustained metronidazole release for the next 8 h. The release of metronidazole after 30 min increases with approximately constant rate which was established by linear regression analysis of the data to zero-order kinetics (resulting correlation coefficient: $R^2 = 0.991$). The burst release is probably due to adsorption of certain fraction of the active substance on the surface of the matrix. Since the macroporous structure of freeze-dried systems allows very fast water uptake by the pores, the immersing the freeze-dried disk in the dissolution medium (acetate buffer) triggers a rapid release of the adsorbed fraction. On the other hand, the rest of metronidazole is incorporated in the dense walls of cryogels which hiders the diffusion of drug molecules and thereafter it is released much more

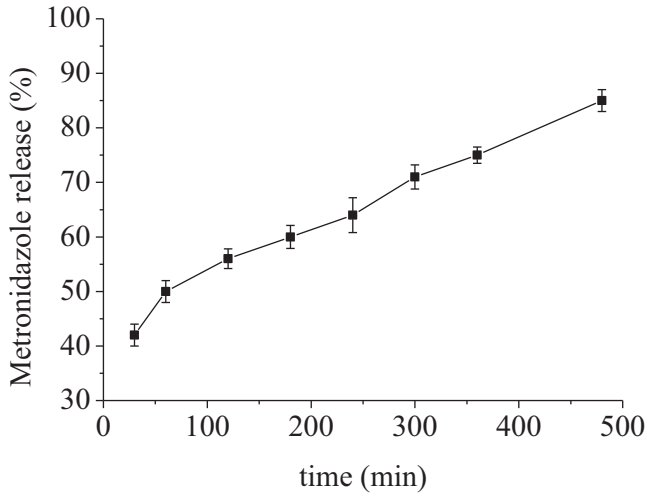


**Fig. 8.** Release of metronidazole from model system based on HEC–CS cryogels (1:0.5 w./w.) obtained with 30 wt.% BisAAm, immersed in 500 ml acetate buffer (pH 4.5) at 37 °C. Mean SD, $n = 6$.

slowly (Kostova et al., 2011). Overall, the release study clearly indicates that HEC–CS cryogels could provide a prolong drug release for a reasonable period of time.

## 4. Conclusions

Combination of cryogenic treatment and irradiation with UV light in frozen state afforded the preparation of sponge-like cryogels from mixed semi-dilute solutions of 2-hydroxyethylcellulose and chitosan. The cryogenic treatment and the addition of crosslinking agent were determinant factors for crosslinking instead of degradation of HEC and CS upon UV irradiation. The incorporation of CS into the polymer co-network resulted in significantly enhanced mechanical strength, pH sensitive swelling properties and bioadhesiveness of material. Taken together, the findings from the presented study and especially the established ability of the tested cryogels to provide prolong drug release give us reason to conclude that the HEC–CS cryogels could be considered a promising drug delivery platforms needing further, more thorough evaluation and optimization.

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