Stiff IPN Hydrogels of Poly(Acrylamide) and Alginate: Influence of the Crosslinking Ion's Valence on Hydrogel's Final Properties

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Abstract

Interpenetrating hydrogels with advanced stiffness were prepared from the iron-alginate and cross-linked poly(acrylamide). Multiple approaches were combined in order to prepare hydrogels with mechanical properties required for various biomedical applications. Final properties of these materials were studied in terms of their water content and elastic modulus. Special attention was paid to describe the relationship between final properties and the nature of alginate crosslinks (bivalent or trivalent ions). Parameters such as concentration and nature of the alginate, crosslinking density of poly(acrylamide) network, IPN formation procedure were studied and adapted for obtaining the desired materials. Compared to the simultaneous synthesis procedure, the two-step process was found to be absolutely necessary for effective miscibility of two highly crosslinked networks.

Keywords: IPN; Hydrogel; Poly(acrylamide); Iron-alginate; Simultaneous or sequential photopolymerization

Introduction

Based on the work of Otto Wichterle [1], in 1959, hydrogels were successfully applied in various fields of the medicine. However, their weak mechanical properties in the swollen state induce some limits for their applications. To offset this effect, the gel could be reinforced by adding another polymer and preparing an interpenetrating polymer networks (IPN) [2]. The IPN hydrogels based on ionically crosslinked alginate and covalent poly(acrylamide) network strongly attract interest of many investigators due to the advanced mechanical properties, water adsorption ability and biocompatibility [3,4]. Alginate is a polysaccharide known for its intrinsic cation-dependent aqueous gelation in the presence of some multivalent metal ions [5]. The influence of the counter-ion's nature and valence on the gelation process of different polysaccharides is widely described across the literature [6-8]. Playing with the nature of crosslinking ions (bivalent or trivalent) [9,10], alginate structure [11,12]. and concentration, as well as a density of crosslinks of poly(acrylamide) network makes possible to tune the water content and stiffness of poly(acrylamide)/alginate hydrogels in a wide range. In this work we have prepared series of hydrogels with various physico-chemical properties. Several approaches were extracted from the literature and applied to get the hydrogels with required stiffness: use of trivalent ions for crosslinking of alginate instead of bivalent [13] (Fe^{2+}, Fe^{3+}); two-step procedure formation of IPN; use of alginate with higher molecular weight and G-blocks content [14,15]. Both simultaneous and sequential polymerization procedures were studied and compared. It was concluded that two-step procedure of IPN formation is absolutely necessary for the effective combination of two highly crosslinked networks and formation of the uniform materials.

Experimental Part

Materials

Sodium alginate, acrylamide, ammonium persulfate (initiator of radical polymerization), calcium sulfate anhydrous, calcium carbonate anhydrous, iron (III) sulfate hydrate, iron (II) sulfate hepta-hydrate (ionic crosslinkers of alginate) and N,N-methylenebisacrylamide (MBAA) (crosslinker of polyacrylamide); N,N,N',N'- tetramethylethylenediamine (TMEDA) (accelerator of radical polymerization) were purchased from Sigma-Aldich, Europe and used as received. Alginate (Algogel 6021, $M_w = 2 \times 10^5$ g/mol) was purchased from Cargill France. The tested mixtures are referred as Alginate 1: 100 wt% of sodium alginate (Sigma - Aldrich) and Alginate 2: 70 wt% of sodium alginate (Sigma - Aldrich) and 30 wt% of Algogel 6021 (Cargill).

Alginate/acrylamide hydrogels synthesis by sequential method

The polymerization process was initiated in the presence of ammonium persulfate as initiator of radical polymerization and N,N,N',N'- tetramethylethylenediamine (TMEDA) as accelerator. Radical polymerization of acrylamide and N,N-methylenebisacrylamide (MBAA) as crosslinker was accompanied by UV irradiation and moderate heating (50°C) to ensure maximal conversion of the monomers. The water solution of acrylamide and alginate was prepared with a fixed ratio of monomers 8:1 respectively and the water content was fixed at 74 wt%. At the first step, 0.094 g of sodium alginate powder were mixed with 0.746 g of acrylamide and MBAA (0.0117 g - 0.0819 g) in 2.18 g of distilled water. When the obtained homogeneous ammonium persulfate 0.12 g (0.044 mol/L) was added to the monomer solution. The solution was stirred under nitrogen bubbling for 10 min and then degassed under vacuum at T=2°C for 10 min. Then, water solution of TMEDA 0.1 g (0.167 mol/L)

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was introduced to the polymeric solution under intensive stirring. Final mixture was filled into the polypropylene cylindrical mold and UV-irradiated for 1h at 50°C in a closed glass box. In a further step, prepared material was swollen to the equilibrium in the cation-saturated solution of Fe_2SO_4 (306 g/L) or $Fe_2(SO_4)_3 \times 7H_2O$ (213 g/L) and the alginate chains were crosslinked.

Alginate/acrylamide hydrogels synthesis by simultaneous method

The water solution of acrylamide and alginate was prepared with a fixed ratio of monomers 8:1 respectively, the water content was fixed at 86 wt%. At first, ammonium per sulfate (1 wt% by acrylamide) and MBAA (0.0006 or 0.0012 MBAA/AAm) was added to the monomer solution. The solution was stirred under nitrogen bubbling for 10 min and then degassed at T=2°C. In a further step, $CaSO_4$ or $CaCl_2/Na_2SO_4$ (from 9.17×10^{-5} to 3.45×10^{-4} moles) and TMEDA (0.0025 wt%) were introduced to the solution under intensive stirring. Final mixture was filled into the PP mold and UV-irradiated for 1h at 50°C in a closed glass box.

Compression test

Elastic modules of hydrogels were obtained from compression test. According to rubber elasticity theory under the assumption of affine network model, the Young's modulus can be determined from the slope of linear dependence:

$$\sigma = \frac{f}{S_0} = Ee$$

where σ is the applied stress in Pa/m², f is the value of measured force, S_0 is the cross section of the unstrained swollen sample and e is the relative deformation of the sample. The structural parameters of the polymer network were also determined from the compressive test. The apparent crosslinking density (n_e) was determined from elastic modulus and swelling ratio data using the following equation [16]:

$$\sigma = RT \left(\frac{\ddot{O}_{p0}}{\ddot{O}_p} \right)^{\frac{2}{3}} \ddot{O}_p n_e e$$

where, Φ_{po} and Φ_p are the polymer volume fractions in anhydrous and swollen state respectively.

Swelling properties of IPN

The samples were weighed (W_d), and then swollen in distilled water for 24 h at 37°C before being weighed in a swollen state (W_s). The equilibrium water content (EWC) was evaluated as follows:

$$EWC = \frac{W_s - W_d}{W_s} * 100\%$$

FTIR spectroscopy

The FTIR spectra were performed on Bruker IFS66 spectrometer (Global source, 32 scans, DTGS detector, resolution 2 cm⁻¹) in transmission mode on KBr pellets prepared with ≈ 2 wt% of powdered dry hydrogel or solid residue.

Results and Discussion

Hydrogels preparation

Several series of hydrogels were synthesized according to the formulations presented in the Table 1. Different procedures

N°	mode	MBAA/ AAm × 10²	Alginate	Cation (source; 2 mol/L)	[Cation], mol.
1 - 4	sequential	0. 76; 2.27; 3.85; 5.26	Alg1	Fe^{2+}, $FeSO_4 \times 7H_2O$	---
5 - 8	sequential	0. 76; 2.27; 3.85; 5.26	Alg1	Fe^{3+}, $Fe_2(SO_4)_3 \times xH_2O$	---
9 - 12	sequential	0. 76; 2.27; 3.85; 5.26	Alg2	Fe^{2+}, $FeSO_4 \times 7H_2O$	---
13 -16	sequential	0. 76; 2.27; 3.85; 5.26	Alg2	Fe^{3+}, $Fe_2(SO_4)_3 \times xH_2O$	---
17 - 21	simultaneous	0.06	Alg1	Ca^{2+}, $CaSO_4 \times xH_2O$	0.92; 1.38; 2.07; 2.76; 3.45
22 - 26	simultaneous	0.06	Alg1	Ca^{2+}, $CaCl_2/Na_2SO_4$	0.92; 1.38; 2.07; 2.76; 3.45
27 -31	simultaneous	0.12	Alg1	Ca^{2+}, $CaSO_4 \times xH_2O$	0.92; 1.38; 2.07; 2.76; 3.45
32 - 37	simultaneous	0.12	Alg1	Ca^{2+}, $CaCl_2/Na_2SO_4$	0.92; 1.38; 2.07; 2.76; 3.45

Table 1: Synthesized IPN hydrogels.

(simultaneous or sequential) and alginate compositions (alginate1 or alginate2) were used with variation of covalent crosslinker concentration (MBAA/AAm) and cation nature ($Ca^{2+}/Fe^{2+}/Fe^{3+}$). Two different sources of Ca^{2+} ions were used for alginate crosslinking through simultaneous procedure. It was noticed that the hydrogels containing more than 2.762×10^{-4} moles of $CaSO_4$ became turbid due to the low solubility of calcium sulfate in the water (0.021 g/L). To overcome this issue, $CaCl_2$ solution was used as a source of Ca^{2+} ions and transparent hydrogels were obtained. However, due to the perfect solubility in the water and high dissociation rate of calcium chloride, the crosslinking rate of alginate chains by Ca^{2+} ions considerably increases. As a result at high concentration of $CaCl_2$ solution (e.g., 811 g/L), the formation of uniform and transparent material in such conditions seems to be impossible [17]. Instantaneous gelation leads to quasi-spontaneous crosslinking of the alginate chains slowing their diffusion in the volume, inducing high and low crosslinked zones. The presence of such an inhomogeneity during the radical polymerization and crosslinking of the acrylamide creates obstructions for propagation of the network and distribution of the crosslinks. Therefore, other routes achieving uniform gel structure are proposed such as the preparation with the use of $CaCO_3$/d-glucono-δ-lactone (GDL) system, a source of ions with low dissociation rate which could increase with the pH [18]. Thanks to the gradual dissociation of carbonate and the consecutive slow crosslinking rate of alginate, homogeneous hydrogel could be prepared. However, due to the presence of carbonate particles, final materials are not transparent. As alternative way of reducing the crosslinking rate of the alginate, we applied the redox system of $CaCl_2/Na_2SO_4$. Comparing with the $CaCl_2$ solution, the dissociation rate of chosen redox system is lower due to the simultaneous ionic-exchange reaction. IPN hydrogels were also prepared by two-step polymerization procedure. This process includes the following successive stages: the synthesis of acrylamide network in the presence of sodium alginate chains leading to the formation of the semi-IPN structure and the further swelling in ion-saturated water solution leading to the crosslinking of alginate chains and the formation of full-IPN structure.

Hydrogels characterization

After the synthesis, all the interpenetrating polymer networks as well as the pure sodium-alginate, poly(acrylamide) (pAAm) and

alginate beads crosslinked with $Ca^{2+}/Fe^{2+}/Fe^{3+}$ ions were analyzed thanks to the FTIR spectroscopy. As expected, the FTIR spectrum of pAAm exhibits the bands of NOH (stretching, 3400-3200 cm^{-1}), C=O (stretching, 1670 cm^{-1}), NH (bending, 1622 cm^{-1}), CH (stretching, 2932 cm^{-1}) and CH (bending, 1500-1300 cm^{-1}). In the case of the spectrum of pure NaAlg, different peaks were observed: broad one assigned to the vibration of –OH group at 3445 cm^{-1}, peaks at 1612 and 1421 cm^{-1} corresponding to the asymmetric and symmetric stretching vibrations of –COO^- group, peak at 1050 cm^{-1} assigned to C-O group, the signal of Na-O appears at 820 cm^{-1} [19]. Spectra of the different sodium alginates were similar. However, comparison of the NaAlg spectrum and the spectrum of alginate crosslinked either by Ca^{2+}, Fe^{2+} or Fe^{3+} shows significant shifts of the –COO^- group and metal–O bands to the higher values (Table 2) giving evidence of its chelation. Those shifted values depend on the metal-carboxylate coordination type [20].

Chelation of two parallel alginate chains with divalent metallic ions leads to a planar geometry configuration, so-called "egg box", while chelation with trivalent metallic ions induces alginate complexe with the coordination type $(COO)_3M$ resulting in a non-planar geometry [14,21]. Such a tridimensional bonding model leads to the extended crosslinking of alginate chains and hence, to a more compact network [9]. The spectra of the synthesized IPNs present characteristic bands of both pure compounds. Peak at 1612 cm^{-1} assigned to –COO^- group of alginate is superposed to the signals of carbonyl and amino groups of pAAm at 1670 cm^{-1} and 1622 cm^{-1}. Band of symmetrical vibration of –COO^- group at 1420 cm^{-1} was superposed with pAAm signals at 1454 cm^{-1} and 1419 cm^{-1}. No shift of the superposed peaks at around 1600-1350 cm^{-1} was observed giving evidence of the formation of two independent pAAm and alginate networks.

Structural and mechanical properties of hydrogels

In order to study the influence of cation's valency and crosslinkers concentrations on the structural properties of poly(acrylamide)/alginate IPN hydrogels, Young's modulus was evaluated. Mechanical properties of hydrogels were investigated by compressive strain-stress measurements. The series of simultaneous IPN hydrogels with various concentrations of Ca^{2+} ions and MBAA/AAm ratio were prepared (Table 1, compositions n° 17 - 37) in order to determine its influence on mechanical properties of the materials. Dependence of the Young's modulus, calculated from the compression measure is given in the Figure 1 as function of the crosslinker concentration.

Whatever the nature or concentration of the crosslinkers $CaSO_4$ or $CaCl_2/Na_2SO_4$ (Figure 1), elastic modulus increases until a maximum around 2.1×10^{-4} mol., then decreases. This behavior could be explained by the rapid increase of viscosity of monomer solution that hinders dispersion and homogeneous distribution of the ions Ca^{2+}. Moreover, gelation rate of the alginate crosslinking increase with the Ca^{2+} ions concentration and temperature [18]. This effect leads to the formation of structurally inhomogeneous and turbid material with low crosslinking density (Table 3) and elastic modulus.

In the same manner as described for the Young's modulus, the apparent crosslinking density increases with the ionic concentration
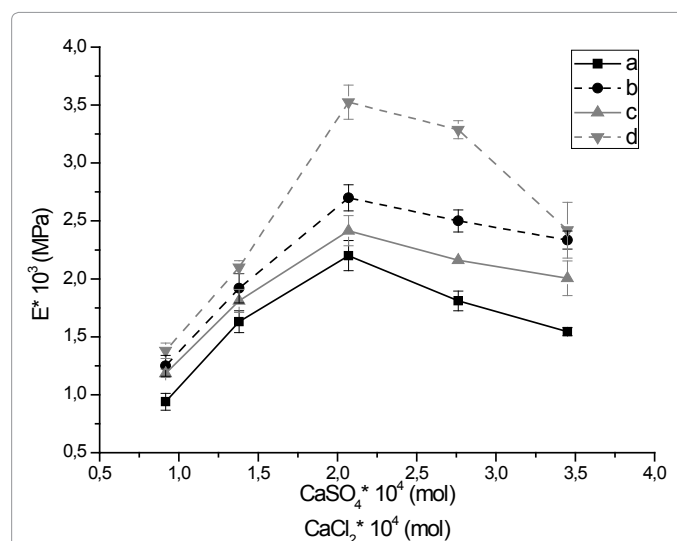

Figure 1: Dependence of the elastic modulus for simultaneous IPN on the concentration of bivalent ionic crosslinker with different ratios of the MBAA and AAm concentrations (a: 6×10^{-4} /$CaSO_4$, and b: 12×10^{-4}/$CaSO_4$; c: 6×10^{-4}/ $CaCl_2$, and d: 12×10^{-4}/$CaCl_2$).

[Cations], mol.		MBAA/AAm × 10⁴	$n_e \times 10^6$, mol/cm³
0.917	Ca^{2+}, $CaSO_4 \times xH_2O$	6	1.91
1.38	Ca^{2+}, $CaSO_4 \times xH_2O$	6	2.99
2.07	Ca^{2+}, $CaSO_4 \times xH_2O$	6	3.55
2.76	Ca^{2+}, $CaSO_4 \times xH_2O$	6	3.16
3.45	Ca^{2+}, $CaSO_4 \times xH_2O$	6	2.84
0.92	Ca^{2+}, $CaSO_4 \times xH_2O$	12	2.3
1.38	Ca^{2+}, $CaSO_4 \times xH_2O$	12	3.41
2.07	Ca^{2+}, $CaSO_4 \times xH_2O$	12	4.28
2.76	Ca^{2+}, $CaSO_4 \times xH_2O$	12	4.27
3.45	Ca^{2+}, $CaSO_4 \times xH_2O$	12	4.12
0.92	Ca^{2+}, $CaCl_2/Na_2SO_4$	6	2.4
1.38	Ca^{2+}, $CaCl_2/Na_2SO_4$	6	3.32
2.07	Ca^{2+}, $CaCl_2/Na_2SO_4$	6	3.90
2.76	Ca^{2+}, $CaCl_2/Na_2SO_4$	6	3.77
3.45	Ca^{2+}, $CaCl_2/Na_2SO_4$	6	3.69
0.92	Ca^{2+}, $CaCl_2/Na_2SO_4$	12	2.53
1.38	Ca^{2+}, $CaCl_2/Na_2SO_4$	12	3.73
2.07	Ca^{2+}, $CaCl_2/Na_2SO_4$	12	5.59
2.76	Ca^{2+}, $CaCl_2/Na_2SO_4$	12	5.61
3.45	Ca^{2+}, $CaCl_2/Na_2SO_4$	12	4.27

Table 3: Apparent cross-linking densities of the simultaneous IPN hydrogels (cation: Ca^{2+}, $CaSO_4 \times xH_2O$, 2 mol/L).

till a maximum and decreases in case of inefficient ionic crosslinking (due to rapid gelation of the alginate chains). We assume that such an instantaneous gelation of alginate creates highly crosslinked zones alginate beads in the monomer solution before the radical polymerization occurs. These highly crosslinked zones of alginate network act as obstructions for distribution of polyacrylamide network in volume leading to structurally inhomogeneous material.

In order to study the routes improving the stiffness of these hydrogels, several approaches were applied. The first one deals with the synthesis procedure since the sequential procedure allows introducing a greater amount of cations for the alginate crosslinking. The second one is focused on the influence of the ionic valence and series of IPN hydrogels were prepared with Fe^{3+} ions and compared to that formed

Ion type	v_{as} COO	v_s COO	v_{M-O}
Na^+	1612	1421	822
Ca^{2+}	1631	1436	825
Fe^{2+}	1628	1441	821
Fe^{3+}	1634	1446	810

Table 2: Infrared frequencies (cm^{-1}) for metal-alginate complexes.

in the presence of Fe^{2+} ions. With the third one, the amount of the covalent crosslinker of acrylamide was increased up to 40 times (Table 1, compositions n° 1 - 16). Alginate chains were crosslinked by the swelling in saturated solutions of iron salts (2 mol/L) for 24h. Stiff hydrogels with elastic modulus up to 0.15 MPa were obtained (Figure 2).

Elastic modulus of hydrogels increases proportionally to the concentration of covalent crosslinking molecules in the composition (Figure 2), which is in accordance with the rubber elasticity theory. If comparing the pure polyacrylamide hydrogel with the semi-IPN pAAm/alginate, we may conclude that there is no influence of interpenetrating alginate chains on the elastic modulus of the system. However, comparison of pure pAAm and semi-IPN pAAm/alginate with full-IPN hydrogels shows that the elastic modulus increases significantly in case of the full-IPN systems. Such increase becomes more important for the hydrogels crosslinked by Fe^{3+} ions. This behavior can be explained by the ability of trivalent ions to bond three carboxylic group of different alginate chains resulting in a tridimensional bonding structure with coordinating number $(COO)_3M$. Calculated values of apparent crosslinking density show that the trivalent bonding structure leads to more compact networks (Figure 2). Such behavior is due to crosslinking of three alginate chains at the same reticulation point. Improvement of the elastic modulus of hydrogels is possible by increase the alginate molecular weight and/or its composition (G-blocks proportion in the structure) [17]. Viscosity of the alginate solution could significantly increase with high molecular weight alginate (Cargill Algogel 6021; $M_w = 2 \times 10^5$ g/mol) resulting in difficulties to blend it with the other components and consequently to form a homogeneous material. In this work, we describe an easy route avoiding alginate solution inhomogeneity caused by high viscosity at the pre-polymerization stage using two types of alginates with different molecular weight which were mixed together and Alginate2 solution prepared (70 wt% of sodium alginate (Sigma - Aldrich) and 30 wt% of Algogel 6021 (Cargill)). However, the analysis of M-blocks to G-blocks ratio is required to make a full conclusion about the chemical structure difference between these two commercial products.

From the Figure 3 showing the elastic modulus of Alginate2 based IPN hydrogels, it appears that these hydrogels possess higher modulus than those based on the alginate (Alginate1) with lower molar mass. For the hydrogels formed in presence of the Fe^{3+}, the curve passes through
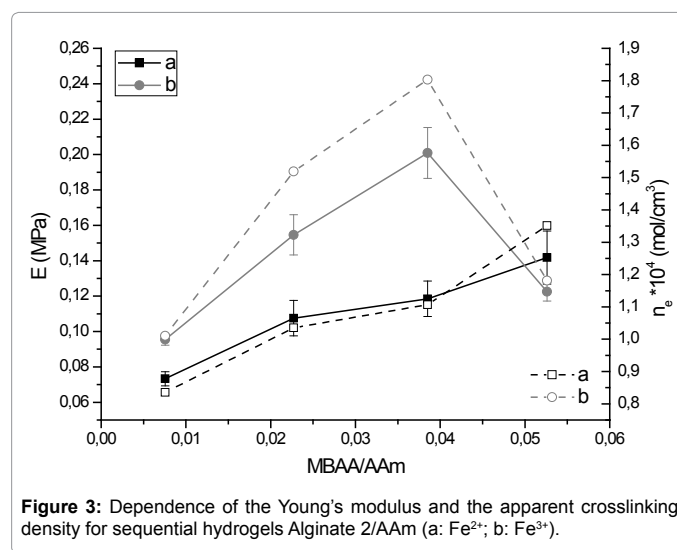
Figure 3: Dependence of the Young's modulus and the apparent crosslinking density for sequential hydrogels Alginate 2/AAm (a: Fe^{2+}; b: Fe^{3+}).

up to maximum of 0.2 MPa at 0.04 MBAA/AAm and then sharply decreases. At this concentration, it is assumed that the covalently crosslinked polyacrylamide network possess too high density that prevent the effective crosslinking of alginate chains penetrating it. However, with the exception of the case of over-crosslinked networks represented by inflection point, hydrogels formed with Fe^{3+} ions are 30% - 100% stiffer. The results obtained for the hydrogels prepared by a two-step procedure show that this procedure combined with the use of trivalent ions and composition of the alginate with high gelation ability is an efficient way to improve the stiffness of polyacrylamide/alginate hydrogels.

Swelling properties

Swelling behavior of simultaneous and sequential hydrogels prepared using different ions ($Ca^{2+}/Fe^{2+}/Fe^{3+}$) was determined in terms of the equilibrium water content (Figures 4 and 5). The water content of the hydrogels prepared in simultaneous route in the presence of Ca^{2+} ions is higher than that of pure pAAm (90%) and lower than with pure alginate (Figure 4). However, with that latter case, the hydrogel is not formed because of the water solubility of the alginate at low crosslinking density avoiding any ionic bonds creation. In this type of preparation process, both polymeric components have a competitive and opposite effect, i.e., the dissolution of the alginate and the non-solubility of the polyacrylamide. Comparing to the apparent network density values, it appears that the water uptake strongly depends on the free volume and so, on the network mesh size. From the Figure 4, we may notice that water uptake decreases in the same manner as the network density rises.

Equilibrium water content was also estimated for IPN hydrogels made in sequential route (Figure 5). We could see that the water content decreases with the density of crosslinks as expected [21-23]. It is remarkable that the water content of hydrogels with Fe^{2+} as crosslinker are 5-7% higher than that of hydrogels with Fe^{3+}. When trivalent ions are used for crosslinking, additional bonds between chains lead to the formation of a three-dimensional structure. The obtained higher density of crosslinks compared to that in case of bivalent ions induces the decrease of the free volume and the water content. No noticeable difference in the water content between compositions with different types of the alginate in the presence of the trivalent ions was found. We consider that in the case of tridimensional bonding structure decrease
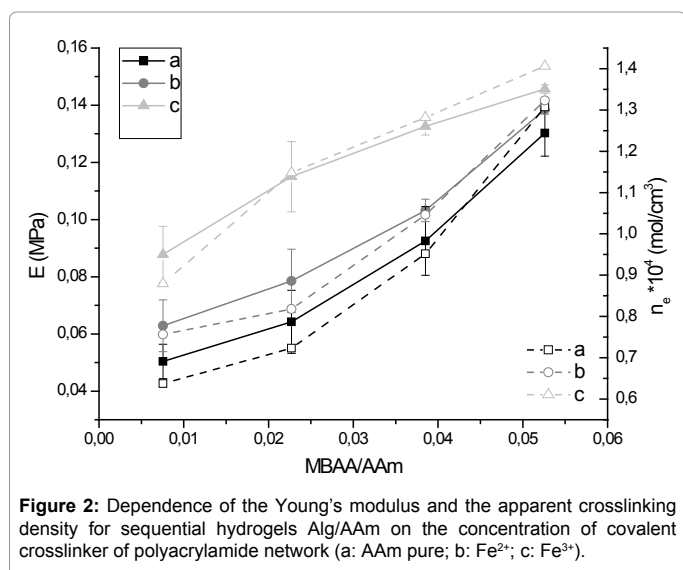

Figure 2: Dependence of the Young's modulus and the apparent crosslinking density for sequential hydrogels Alg/AAm on the concentration of covalent crosslinker of polyacrylamide network (a: AAm pure; b: Fe^{2+}; c: Fe^{3+}).
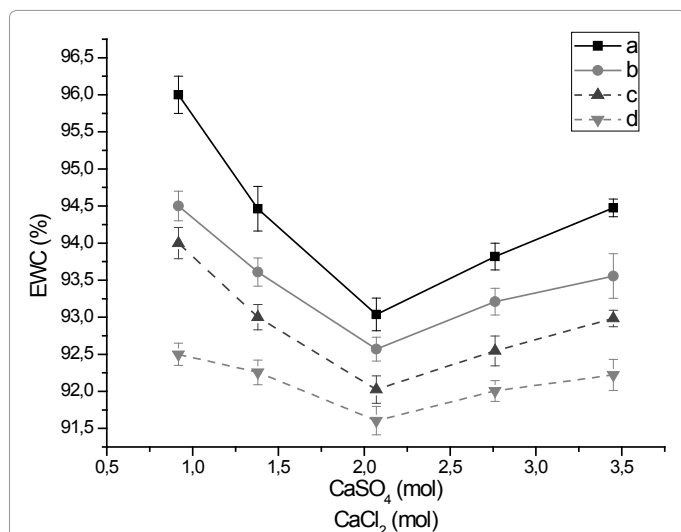
Figure 4: Dependence of equilibrium water content at 37°C for simultaneous IPNs on the concentration of bivalent ionic crosslinker with different ratio of the MBAA and AAm concentrations (a: 6×10^{-4}/CaSO$_4$, b: 12×10^{-4}/CaSO$_4$; c: 6×10^{-4}/CaCl$_2$, and d: 12×10^{-4}/CaCl$_2$).
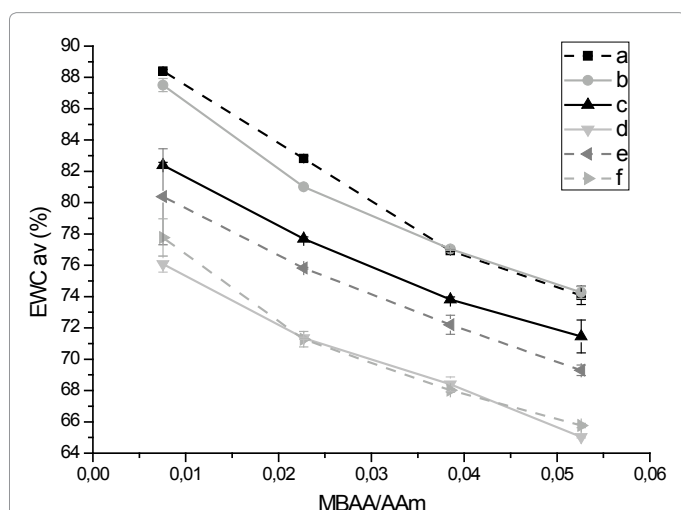

Figure 5: Dependence of equilibrium water content at 37°C for sequential hydrogels Alg/AAm on the concentration of covalent crosslinker of polyacrylamide network (a: AAm pure; b: Alg.1/AAm; c: Alg.1/AAm Fe^{2+}; d: Alg.1/AAm Fe^{3+}; e: Alg.2/AAm Fe^{2+}; f: Alg.2/AAm Fe^{3+}).

of free volume neglects the effect of alginate on the water content. For these hydrogels, the free volume becomes limiting factor of the water uptake and EWC decrease proportional to reduction of it. However, about a 2%-3% higher EWC was obtained for the Alginate1/Fe^{2+} hydrogels comparing to the Alginate2/Fe^{2+} indicating the effect of the alginate composition.

Conclusion

IPN hydrogels based on ionically crosslinked alginate and covalently crosslinked polyacrylamide were prepared using two different processes and various multivalent ions. Swelling and mechanical properties of the prepared hydrogels were studied depending on the ions valence and crosslinking density of each network. Several approaches were combined to obtain stiff hydrogels required for certain biomedical applications. It was shown that two-step polymerization process is

absolutely necessary to combine two highly crosslinked polymer networks. Use of trivalent ions for crosslinking of adapted alginate composition, combined with dense polyacrylamide network by two-step synthesis process allows obtaining highly stiff IPN hydrogels. On the other hand, it was not possible to prepare same hydrogels by simultaneous route because of the instantaneous character of alginate crosslinking resulting in the structurally inhomogeneous materials.

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