Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 1, January - March 2013, p. 101 - 110

GELATIN HYDROGELS: EFFECT OF ETHYLENE OXIDE BASED SYNTHETIC CROSSLINKING AGENTS ON THE PHYSICO-CHEMICAL PROPERTIES

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In this paper, different kinds of bifunctional crosslinkers were chosen in order to study the effects of both the crosslinker composition and size on the characteristics of methacrylated gelatin hydrogels. In the first step, crosslinked gelatins were synthesized using photoinitiated network formation. Water uptake capacity tests were performed. It was found that the internal arrangement of hydrogels' components is strongly affecting the hydrophilicity of the materials. Also the thermal properties of gelatin are improved by the presence of the synthetic crosslinker. The rheological measurements have shown that the presence of longer crosslinkers lead to more viscous materials when compared with gelatin hydrogels.

(Received December 11, 2012; Accepted December 20, 2012)

Keywords: hydrogel; PEGDMA; matrice, ethylene oxide.

1. Introduction

In recent years, hydrogels have attracted extended attention in food, packaging and for a wide range of biomedical applications [1-3]. Hydrogels can be obtained from natural polymers as well as from synthetic polymers or combinations of both. Among the natural polymers, gelatin, obtained by partial denaturation of collagen has gained extended attention due to its abundance and its properties that recommend it as a suitable for many applications [4]. It is well known that gelatin is one of the most used biomaterials due to properties such as biological interaction capabilities and numerous functional groups' modification. Also it is unique among hydrocolloids in forming thermo-reversible with gel-sol temperature close to the physiological temperature [5]. When photo-crosslinkable groups are introduced on the gelatin backbone, gelatin networks obtained through UV curing can be obtained that present stability when exposed at body temperature. Van Den Bulcke et al [6] have modified gelatin through the direct reaction with methacrylic anhydride to introduce photo-crosslinkable moieties.

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It was noticed that using photocroslinking of gelatin, the mechanical integrity of the constructs was enhanced, but for some specific applications, the mechanical properties required are not sufficiently improved [7].Addition of a plasticizer within the gelatin networks aims to overcome the brittleness of the materials in their dry form, to improve flow and flexibility and to increase toughness of hydrated hydrogels but also to prevent the materials from cracking during packaging, transportation and usage [8-9]. The effect of various plasticizers on the properties of gelatin films was studied. There have been various products applied containing different chemical compositions, sizes and shapes, such as oligosaccharides, organic acids and polyethylene glycols with a wide range of molecular weights [4]. As a synthetic hydrogel, polyethylene glycol (PEG) has been very popular due of its many desirable properties. PEGs are intensively studied for biomaterials applications due to their biocompatibility, hydrophilicity, lack of toxicity, favorable to nutrient and oxygen transport, robust mechanical properties and, nevertheless, absence of interaction sites for proteins [3, 10-11].

In the present study, we have developed photo-crosslinkable hydrogels based on methacrylamide modified gelatin (MAG) and different types of crosslinkers: ethylene glycol dimethacrylate (EGDMA), tetraethylene glycol dimethacrylate (TEGDMA) and poly(ethylene glycol) dimethacrylates (PEGDMA I with Mw 550 g/mol and PEGDMA II with Mw 750 g/mol). Bifunctional crosslinkers with different chain lengths have been used in order to investigate their influence over some key physical properties of MAG-based hydrogels. Due to the fact that the gelatin used in this study presents methacrylamide groups, it is expected that during the photoinitiated network formation to obtain materials with complex internal structure; this would represent a key element for the hydrophilic behaviour that later influences the rheological properties of the novel materials.

2. Experimental

Materials

Gelatin (type B), isolated from bovine skin by the alkaline process, was supplied by SKW Bio-systems (Ghent, Belgium). 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one] (Irgacure®2959) was purchased from Ciba Speciality Chemicals. Ethylene glycol dimethacrylate 98% (EGDMA), tetraethylene glycol dimethacrylate ≥90% (TEGDMA), poly(ethylene glycol) dimethacrylate with Mn ≈ 550 g/mol (PEGDMA I) and with Mn ≈ 750 g/mol (PEGDMA II), and methacrylic anhydride 94% (MA) were purchased from Sigma-Aldrich and used as received.

Gelatin was chemically modified with methacrylamide side groups, resulting in a methacrylamide-modified gelatin derivative (MAG) with a degree of substitution (DS) of 55%, as described in a previous work [12]. The DS has been determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and UV-VIS spectroscopy. MAG was used as a 10% w/v solution in double distilled water (ddw).

Methods

In order to obtain hydrogels, a photoinitiated network-forming polymerization technique was used.

A MAG aqueous solution (10% w/v) and the crosslinking agents (CAs) (**EGDMA, TEGDMA, PEGDMA I, PEGDMA II)** were mixed using 1:1 molar ratio between the methacrylamide side groups from MAG and the "C=C" double bonds from the CAs (see Table 1) in the presence of a photo-initiator (Irgacure®2959) with a concentration of 2.1 mol % with respect to total C=C bonds. Subsequently, the polymerization solutions were degassed for 15 minutes using a vacuum pump, followed by injection of the solutions between two parallel glass plates, separated by a 1 mm thick silicone spacer. Finally, the hydrogel was irradiated with UV-light (at a wavelength of 276 nm, 10 mW/cm^2) for 60 minutes on both sides. Crosslinked materials were stored at 5°C until their evaluation. The MAG – crosslinker hydrogels with various compositions were obtained and further denoted as indicated in Table 1. A control hydrogel consisting of crosslinked MAG (P0 in Table 1) was synthesized using the same photo-initiation procedure as described above.

Table 1. Composition of the initial mixtures.

Material	**Components**
P0	MAG
P1	MAG – EGDMA
P2	MAG – TEGDMA
P3	MAG – PEGDMA I
P4	MAG – PEGDMA II

Characterization

Gel fraction analysis

The gel fraction (GF, %) was determined gravimetrically using the following equation (1):

$$GF\ (\%) = \left(m_f/m_0\right) \times 100\% \tag{1}$$

with m_0 being the mass of the dried materials as they result from the reaction without purification and m_f being the mass of the dried scaffolds after extraction of the soluble fraction in ddw at 40°C for 48 hours.

Water uptake behaviour

The swelling behaviour was assessed in ddw, at 40 °C. The gravimetric method was used to estimate the swelling degree (SD) at predetermined time intervals, *t*, using the well known equation (2):

$$SD\ (\%) = (m_t - m_0)/m_0 \times 100\% \tag{2}$$

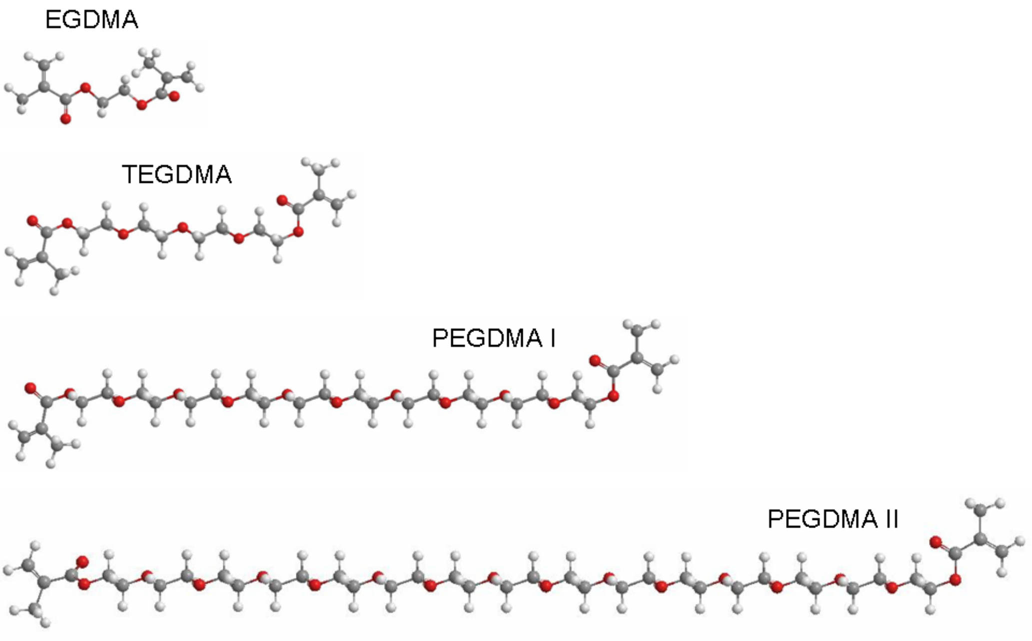
with m_t being the mass of the wet sample at t while m_0 represents the mass of the dried sample before incubation in water. At different incubation intervals, the samples removed from ddw were blotted to gently remove solvent excess and weighted to evaluate the wet weight (mt). After measuring, samples were reimmersed in ddw. The maximum swelling degree (MSD) was estimated as the equilibrium value of SD.

Rheology experiments

The mechanical properties of the hydrogel films were evaluated using a rheometer type Physica MCR-301 (Anton Paar, Sint-Martens-Latem, Belgium). The oscillation measurements were performed using two parallel plates, with an upper plate diameter of 50 mm. The temperature dependence of the elasticity modulus G' or the viscosity modulus G'' was measured by oscillation shear measurements during heating in the range of 21°C to 40°C at a constant frequency (1 Hz), deformation (0.05% strain) and normal force (1N).

3. Results and discussion

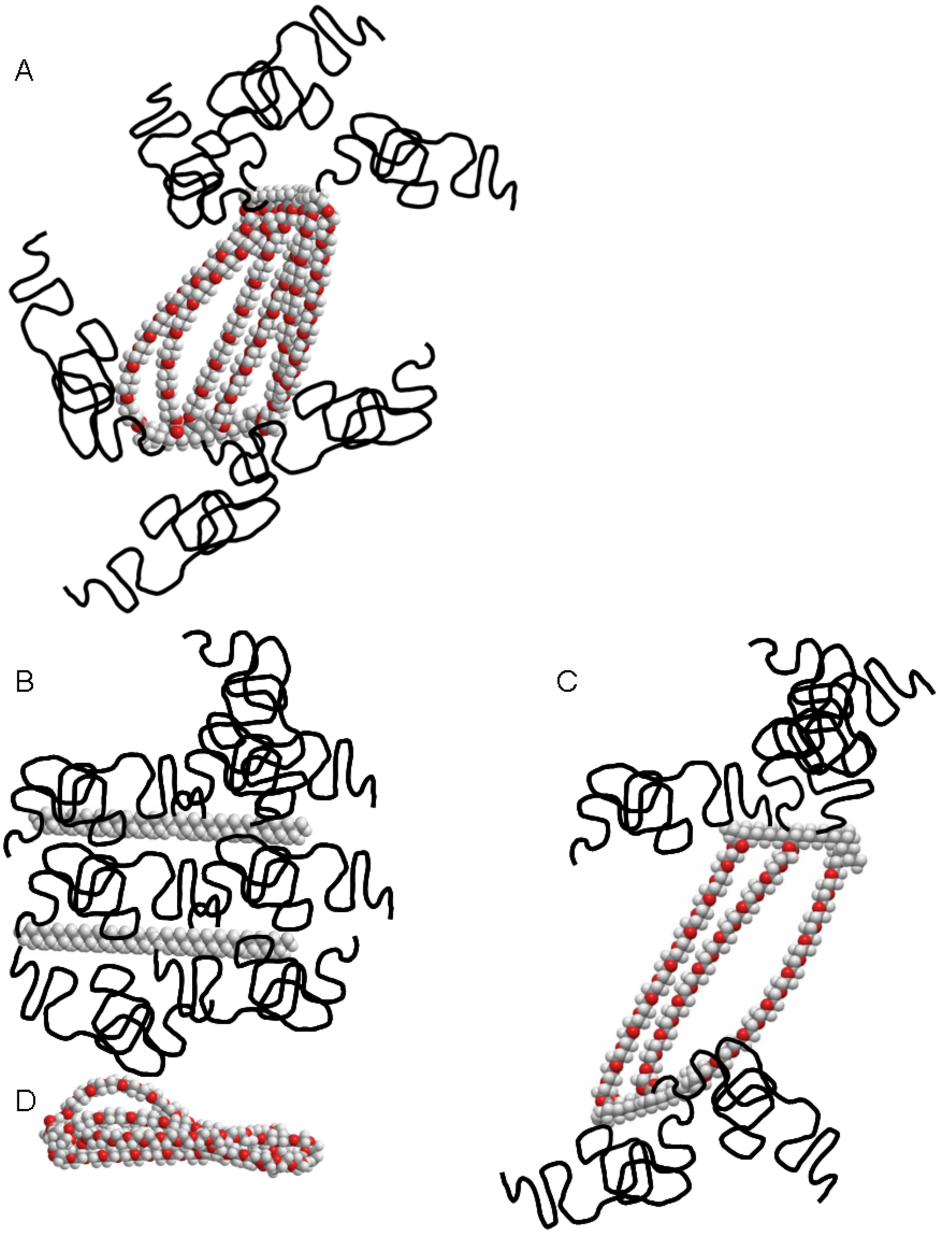
In this study, we report on the synthesis and the physico-chemical characterization of crosslinked hydrogels based on MAG and different crosslinking agents (see Scheme 1). The control of the properties of the resulting hydrogels through the use of shorter (EGDMA and TEGMA) or longer (PEGDMA II) crosslinkers was investigated.



Scheme 1. Crosslinking agents used to obtain MAG hydrogels.

We anticipate that different internal organization of the resulting materials may occur, as depicted in Scheme 2, for the hydrogel obtained with the longest CAs. Thus, it is possible to obtain hydrogels with homogeneously distributed crosslinks according to the used recipe (A in Scheme 2). However, we do not exclude the formation of MAG-MAG domains (B in Scheme 2) and self-polymerized crosslinker (C in Scheme 2), coexisting with MAG-CAs hydrogels (D). Scheme 2 depicts these structures as schematically obtained for MAG and PEGDMA II. Based on this schematic representation the other structures for EGDMA, TEGDMA and PEGDMA I, respectively, can be easily anticipated.

In a first step, the success of the synthesis was assessed through GF estimation. Since the systems are intended to be applied for potential biomedical purposes for soft tissue engineering, the materials were analyzed with respect to the influence of the crosslinker on the swelling behaviour and the rheological performance.



Scheme 2. Hydrogel constituents for MAG and PEGDMA II: A – hydrogel obtained through homogeneous crosslinking of MAG and PEGDMA II according to the receipe used; B – MAG-MAG hydrogel; C – PEGDMA II self-polymerized domains; C –hydrogel with lower density of crosslinks with respect to the original receipe. Two C-C polymer chains are represented for each possible structure. Polypeptide chains are shown as black entangled chains.

Gel fraction analysis

Gelatin solutions stored below 30°C form reversible physical gel networks. Incubation at 37°C causes destruction of the physical network of gelatin, since the protein chains change from helix to random coil when heated above the sol-gel transition temperature. This behaviour is also a key feature of the gelatin C=C - modified derivative, MAG. This is why the investigation of the complex MAG-CAs hydrogels developed in this study started with the estimation of the GF. This parameter quantifies the success of the reaction while indicating the quantitative migration of all hydrosoluble species in water. Therefore, the materials have been incubated at 40°C in double distilled water for 48 hours. The results were estimated using equation (1) and the obtained GF values are summarized in Fig. 1. All the synthesized hydrogels are characterized by high GF values (between 82% ± 0.84% and 88% ± 1.35%) indicating that the new synthesized hydrogels are water insoluble, and the fact that mass conserves during the chemical treatment. This applied also for the control sample, proving the efficiency of the applied photo-polymerization with respect to the synthesis of the MAG hydrogel.

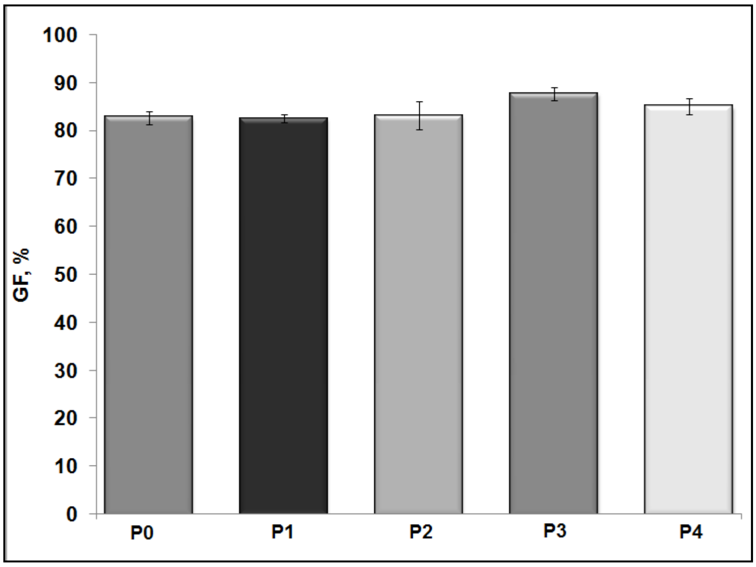


Fig. 1. GF average values for the new types of hydrogels: P0 – MAG; P1 – MAG-EGDMA; P2 – MAG-TEGDMA; P3 – MAG-PEGDMA I; P4 – MAG-PEGDMA II.

Water uptake capacity

MAG based hydrogels are thought to possess a complex combination of hydrophilic and hydrophobic network properties due to the formation of entangled >C–C< polymer chains (see Scheme 1) with polypeptidic branches. The water affinity can be mainly attributed to the gelatin sequences as major hydrogel component. However, the crosslinking through carbon-carbon chains leads to the modulation of this property.

Adding bifunctional CAs results in a modification of the water-absorption capacity, depending on the compatibility of these molecules with MAG and on their water affinity, respectively. Furthermore, in addition to the carbon-carbon chains, new branches from the crosslinker structural units are formed, and this leads to an even more complex modulation of the hydrophilic-hydrophobic balance within the hydrogel structures.

In this context, the water uptake capacity of all types of hydrogels developed was studied through incubation in ddw, at 40°C, while monitoring the water absorption at regular time points. The results are summarised in Fig. 2. SD was calculated using equation (2).

As anticipated, the MAG homopolymer is the most hydrophilic material. The swelling capacity of MAG is very high (MSD = 6857%). After hydration, the MAG-MAG hydrogels are soft and flexible. This behaviour is easily explained through the structure of the MAG-MAG hydrogels, consisting out of (i) C-C polymer chains formed through the polymerization of the C=C groups from the methacrylamide side chains in MAG and (ii) polypeptide sequences from gelatin (panel C in scheme 2). The latter are responsible for the high water uptake due to their hydrophilicity. The other four synthesized networks have a considerably lower capacity to absorb water in comparison to MAG-MAG, being dependent on the composition of the initial polymerization mixtures. Thus, the association of MAG with a synthetic crosslinker, even in a low concentration, limits the swelling of the materials. The MSD for MAG-EGDMA is 5733%, which is significantly higher than the MSD for MAG-TEGDMA (3927%). This result is rather intriguing if considering the length of the two CAs and their water compatibility. Therefore, we speculate the formation of self-polymerized synthetic polymer domains, affecting the overall water uptake capacity (Scheme 3). On the other hand, the dimension of the mesh is variable in the studied hydrogels, also affecting the polymer hydration capacity. Furthermore, in the case of MAG-PEGDMA I and II, the materials present very similar water affinity. The MSD values are high and comparable: 4903 % and 4569 %, respectively. These values confirm a higher water affinity of these crosslinked materials with respect to TEGDMA-containing hydrogels. This behaviour is normal since PEGDMA is more hydrophilic when compared to TEGDMA. However, this is intriguing when compared to EGDMA-containing scaffolds, the latter conferring even less hydrophilicity, at least theoretically. The obtained results are difficult to explain. They may be due to the fact that a lower amount of double bonds react since they are more closely located next to one another. Lower conversion could lead to higher swelling. We could also speculate the possible formation of CAs agglomerations (see Scheme 3). EGDMA, due to its low dimension, forms small domains of lower hydrophilicity and allows for high swelling of MAG-MAG chains without significant modification of the MSD when compared to MAG-MAG hydrogels. All these aspects prove important variations of the swelling behaviour of the hydrogels upon modification of the composition with different CAs.

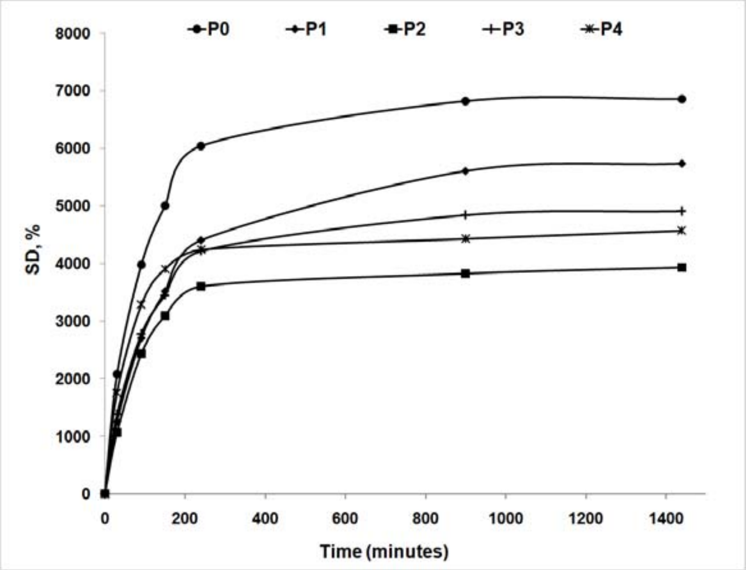
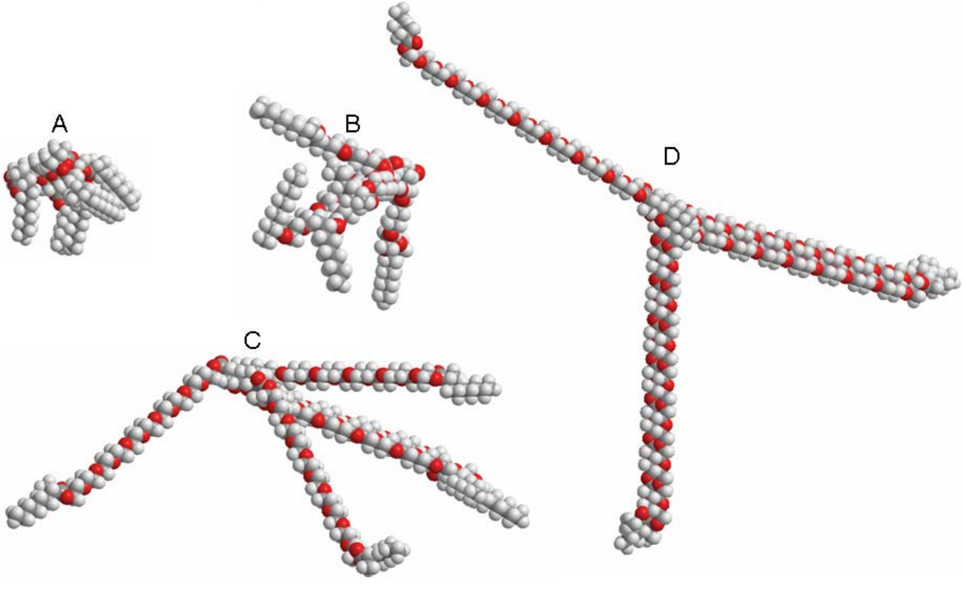


Fig. 2. Water uptake capacity expressed as SD (%) as a function of the incubation time, for the hydrogels P1–P4, when compared to the control sample, P0.



Scheme 3. Crosslinker-rich domains in the hydrogel matrices (five molecules associated through C-C chains): A – EGDMA; B – TEGDMA; C - PEGDMA I; D - PEGDMA II.

Rheological evaluation of hydrated hydrogel films

Viscoelasticity represents another important characteristic of the materials and it is influenced by the introduction of the CAs in the MAG system.

The viscoelastic properties of the hydrogels were determined in a swollen state, as obtained after the synthesis. The values were compared to the properties of a crosslinked MAG network (as depicted in Fig. 3).

In the first part of the experiment, mechanical spectra were recorded at a constant temperature of 21°C, plotting the storage *G'* and loss *G''* moduli as functions of time. Both moduli exhibit a pronounced plateau for the applied temperature. Furthermore, for all samples *G''* is more than 21 times smaller than *G'*, indicating that well established elastic networks were formed.

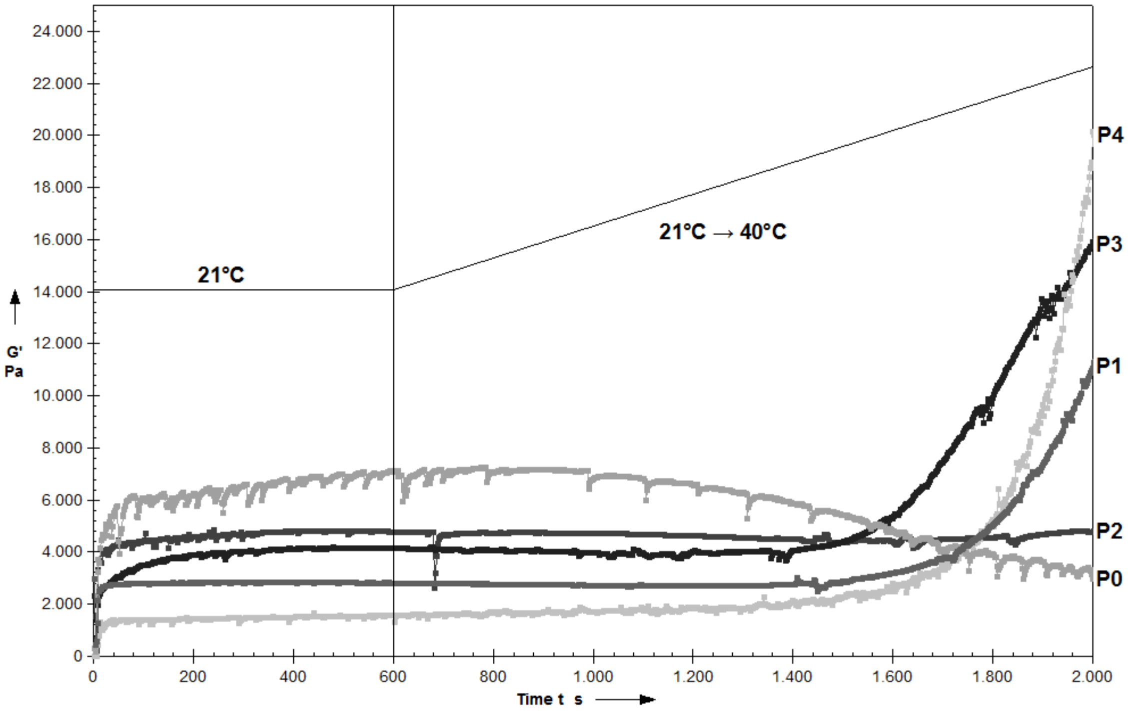


Fig. 3. Mechanical properties of hydrogel films (as recorded using a NF of 1 N). The films were UV irradiated for 60 minutes prior to investigation.

In a subsequent step, the temperature applied was varied from 21°C to 40°C. In the temperature range of 21°C ÷ 32°C, all the obtained networks presented a plateau for both *G'* and *G''*. When the temperature exceeds 32°C, P0 presents a decrease of *G'*. This result is attributed to the thermosensitive nature of MAG, still presenting the gel-sol transition characteristic for the polypeptidic chains interconnected through C-C polymer sequences formed within the MAG network.

Furthermore, the results recorded for the hydrogels obtained in the presence of the synthetic crosslinking agents indicated that the thermosensitivity of the MAG sequences can be influenced by both the crosslinker nature and its density in the network. In this context, the networks obtained through the addition of CAs, when applying temperatures exceeding 32°C, both *G'* and *G''* present significant increase until reaching 40°C. The only sample that has a stable rheological behavior is sample P2. For all the other materials, the increase of temperature also leads to a smaller ratio between the *G'* and *G''* (see Fig. 4); being typical for more viscous systems.

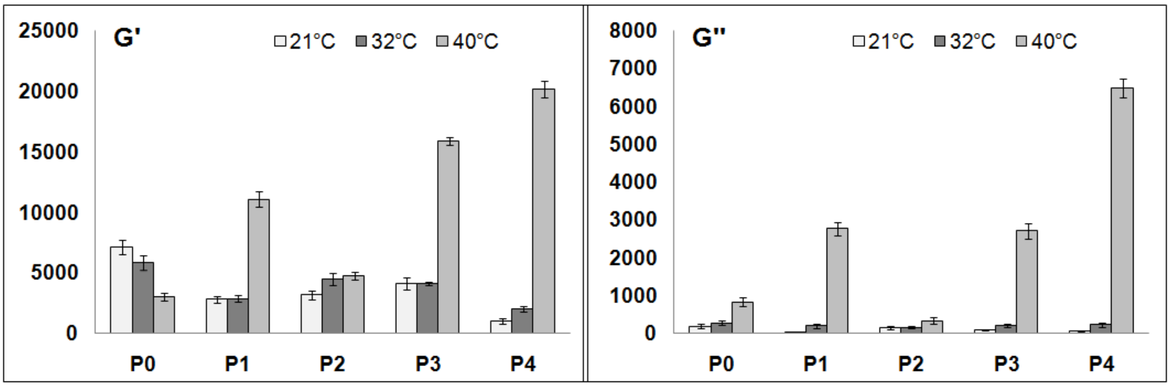


Fig. 4. Storage moduli (left) and loss moduli (right) recorded at 21, 32 and 40 °C for all obtained materials.

Concerning the viscoelastic properties of the swollen materials, all the synthesized MAG-CAs systems present the specifications for continuous and flexible solids. Also the presence of all crosslinkers enhances the thermal behaviour of MAG when referring to the improvement of the mechanical resistance with higher temperature. Thus, all the materials present a real potential for a fine control over their rheological properties.

Conclusions

This work presents the influence of four synthetic crosslinking agents on the characteristics of gelatin-based hydrogels and tries to further correlate the specific behavior with the chemical composition and the internal arrangement of the hydrogel components. The most interesting systems include MAG-PEGDMA I and II because they have a different rheological behaviour when compared with the other materials. Thus, when increasing the temperature of the systems, during the rheological measurements, from room temperature to 40°C, the mechanical resistance presents a significant increase. Also, these types of hydrogel are dimensionally more stable by comparison to MAG. These will therefore be the subject of further research.

Acknowledgement

The financial support through the Human Resources project Study of the biomimetic mineralization using specifically functionalized 3D hydrogels, PN-II-RU-TE-2009-10, code TE-80/2010 is gratefully acknowledged.

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