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NEW HYDROGELS BASED ON GELATIN AND ACRYLAMIDE

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The study presents the synthesis and characterization of bicomponent polymer systems based on modified gelatin and polyacrylamide in the aim of obtaining materials with superior properties with respect to the individual components. The synthesis of the hydrogels was carried out through the polymerization of C=C groups of the synthetic monomer and of the modified gelatin using photoinitiation. Five bicomponent systems with different ratios between the individual components have been obtained, using methacrylamide modified gelatin of two different substitution degrees. Subsequently, the materials have been characterized in order to establish the relationship composition - properties.

Keywords: gelatin, acrylamide, bicomponent polymer system

1. Introduction

Hydrogels are a class of biomaterials that have demonstrated high potential for biological and medical applications. Significant interest has been shown in the use of natural, synthetic, and hybrid hydrogels as biomaterials and carriers for drug delivery [1].

Gelatin represents an attractive candidate as starting material for preparing hydrogels due to its biocompatibility, biodegradability and gelling properties. However, gelatin has a relatively low melting point, thus leading to unstable gels at room temperature [2]. Over the years, several compositions based on gelatin have been obtained in order to be used in the biomedical field as wound dressing, drug delivery systems [3], scaffolds for tissue engineering [4], as well as in other

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applications ranging from food related ones to photographic, or technical products [5]. On the other hand, polymers and copolymers of acrylamide (AA) are the most commonly used hydrogels in a wide range of applications. Hydrogels based on poly(acrylamide) (pAA) can absorb large amounts of water, but have poor mechanical strength [6] . Recently, we have demonstrated that interpenetrating polymer networks (IPNs) based on gelatin, alginate and crosslinked pAA present interesting properties as superabsorbent materials for soft tissue engineering [7]. We have shown that in addition to physical properties dependent on the AA content, the internal organization of the tricomponent hydrogels might be modulated through the amount of initially used AA [7]. It is well known that AA presents very high rate constant for the propagation; this fact may be explained by a pre-organization of the monomer leading to a pseudo-lattice. Therefore, one may expect the corresponding polymer to form an initial framework in the presence of gelatin or alginate, further supporting the development of more complex hydrogel structures.

In this context, the present work describes the first attempts to synthesize a bicomponent hydrogel based on methacrylamide modified gelatin (MAG) as natural component and pAA as synthetic polymer, through simultaneous polymerization and cross-linking, using photo-initiation. The study aims the investigation of the success of the reaction as well as the characterization of the obtained hydrogels.

1. Materials and methods

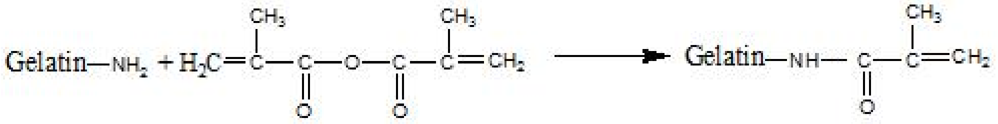
2.1. Materials

Methacrylic anhydride (94%), gelatin from bovine skin and acrylamide (all supplied by Sigma-Aldrich) were used as such. Na_2HPO_4, NaOH, HCl (Sigma-Aldrich) and KH_2PO_4 (Fluka) were used in order to prepare the buffer solution (pH 7,8). o-phtalic aldehyde was supplied by Acros Organics. n-butylamine (Sigma) was diluted with double distilled water to various concentrations. The initiator, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one] (Irgacure 2959) was purchased from Ciba Speciality Chemicals and used as 0.8% (w/v) aqueous solution. Coomassie protein assay reagent, methanol and acetic acid were supplied by Sigma Aldrich. Dialysis membranes used were produced by neoLab Migge Laborbedarf.

2.2. Methods

Synthesis of modified gelatin

MAG was prepared by the direct reaction of gelatin with methacrylic anhydride according to the protocol set up by Van Den Bulcke [2]. Two solutions with different concentrations of methacrylic anhydride were prepared. Subsequently, a 10% solution of gelatin was prepared through the dissolution of the polypeptide in phosphate buffer (pH 7.8; 0.1 M) at 40°C. The appropriate amount of methacrylic anhydride was added to this solution and the reactions took place under vigorous stirring, at 40°C. The final solutions were subjected to dialysis against distilled water at 40°C for 24 hours; water was changed five times in 24 hours. Finally the resulted solutions were poured into plastic dishes and freeze-dried.



Scheme 1: Synthesis of MAG

Characterization of MAG through UV-VIS

The reaction products were subjected to UV-VIS analysis in order to calculate the degree of substitution (DS) of the prepared MAG. For this purpose, the method of primary amine detection through the reaction with o-phtalic aldehyde (OPA) has been used. UV-VIS spectroscopy was performed on a CINTRA 101 spectrometer, at a fixed wavelength of 340 nm.

DS is usually defined as the ratio of incorporated methacrylamide groups with respect to free amine groups available before modification of native gelatin.

$$DS\% = \frac{(initial-NH_2\ groups\ in\, gelatin)-(non\, reacted-NH_2\ groups\ in\, MAG)}{(free\ -NH_2\ groups\ in\ gelatin)} \times 100 \qquad (1)$$

Gelatin and MAG aqueous solutions with a concentration of 10 mg/mL were then prepared. The amine detection protocol is described by Greg T. Hermanson [8]. n-butylamine solutions of known concentrations (in the range of 1-10 mM) were used to draw a calibration curve. The specific absorbance was measured after 15 minutes of reaction. This experiment was performed in triplicate, at room temperature.

The recorded values were further converted into amount of non-reacted $-NH_2$ groups and DSs were subsequently estimated using the above equation. The obtained values were 41% and 55%. For simplicity, MAG 1 states for DS 55% while MAG 2 for DS 41%, respectively.

Hydrogel synthesis

In order to obtain bicomponent hydrogels, combined processes of polymerization and cross-linking were performed using photoinitiation. 10% (w/v) aqueous solution of MAG 1 and 15% (w/v) MAG 2, respectively, were mixed at different ratios of AA (as indicated in table 1) in the presence of the photo-initiator whose concentration was 2 mol% with respect to methacrylamide side groups in MAG and 0.1 mol% with respect to C=C groups from AA.

Table 1

Reaction mixtures used to prepare bicomponent hydrogels.

Probe	X : Y*	% weight			
		MAG 1	MAG 2	AA	Irgacure 2959
P0	0:1	9.88	-	-	0.01
P1	50:1	6.84	-	9.11	0.03
P2	100:1	6.39	-	9.59	0.04
P3	500:1	1.87	-	14.08	0.05
P4	1000:1	1	-	14.95	0.05
P5	0:1	-	14.99	-	0.01
P6	50:1	-	9.6	5.38	0.02
P7	100:1	-	7.05	7.92	0.03
P8	500:1	-	2.27	12.69	0.04
P9	1000:1	-	1.23	13.72	0.05

* X – initial number of AA moles
Y - initial number of double bonds in MAG

The polymerization mixtures were poured in glass moulds and cured for 60 minutes using a UV transiluminator ECX-F26 at 312 nm wavelength. The resulting materials were intensively washed with double distilled water (ddw), at room temperature to assure the complete removal of the unreacted species.

Gel fraction analysis

The hydrogel samples were kept in distilled water at 40°C, in order to remove all the soluble fractions. After 72 hours the gel samples were dried to constant weight at 40°C and the gel fraction (GF) characteristic to each of the samples was gravimetrically measured using the following equation:

$$GF,\% = \frac{w_g}{w_0} \times 100 \qquad (2)$$

Where: w_g - is the weight of the dry gel after extraction from water w_0 - is the initial weight of dry gel.

Homogeneity study through Fourier Transform Infrared Spectroscopy (FT-IR)

The bicomponent hydrogels have been submitted to FT-IR analysis using a JASCO 4200 spectrometer equipped with a Specac Golden Gate attenuated total reflectance (ATR) device in the 4000-600 cm^{-1} wave number region. Spectra recorded in multiple points on the two sides of the hydrogels were compared to assess the homogeneity of the materials. Gelatin and pAA were used as control samples.

Coomassie Blue Staining Method

Thin samples were cut from each probe and immersed into Coomassie reagent for 30 minutes, changing the solution at 15 minutes, as indicated in the data sheet of the product. Subsequently, the samples were immersed into the washing mixture (methanol, acetic acid and distilled water) so that to remove non-reacted reagent.

Determination of swelling degree

The gel samples were immersed in distilled water at 40°C. Hydrogel samples were periodically weighed after removing excess water from the surface of the gel with a filter paper. The swelling degree (SD) was calculated using the following relation:

$$SD,\% = \frac{w_t - w_0}{w_0} \times 100 \tag{3}$$

Where: w_t - is the weight of swollen gel sample at time t
w_0 - is the initial weight of dry gel samples submitted to swelling.

Evaluation of mechanical properties

Evaluation of mechanical properties of MAG-pAA hydrogels was performed using a CT3 texture analyser with a 4500 grams cell load (Brookfield Engineering) in compression mode. The sizes of samples were 10 x 10 x 5 mm. A probe with the diameter of 24.5 mm was used to compress the samples to a deformation of 20% for 3 seconds. The tests were performed in triplicate. A stress-deformation curve was ploted and hardness and elasticity modulus were calculated for each composition.

2. Results and discussion

2.1. Characterization of the hydrogel films

Gel fraction analysis

In order to estimate the polymerization efficiency of the developed hydrogels, the GF study was performed. This quantifies the yield of the reaction while indicating the migration of hydrosoluble species in water. Therefore, the materials have been incubated at 40°C in ddw for 72 hours. The results were estimated using the equation (2).

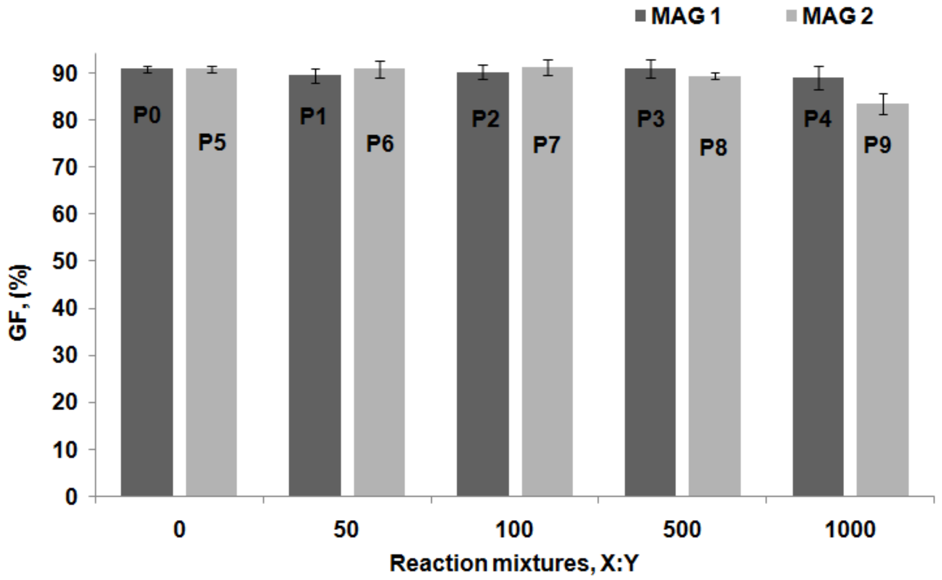


Fig. 1. Average values of GF obtained after extraction in distilled water at 40°C, after 72 hours.

Fig. 1 shows GF values for the compositions obtained both with MAG 1 and MAG 2, respectively. Both types of compositions show high GF values when immersed into distilled water at 40°C for 72 hours. GF values ranging from 88.96 ± 2.39% to 90.96 ± 1.94 are characteristic for the hydrogels obtained with MAG 1, while GF values ranging from 83.42 ± 2.13 to 91.09 ± 1.66 are obtained when MAG 2 was used. These results indicate that all the hydrogels present an excellent mass conservation during the chemical reaction; nevertheless, GF values prove that the obtained bicomponent systems are water insoluble.

These findings represent the first proof that the synthesis of the hydrogels is successful and they also demonstrate that the materials present stability at 40°C.

Another important conclusion is that the ratio between the AA and MAG components does not affect the success of the polymerization reaction.

Homogeneity study through FT-IR Spectroscopy

Since undesired separation of the two polymeric components may occur during the synthesis of the hydrogels, the homogenity of the obtained materials has been studied through FT-IR investigation. No differences have been noticed between the spectra recorded on the upper and lower side of the samples (Fig. 2); this fact indicates that the resulted hydrogels exhibit an homogeneous distribution of the natural and synthetic components.

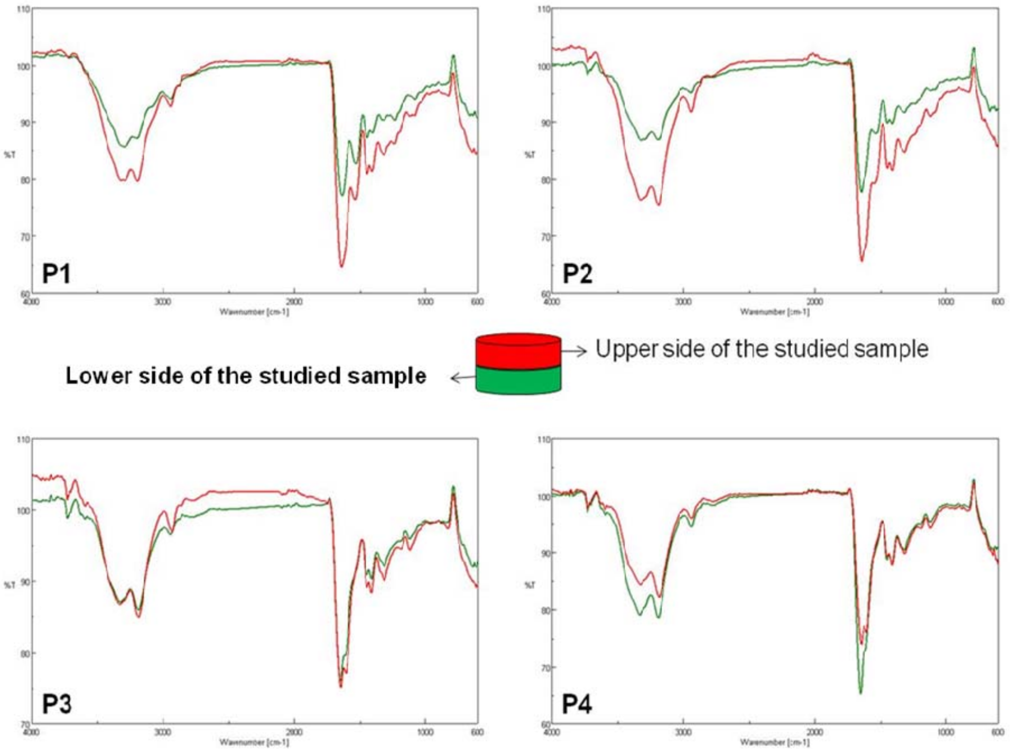


Fig. 2: FT-IR spectra for samples P1÷P4

Furthermore, it was expected that the vibrations characteristic to the two polymer components to be identified in the spectra of the bicomponent materials. The spectrum of gelatin presents as main characteristic signals broad with spike at 3290 cm^{-1} (common signal for O-H and N-H stretching), 3037 cm^{-1} (N-H), 2943 cm^{-1} (saturated C-H stretch), 1630 cm^{-1} (amide I) and 1530 cm^{-1} (amide II). On the other hand, pAA is characterized by one broad with two spikes at 3328 cm^{-1} and 3187 cm^{-1} (N-H stretch) and a much stonger C=O stretch at 1647-1612 cm^{-1} [7]. In addition, Fig. 2 proves: 1) the presence of the two components in all the bicomponent materials (P1-P4) and 2) increasing pAA content from P1 to P4. With respect to the first statement, the spectra of all the hydrogels exhibit a combination of broad O-H and N-H vibrations of mixed origin (gelatin, pAA). Increasing amount of pAA is associated with a shifting of the broad peak from 3297 cm^{-1} in P1 to 3333 cm^{-1} in P4. Furthermore, in the wavenumber interval 1530 cm^{-1} to 1640 cm^{-1}, the vibrations characteristic to amide I and II from gelatin and to C=O from pAA are noticed. A combination of amide I and C=O vibrations has been noticed, increasing in intensity from P1 and P4, according to the increase in pAA content. This occurs simultaneoulsy with a weak shifting of the corresponding maximum from 1637 cm^{-1} to 1651 cm^{-1}. Nevertheless, due to the decrease in the concentration of gelatin, the peak corresponding to amide II (1530 cm^{-1} in P1) can be noticed as a shoulder in P3 and P4.

Coomassie Blue Staining Method

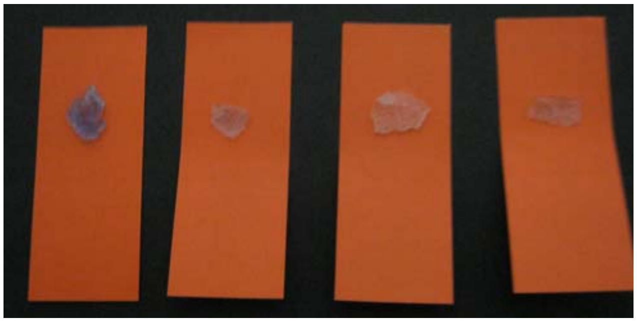


Fig. 3: Hydrogel samples after immersion in Coomassie reagent (from left to right: P1 to P4)

Then, a colorimetric study based on protein detection through Coomassie reagent was performed. According to the homogeneity of the blue color resulted from the Coomassie treatment, it may be concluded that the protein is uniformly distributed in the hydrogels. Furthermore, the intensity of the colour is directly dependent on the amount of protein in each sample: the more intense color corresponds to the hydrogels with the highest gelatin content (Fig. 3).

Swelling degree

As the new materials are intended to be used as superabsorbent media in biomedical applications, the affinity against body fluids and the capacity to transfer nutrients represent key elements. Therefore the SD has been studied through incubation in ddw at 40°C, while monitoring the water uptake at regular time points. SDs at different time intervals were calculated using equation (3). The equilibrium value is considered the maximum swelling degree (MSD) and this parameter is strongly influenced by the composition of the hydrogels (Fig. 4). Fig. 4 indicates that lower DS in MAG is responsible for a less crosslinked intrinsic network in the resulting hydrogel (P5), leading to higher swelling when compared to the more substituted protein (P0).

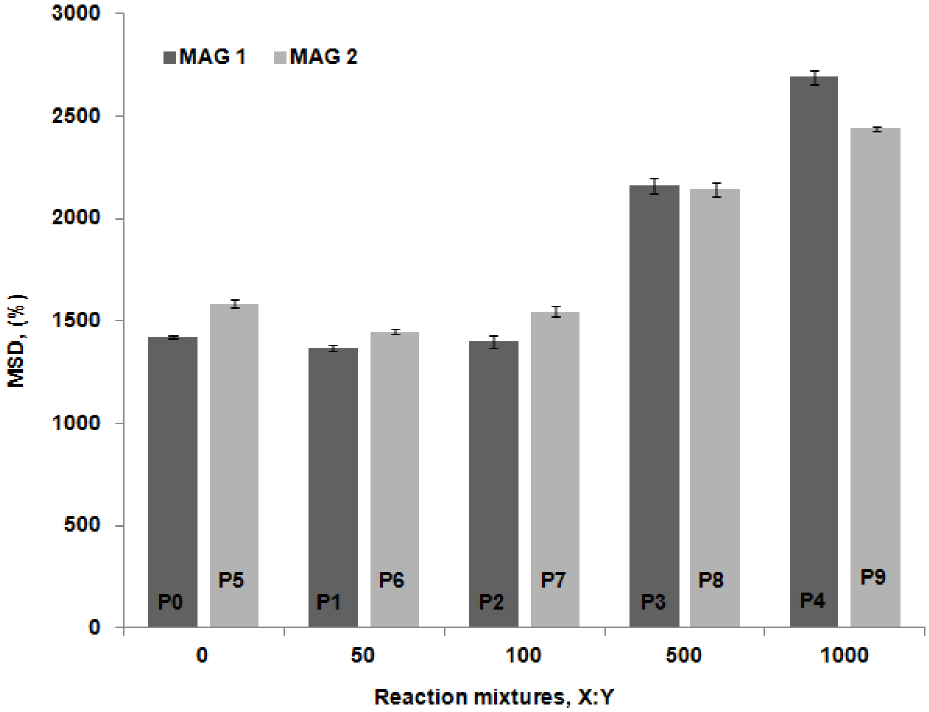


Fig. 4: Average values of the maximum SD obtained after extraction in distilled water, at 40^{o}C, after 98 hours

Water uptake capacity increases with the amount of pAA, especially when the ratio ”C=C”$_{AA}$/”C=C”$_{MAG}$ exceeds 100 (P3, P4, P8 and P9). Also, it can be noticed that at a ratio ”C=C”$_{AA}$/”C=C”$_{MAG}$ = 1000:1, the samples obtained by the use of MAG with DS 55% have higher water uptake capacity (P4).

Mechanical properties

Collagen is widely known as the most abundant protein of the extracellular matrix (ECM) of vertebrates. This is why in the present study gelatin (as MAG) was used as proteic component of collagen origin. ECM of connective tissues consists in fibrilar components and an aqueous matrix containing soluble macromolecules [9]. Particularly, ECM is mainly responsible for specific physiological and mechanical characteristics. It is widely recognized that the macroscopic mechanical behaviour of connective tissues strongly depends on their composition and microstructure. However, the mechanical properties of connectives are known to be non linear and anisotropic, and to vary with the body location and age [9]. Most of these natural constructs present complex fibrilar or/and layered structures leading to mechanical properties dependent on their orientation and alignment. In this context, the materials developed in the present work exhibit very interesting mechanical properties. The MAG-pAA hydrogels are insoluble homogeneous bicomponent systems based on covalently bound gelatin and pAA sequences.

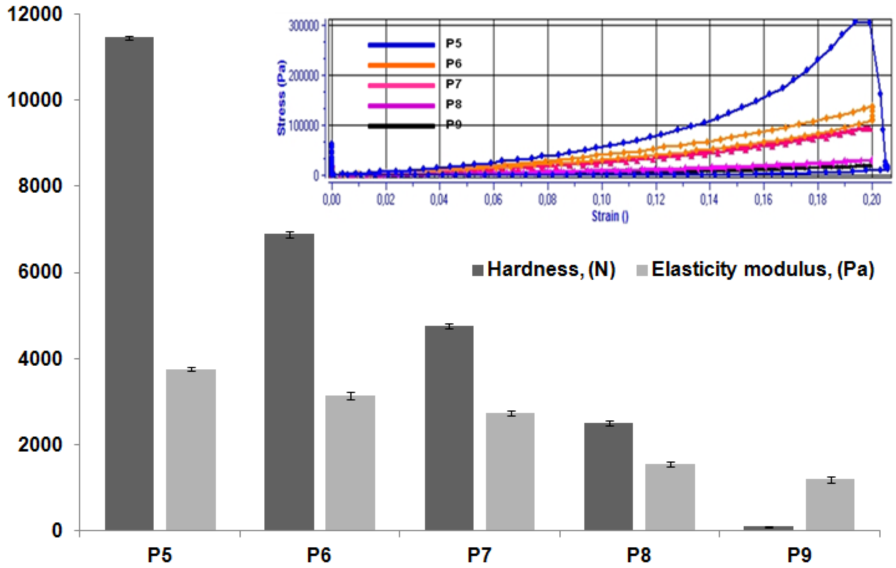


Fig. 5. Average hardness and elasticity modulus as computed from the stress-strain curves calculated for the linear region of the curves, at 2% deformation. Inset – uniaxial stress-strain curves; cyclic loading and unloading up to 0.2 value of the strain.

$$E = \frac{\sigma}{\varepsilon} \tag{4}$$

Where: E – elasticity modulus, Pa
σ – is the compressive stress, Pa
ε - is the compressive strain

Compression experiments were performed on the water saturated hydrogels at a deformation of 20%. Hardness and elasticity modulus, which were used to define the mechanical properties of the hydrogels, were calculated. Hardness represents the maximum force required to compress the samples at 20% deformation and was calculated by the dedicated software (TexturePro CT V1.3 Build 14). The stress – strain curve (inset in Fig. 5) was ploted and the elasticity modulus was calculated using equation (4) for the linear part of the stress-strain curve, at 0.02 strain. Fig. 5 summarizes the obtained results. It can be noticed that increasing pAA component is associated with increasing elasticity. These results are in good correlation with the water swelling experiment and demonstrate that augmenting pAA is associated with increased water uptake and results in higher elasticity.

On the other hand, hysteresis noticed during compressive cycles could result from changes in the orientation of the macromolecular network. The area of the hysteresis loops decreases with augmenting pAA content in the hydrogels (inset in Fig. 5). Stress level softening (known as preconditioning) and the hysteresis in compression are indications of the typical viscoelastic behaviour of these hydrogels.

4. Conclusions

Bicomponent hydrogels based on MAG as natural component and pAA as synthetic polymer were synthesized through photo-initiation. The obtained materials are water insoluble and present a good mass conservation during incubation in water at 40^{o}C. It was demonstrated that the hydrogels studied here are compositionally homogeneous and do not exhibit separation between the natural and the synthetic components. Furthermore, the estimation of the swelling degree proved that the water uptake capacity increases with the amount of pAA, especially when the ”C=C”$_{AA}$/”C=C”$_{MAG}$ exceeds 100. It was also noticed that at a ratio ”C=C”$_{AA}$/”C=C”$_{MAG}$ = 1000:1, the samples obtained by the use of MAG with a higher DS, have higher water uptake capacity. Moreover, the compression tests showed that increasing pAA component is associated with increasing elasticity. These results indicate the potential of MAG-pAA hydrogels as materials for soft tissue applications.

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