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Crosslinking of cellulose derivatives and hyaluronic acid with water-soluble carbodiimide

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Abstract

Hydrogels with good mechanical stability are generally obtained by chemical crosslinking. However, the compounds used as crosslinking agents are often highly toxic, and thus they are not recommended as starting materials in view of an environmentally safe production process and a biocompatible product.

In this work chemically crosslinked superabsorbent hydrogels have been synthesized from water solutions of hydroxyethylcellulose (HEC), carboxymethylcellulose sodium salt (CMCNa) and hyaluronic acid (HA), using a non-toxic water-soluble carbodiimide (WSC) as crosslinking agent. Sorption capacity both in water and in water solutions at different ionic strength has been investigated, taking into account the effect of different chemical compositions. Moreover, uniaxial compression tests and dynamic-mechanical measurements on water swollen samples have been performed to probe the elastically effective degree of crosslinking of the polymeric network.

In vivo tests on humans for the application of this material as a bulking agent in dietary regimes are in process and preliminary results are encouraging.

Keywords: Hydrogel; Crosslinking; Carbodiimmide

1. Introduction

Hydrogels are three-dimensional polymeric networks, that are able to absorb and retain large volumes of water. Chemical or physical crosslinks are required to avoid dissolution of the hydrophilic polymer chains into the aqueous phase. Because of their sorption capacity, superabsorbing hydrogels have been extensively used as water retaining devices, mainly in the field of personal hygiene products and in agriculture [1–4]. Moreover, in the recent years the possibility to modulate their sorption capabilities by changing the external conditions (e.g. pH, ionic strength, temperature) have suggested their innovative application as smart materials, drug delivery devices, actuators and sensors [5–14].

The most commonly available hydrogels on the market are polymers of acrylamide and potassium acrylate and, as such, non biodegradable. However, because of the increasing interest of Institutions and public opinion in environmental protection issues, research has recently focused on the development of biodegradable superabsorbents, which should be preferentially obtained from harmless precursors, in view of an environmentally safe production process [15–17].

As reported in the literature [15,16], biodegradable cellulose based hydrogels have been chemically crosslinked by using the small difunctional molecule of divinylsulfone (DVS) to create intermolecular covalent bonds among polymer chains. What is noticeable is the fact that the swelling properties of these materials are comparable with those displayed by acrylate based products. More recently, cellulose derivatives-hyaluronic acid based hydrogels, which are potentially biodegradable as well as biocompatible, have been also synthesized with DVS as crosslinking agent [17]. Nevertheless, due to its toxicity, DVS requires appropriate safety measurements during the production process and strict quality controls on the final product (i.e. all unreacted DVS has to be extracted from the gel before it can be applied).

The aim of this work is to synthesize a chemically crosslinked superabsorbent hydrogel from hydroxyethylcellulose (HEC), carboxymethylcellulose sodium salt (CMCNa)

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and hyaluronic acid (HA), through the use of a non-toxic carbodiimide (WSC) as a crosslinker.

The presence of the polyelectrolyte CMCNa in the hydrogel network provides a Donnan equilibrium with the external solution, thus modulating material's sorption capacity in relation to the external solution ionic strength and pH; HEC, in turn, promotes intermolecular rather then intramolecular crosslinking, contributing to the chemical network stabilization [15].

Hyaluronic acid (HA), a naturally occurring polysaccharide which is widely distributed in animal tissues, is a key precursor for most novel biomaterials, that are finding application mainly as drug delivery vehicles, tissue engineering scaffolds and tissue fillers [18,19]. The combination of HA with cellulose derivatives has a double effect. On one hand, the mechanical properties and the stability of hyaluronan hydrogels, together with their sensitivity to external stimuli, are improved by the chemical stabilization of HA in the crosslinked structure with cellulose derivatives. On the other, the presence of HA in the cellulose based superabsorbents improves significantly their biocompatibility [17].

The choice of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC) as crosslinking agent is related to its well known ability to induce crosslinking of HA and other polysaccharides, without taking part in the linkage but simply changing to a water soluble urea derivative, which has been proved to be non-toxic [20–24]. The crosslinking reaction of HA and cellulose derivatives that occurs in the presence of WSC will be discussed in the following.

In this study we have first considered the effect of the chemical composition on both the crosslinking reaction and the final hydrogel sorption properties. For this reason, the swelling properties of various samples, differing for the HA/CMCNa/HEC ratio, have been evaluated both in water and in water solutions at different ionic strength.

Secondly, we have focused our attention on the degree of crosslinking, which directly affects the entropic elasticity of the hydrogel network, and, as a result, its swelling and mechanical properties. Two different techniques, based on uniaxial compression tests and dynamic-mechanical analysis (DMA) on water swollen samples, have been applied to evaluate the elastically effective degree of crosslinking, according to classical rubber elasticity theory.

Finally, a morphological analysis on acetone-dehydrated samples has been performed by means of electron scanning microscopy (SEM) to assess the presence of a microporous structure.

One application of this non-toxic superabsorbent is in the field of hypocaloric dietary regimes; in this case, the product will be used as a bulking agent in the stomach to reduce hunger and help patients to eat low amount of food. Combined properties of high swelling capacity in water solutions and potential non toxicity allow the use of this material in other biomedical applications such as drug delivery substrates, absorbent medications and scaffolds for tissue engineering.

2. Materials and methods

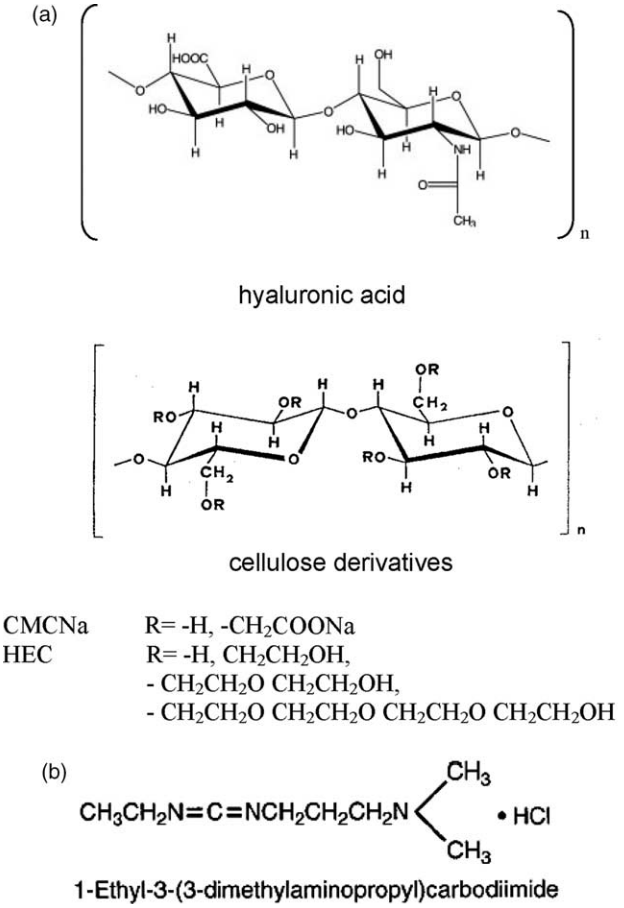
2.1. Chemical reagents

The hydrogels described in this work have been obtained by crosslinking different mixtures of HA, CMCNa and HEC using WSC as crosslinking agent in water solution. CMCNa (MW 700.000, DS 0.9, viscosity 3400 cm/s [$C=1\%$, H_2O at 25 °C]), HEC (MW 250.000, MS 2, viscosity 80–125 cm/s [$C=2\%$, H_2O at 25 °C]) and WSC were purchased from Aldrich Chimica s.r.l., Milano and used as received. Hyaluronic acid was purchased from FAB s.r.l., Abano Terme (PD), Italy. The chemical structures of HA, CMCNa, HEC and WSC are represented in Fig. 1(a) and (b).

2.2. Preparation of hydrogels

As reported in Table 1, various hydrogels, differing for the HA/CMCNa/HEC weight ratio as well as for polymer concentration in the starting water solution, have been synthesized.

Mixtures of HA, CMCNa and HEC, with the desired weight ratio, were dissolved in distilled water by stirring gently at room temperature. The final polymer weight fraction in the solution was either 3 or 5%. After the mixing stage,



CMCNa R= -H, $-CH_2COONa$
HEC R= -H, CH_2CH_2OH,
- $CH_2CH_2O\ CH_2CH_2OH$,
- $CH_2CH_2O\ CH_2CH_2O\ CH_2CH_2O\ CH_2CH_2OH$

$$CH_3CH_2N{=}C{=}NCH_2CH_2CH_2N(CH_3)_2 \cdot HCl$$

Fig. 1. The HA and cellulose derivatives repeating units (a) and the WSC molecule (b).

Table 1
Crosslinked samples differing for HA, CMCNa and HEC weight ratios and for polymer concentration

Chemical composition	Sample
HA/CMCNa/HEC weight ratio 2/1/1 5 wt% WSC concentration 5 wt% polymer concentration	C1
HA/HEC weight ratio 1/1 5 wt% WSC concentration 5 wt% polymer concentration	C2
CMCNa/HEC weight ratio 1/1 5 wt% WSC concentration 5 wt% polymer concentration	C3
CMCNa/HEC weight ratio 1/1 5 wt% WSC concentration 3 wt% polymer concentration	C4

the crosslinking agent was added and allowed to dissolve and distribute homogeneously throughout the solution. A fixed crosslinking agent concentration, equal to 5 wt%, was always used. An aqueous solution of citric acid 1 wt% was then added as catalyst to the polymer solution, in a 5% vol/vol amount. The crosslinking reaction occurred in few hours at room temperature.

In order to remove the unreacted chemicals, the partially swollen hydrogel samples were soaked in distilled water to equilibrium before being desiccated. As explained in the following, these equilibrium swelling data have been used for both the determination of the elastically effective degree of crosslinking and the evaluation of the hydrogel sorption properties.

After the washing stage, hydrogel samples were desiccated by phase inversion in acetone, according to a procedure reported elsewhere [16]. Briefly, being acetone a non-solvent for the polymer, the swollen hydrogel sample undergoes phase inversion when immersed in acetone, thus precipitating as a white glassy xerogel. Moreover, the rapid extraction of water by acetone induces the formation a connected microporous structure within the material, which enhances its swelling capability due to capillarity effects as well as to the fact that part of the water condensates in the microvoids whose dimension further increases after polymer swelling [16].

2.3. Swelling measurements

Hydrogel sorption properties have been evaluated in terms of swelling ratio Q, defined as follows:

$$Q = \frac{W_{\text{swollen}}}{W_{\text{dry}}} \tag{1}$$

where W_{swollen} is the weight of the completely swollen sample, i.e. after the washing stage, and W_{dry} is the weight of the dry sample, i.e. after desiccation by phase inversion in acetone.

Reported values of equilibrium water uptakes for each different composition have been averaged over five measurements.

Equilibrium swelling studies at different ionic strength were also performed, by preparing four water solutions of NaCl at concentrations of 10^{-3}, 10^{-2}, 10^{-1}, 1 M. The dry samples were weighed and immersed in the water solution until equilibrium attainment. Also in this case the reported values of equilibrium water solution uptake have been averaged over five measurements for each different sample and water solution composition.

Equilibrium swelling measurements both in distilled water and water solutions at different ionic strength have been carried out for all samples using an electronic microbalance (Sartorius) with an accuracy of $\pm 10^{-5}$ g.

2.4. Degree of crosslinking

The degree of crosslinking dc is defined as the number density of junctions joining the chains into a permanent structure. According to this definition the degree of crosslinking is given by:

$$\mathrm{dc} = \frac{v}{2V} \tag{2}$$

where $v/2$ is the total number of chemical crosslinks and V is the total volume of polymer. In the case of a perfect network with no dangling ends, loops and entanglements, the concentration of elastically effective chain elements, $\rho_x = v_e/V$, corresponds to the concentration of all chemically crosslinked polymer segments v/V:

$$\rho_x = \frac{v_e}{V} = \frac{v}{V} = \frac{1}{\bar{v}\bar{M}_c} \tag{3}$$

where $\bar{v}$ is the specific volume of the polymer, $\bar{M}_c$ is the average molecular weight between crosslinks and v_e/V are the moles of elastically effective chains per unit volume of network.

Generally, the elastically effective crosslinking density can be evaluated by performing uniaxial compression tests on swollen networks or by direct measurements [25–27]. Making the basic assumptions that the network is perfect (i.e. all crosslinks in the network are elastically effective) and that no volume change occurs upon compression, Flory derived a relationship between the compressive stress and the compressive deformation for the case of a swollen crosslinked polymer [25]:

$$\sigma = RT\frac{v_e}{V_0}\phi_{2,r}^{2/3}\phi_{2,s}^{1/3}\left(\alpha - \frac{1}{\alpha^2}\right) = G\left(\alpha - \frac{1}{\alpha^2}\right) \tag{4}$$

where σ is the uniaxial compressive stress, $\alpha = L/L_i$, with L the actual thickness of the compressed swollen sample and L_i the initial thickness of swollen sample, R is the universal gas constant, T is the absolute temperature, $\phi_{2,s}$ is the polymer volume fraction in the swollen state under compression, which is assumed to be equal to the value for the swollen undeformed gel (it is equal to $1/Q$); $\phi_{2,r}$ is the polymer volume fraction in the reaction mixture before full swelling in distilled water (polymer volume fraction in the 'relaxed' network), v_e/V_0 are the moles of elastically effective chains per cm^3 of dry polymer network and G is the shear modulus of the swollen network.

Since some water is squeezed out of the swollen sample during the compression, the assumption of constant volume is not always verified. However, the above expression for G is generally used to interpret gel deformation behaviour and to evaluate the crosslink density in the case of small deformations ($\alpha \rightarrow 1$).

In this study the shear modulus G, to be used to determine the degree of crosslinking, has been evaluated through compression and dynamic mechanical tests, as described in the following.

2.5. Mechanical measurements on swollen gels

The mechanical properties of hydrogels were evaluated by performing both uniaxial compression and dynamic mechanical tests on a parallel plate rheometer (ARES, Scientific Rheometric) at 25 °C. For each type of sample the experiments were repeated five times.

In the compression tests thin dishes of swollen gel were accurately positioned between the parallel plates. The gap was adjusted starting from the original sample height; gap sizes for swollen gels were generally between 3.0 and 4.0 mm. A constant velocity equal to 0.005 mm/s was then imposed to the upper plate to start the measurement. The evolution of the normal force was recorded as a function of the gap between the plates.

In the dynamic mechanical tests, the parallel plate surface was properly modified to prevent hydrogel slipping. A strain sweep test was first performed to select an appropriate strain amplitude at which the linear viscoelastic behaviour is observed. Measurements were then conducted in a constant strain (0.02) mode as a function of frequency, from 0.1 to 100 rad/s. The results were recorded in terms of storage and loss moduli, respectively G' and G'', and of loss factor $tg\delta$.

2.6. Morphological analysis

The morphology of acetone dehydrated samples was examined by means of scanning electron microscopy (SEM), with the aim of detecting qualitatively the presence of a connected microporosity. Micrographs of gel surfaces were obtained using a JEOL JSM-6500 F scanning electron microscope.

3. Results and discussion

3.1. Hydrogel crosslinking

The carbodiimide WSC has been chosen as crosslinking agent because of its ability to induce crosslinking of HA and other polysaccharides [20,21]. As reported by Ikada et al. [20], WSC does not chemically bind to polysaccharide molecules, but seems to mediate ester bonds formation between carboxyl and hydroxyl groups belonging to different polysaccharide molecules. Remarkably, WSC can be found as a by-product of the reaction, in the form of an urea derivative, which displays a very low degree of cytotoxicity [21–24]. For this reason, WSC can be considered as a non-toxic crosslinking agent.

The reaction scheme of WSC with polysaccharide molecules is reported in Fig. 2 and can be summarized as follows: firstly the presence of WSC induces the intramolecular or intermolecular formation of an acid anhydride between two carboxyl groups, changing the WSC itself into an urea derivative; this anhydride is then responsible for the reaction with a hydroxyl group, to yield an ester bond between two polysaccharide molecules. However, because of the high instability of the acid anhydride in water solutions at room temperature, the reaction can not take place if any hydroxyl groups do not quickly encounter the acid anhydride [21]. Therefore, the crosslinking reaction is markedly affected by the chemical composition of the starting polymer solution [20,21]. Moreover, the initial reaction of WSC with carboxyl groups is dependent on pH, the optimal pH ranging from 3.5 to 4.5, as reported in the literature [21].

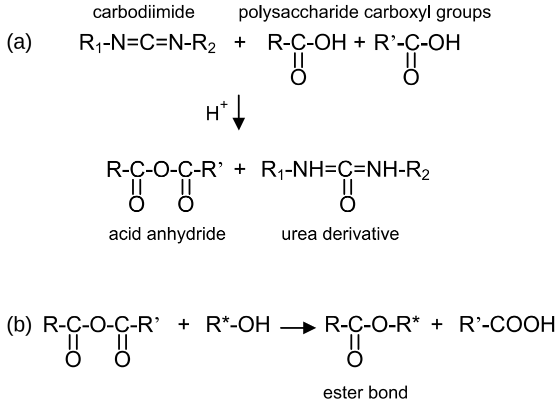


Fig. 2. Reaction scheme of WSC with polysaccharide molecules: (a) WSC-mediated intramolecular or intermolecular formation of an acid anhydride between two carboxyl groups, with a non-toxic urea derivative as by-product; (b) anhydride-mediated formation of an ester bond between two polysaccharide molecules.

In our study we assume that cellulose derivatives can be crosslinked by WSC through the same reaction scheme reported for HA alone [20]. In fact in acid water solution the CH_2COO^- anions provided by the CMCNa react with the H^+ ions, leading to carboxyl groups formation and subsequently to the crosslinking reaction. However, we found that, for a WSC concentration of 5 wt%, hydrogel formation does occur when polymer concentration in the starting solution is at least 3 wt% (unreported data), confirming that the chemical composition of the starting solution is fundamental to achieve a good crosslinking [20,21].

As shown in Table 1, four types of hydrogels have been synthesized, differing for both the HA/CMCNa/HEC weight ratio and the polymer concentration. In particular, samples C1 and C2 have been obtained in the presence of HA, at a fixed polymer concentration (5 wt%). Samples C3 and C4 have been crosslinked starting from mixtures of CMCNa and HEC at a polymer concentration equal to 5 and 3 wt% respectively.

3.2. Swelling measurements

Swelling tests have been performed in distilled water for each crosslinked sample, with the aim of investigating the effect of different chemical compositions on the hydrogel

Table 2
Hydrogels equilibrium swelling properties in distilled water

Sample	Swelling ratio $Q(W_{\mathrm{swollen}}/W_{\mathrm{dry}})$
C1	120 ± 15
C2	85 ± 12
C3	145 ± 16
C4	425 ± 27

Reported values of equilibrium water uptake have been averaged over five measurements and are expressed as mean ± standard deviation.

sorption properties. The results are shown in Table 2. As expected, the hydrogel crosslinked in the presence of the polyelectrolyte CMCNa (sample C1) displays a higher sorption capacity if compared with the samples crosslinked only with HA and HEC (sample C2). This is ascribed to the increased osmotic pressure due to the Donnan effect, related to the presence of fixed charges on the CMCNa backbone.

However, higher swelling ratios are obtained in the absence of HA (samples C3 and C4). Precisely, a decrease of the polymer concentration in the starting water solution, from 5 to 3% by weight, results in a remarkable increase of the equilibrium water uptake. Because of its good sorption properties, similar to those displayed by acrylate based products, sample C4 seems to be a biodegradable and environment-friend alternative to commercially available hydrogels.

The effect of the ionic strength of the external solution on the equilibrium sorption properties of the hydrogels is shown in Fig. 3. The equilibrium solution uptake always diminishes for higher values of the ionic strength. However, it can be observed that the samples crosslinked in the presence of CMCNa display a higher sensitivity to ionic strength variations. In fact, the osmotic pressure related to the Donnan effect is proportional to the difference in concentration of charges between those contained in the gel and those in the external solution. Being a polyelectrolyte, the CMCNa provides an overall higher ion concentration in the gel, and thus a higher Donnan-type contribution to polymer swelling. Obviously increasing the ionic strength of the external solution decreases the difference between the concentration of ion species in the gel and in the external solution and, as a result, the water uptake.

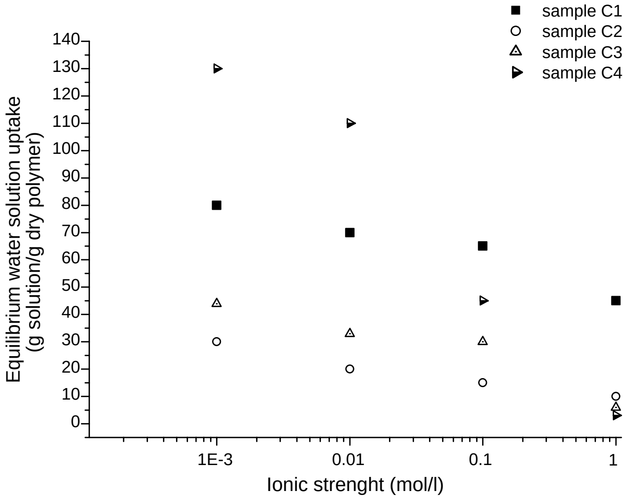


Fig. 3. Hydrogels equilibrium swelling properties in water solution at different ionic strength. Reported values have been averaged over five measurements; average values are plotted.

Table 3
Linear fittings, in the form $Y=A+BX$, of the plot of uniaxial compressive stress σ as a function of $((1/\alpha^2)-\alpha)$, for equilibrium swollen hydrogels

Sample	A (Pa)	B (Pa)
C1	−412.5	5065.2
C3	−591.9	2519.3
C4	−225.3	2716.7

According to Eq. (4), the slope of this plot represents the shear modulus G. Reported values have been averaged over five measurements.

3.3. Mechanical measurements and degree of crosslinking

Compression and dynamic mechanical measurements have been performed for all of the crosslinked hydrogels, with the exception of sample C2, due to its poor mechanical properties.

The data collected from uniaxial compression tests have been used to plot the compressive stress σ as a function of $(\alpha-(1/\alpha^2))$, where α is the deformation ratio. According to Eq. (4), the slope of this plot represents the shear modulus G, to be used to calculate the elastically effective degree of crosslinking. Table 3 reports the coefficients of the linear fittings, $Y=A+BX$, of the plots of σ vs. $((1/\alpha^2)-\alpha)$, obtained for samples C1, C3 and C4.

The shear modulus G has been evaluated also from dynamic experiments. The mechanical spectra obtained for all samples, reported in Fig. 4(a)–(c), denote a prevalently elastic behaviour, being the elastic modulus G' significantly higher than the viscous modulus G''.

The value of $|G^*|$, calculated at a fixed frequency (10 rad/s), has been used to evaluate the degree of crosslinking, according to Eq. (4).

The values of $\phi_{2,\mathrm{s}}$ and $\phi_{2,\mathrm{r}}$, which have been used in the calculations, are listed in Table 4.

The correspondent values obtained for the crosslink density from both uniaxial compression and DMA are reported in Table 5. It can be observed that the values calculated from both techniques are quite similar to each other, except for sample C1. However, we assumed that the values of the degree of crosslinking obtained from compression tests are more likely to approximate the real ones. In fact the modification of the parallel plate surface during dynamic measurements may introduce some microcracks in the hydrogel structure; for this reason, the measured values of $|G^*|$, and consequently the correspondent values of crosslink density, always result lower than those obtained from compression experiments. Sample C1, crosslinked in the presence of HA, is fragile with respect to the other crosslinked samples, thus compromising the results obtained from dynamic tests.

Taking everything into account, sample C1 displays a higher degree of crosslinking if compared with sample C3, suggesting that, at a fixed polymer concentration, the presence of hyaluronan may promote the crosslinking reaction.

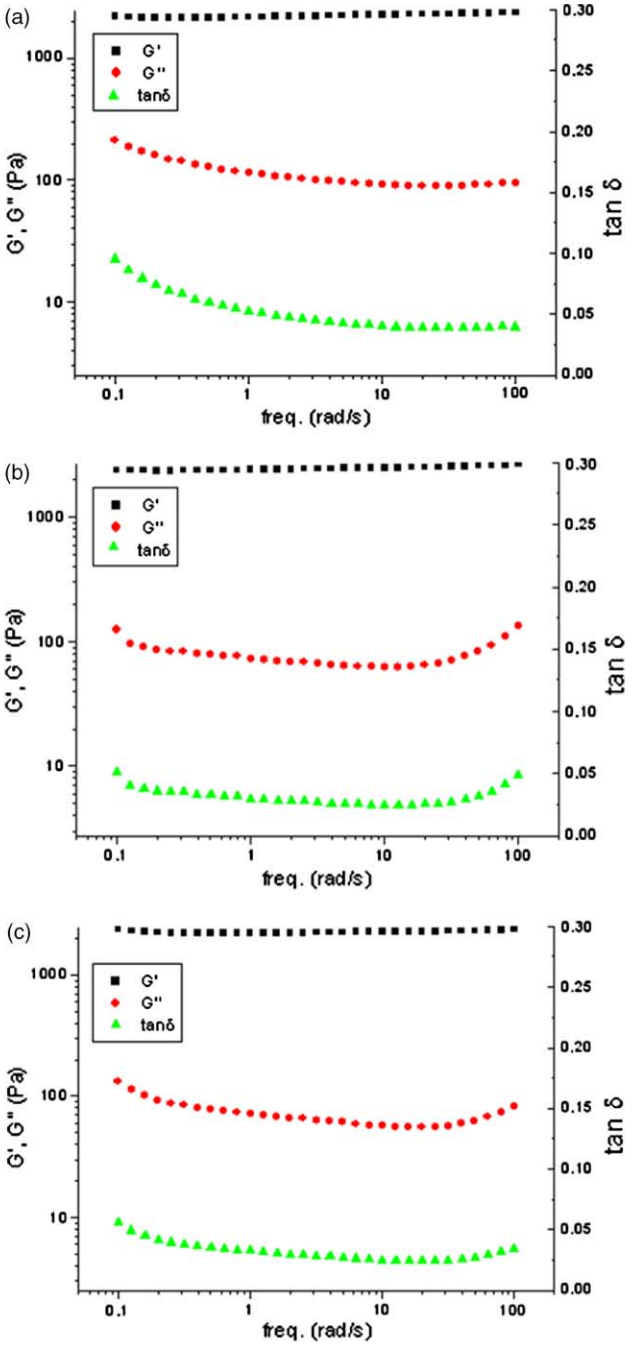


Fig. 4. Dynamic mechanical spectra for (a) sample C1, (b) sample C3, (c) sample C4. The modulus $|G^*|$ has been calculated at 10 rad/s.

3.4. Morphological analysis

The process of dehydration by means of phase inversion in acetone is well known to induce an interconnected microporosity in cellulose based hydrogels crosslinked with DVS [16]. To confirm the presence of a microporous structure within the newly synthesized hydrogels, a morphological analysis has been performed on desiccated gel surfaces. As examples, Fig. 5(a) and (b) reports the electron scanning micrographs obtained from samples C2 and C4 respectively. In both cases, it can be observed that the xerogel structure is quite similar to that reported in the literature for microporous hydrogels desiccated through the same procedure. The numerous foldings on the gel surfaces suggest that a connected microporosity is likely to be present.

Table 4
Values of $\phi_{2,r}$ and $\phi_{2,s}$ used for the calculation of the elastically effective degree of crosslinking

Sample	$\phi_{2,r}$	$\phi_{2,s}$
C1	0,1	0,00833
C3	0,1	0,00667
C4	0,08	0,0025

Table 5
Values of the degree of crosslinking as evaluated through uniaxial compression and dynamic-mechanical experiments

Sample	ρ_x uniaxial compression (mol/cm^3)	ρ_x DMA (mol/cm^3)
C1	$5.2\times10^{-5}\pm0.7\times10^{-5}$	$1.9\times10^{-5}\pm0.3\times10^{-5}$
C3	$4.1\times10^{-5}\pm0.7\times10^{-5}$	$3.4\times10^{-5}\pm0.5\times10^{-5}$
C4	$3.1\times10^{-5}\pm0.6\times10^{-5}$	$2.7\times10^{-5}\pm0.5\times10^{-5}$

Reported values have been averaged over five measurements and are expressed as mean ± standard deviation.

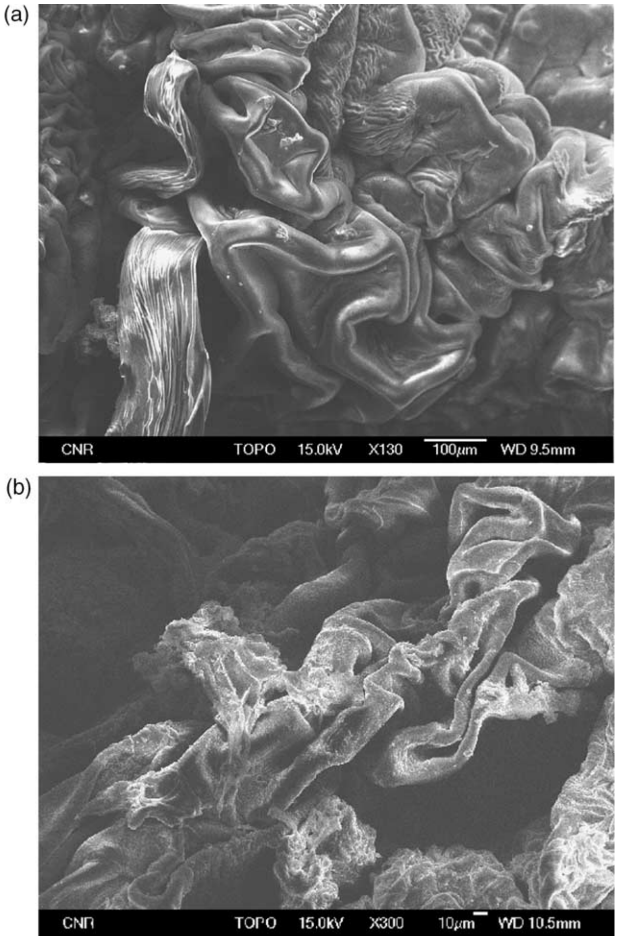


Fig. 5. SEM pictures of dehydrated hydrogels: (a) sample C2; (b) sample C4.

4. Conclusions

Cellulose derivatives-hyaluronic acid based hydrogels have been synthesized using a non-toxic water soluble carbodiimide as crosslinking agent. The resulting hydrogels are potentially biodegradable and biocompatible, and obtained from non-toxic precursors, in view of an environmentally safe production process.

A detailed study of the swelling properties of these hydrogels has been performed. Chemical composition is found to significantly affect both the crosslinking reaction and the equilibrium water uptake. Moreover, for a fixed chemical composition, the hydrogel swelling capability can be modulated by changing the ionic strength of the external solution.

The dehydration procedure by phase inversion in acetone seems to affect the sorption properties of the hydrogels, because it does induce a microporous structure within the material.

Finally, the elastically effective degree of crosslinking has been evaluated by means of uniaxial compression and dynamic mechanical measurements, according to classical rubber elasticity theory.

The reported results evidence that, at a fixed polymer concentration, the highest degree of crosslinking is obtained in the presence of hyaluronan.

References

[1] Masuda F. Superabsorbent polymers. Kyoritsu Shuppann: Japan Polymer Society; 1987.
[2] Chambers DR, Fowler HH, Fujiura Y, Masuda F. Patent US 5,145,906; 1992.
[3] Urban A. Diaper trends—historical and future perspective. IDEA 1992 book of papers. Cary, NC: INDA Association of Nonwoven Fabrics Industry; 1992.
[4] Bakass M, Mokhlisse A, Lallemant M. J Appl Polym Sci 2002;83: 234–43.
[5] Livingston T, Ducheyne P, Garino J. J Biomed Mater Res 2002;62: 1–13.
[6] Sepulveda P, Bressiani AH, Bressiani JC, Meseguer L, Koenig Jr B. J Biomed Mater Res 2002;62:587–92.
[7] Barralet JE, Grover L, Gaunt T, Wright AJ, Gibson IR. Biomaterials 2002;23:3063–72.
[8] Baker JP, Stephens DR, Blanch HW, Prausnitz JM. Macromolecules 1992;25:1955.
[9] Hirokawa Y, Tanaka T, Sato E. Macromolecules 1985;18:2782.
[10] Tanaka T, Fillimore D, Sun ST, Nishio I, Swislow G, Shah A. Phys Rev Lett 1980;45:1636.
[11] Siegel RA, Firestone BA. Macromolecules 1988;21:3254.
[12] Katayama S, Hirokawa Y, Tanaka T. Macromolecules 1984;17:2641.
[13] Ni C, Zhu X. Eur Polym J 2004;40:1075–80.
[14] Adhikari B, Majummdar S. Prog Polym Sci 2004;29:699–766.
[15] Anbergen U, Opperman W. Polymer 1990;31:1854.
[16] Esposito F, Del Nobile MA, Mensitieri G, Nicolais L. J Appl Polym Sci 1996;60:2403–7.
[17] Sannino A, Madaghiele M, Conversano F, Mele G, Maffezzoli A, Netti PA, et al. Biomacromolecules 2004;5:92–6.
[18] Weiss C. In: Laurent T, editor. The chemistry, biology and medical applications of HA and its derivatives. London: Portland Press; 1998. p. 255–66.
[19] Larsen NE. In: Laurent T, editor. The chemistry, biology and medical applications of HA and its derivatives. London: Portland Press; 1998. p. 267–81.
[20] Tomihata K, Ikada Y. J Biomed Mater Res 1997;37:243–51.
[21] Nakajima N, Ikada Y. Bioconjugate Chem 1995;6:123–30.
[22] Choi YS, Hong SR, Lee YM, Song KW, Park MH, Nam YS. Biomaterials 1999;20:409–17.
[23] Park SN, Park JC, Kim HO, Song MJ, Suh H. Biomaterials 2002;23: 1205–12.
[24] Xu JB, Bartley JP, Johnson RA. J Membr Sci 2003;218:131–46.
[25] Flory PJ. Principles of polymer chemistry. Ithaca, NY: Cornell University Press; 1953.
[26] Erman B. Superabsorbent polymers. In: Buchholz FL, Peppas NA, editors. ACS symposium series, vol. 573; 1994, 1994. p. 50–63.
[27] Lenzi F, Sannino A, Borriello A, Porro F, Capitani D, Mensitieri G. Polymer 2003;44:1577–88.