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Novel thermo-responsive membranes composed of interpenetrated polymer networks of alginate-Ca^{2+} and poly(*N*-isopropylacrylamide)

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Abstract

In this work, interpenetrated polymer networks (IPN) composed of alginate-Ca^{2+} and poly(*N*-isopropylacrylamide), PNIPAAm, were synthesized and their water uptake capability was measured at temperatures from 25 to 40 °C and compared to that of pure alginate-Ca^{2+} hydrogels without PNIPAAm. A sharp decrease of WU was observed when IPN hydrogels are heated above 32–33 °C. The phenomenon is associated to a drastic shrinking of hydrogels. At temperatures above 32 °C the PNIPAAm chains collapse, contracting their network and pulling back the alginate-Ca^{2+} network. The rate of shrinking depends of the heating rate. The phenomenon is more effective and faster in IPN containing lower amount of alginate-Ca^{2+}. The shrunken IPN hydrogels can be re-swollen but the expansion is slower than the shrinking. The diffusion of Orange II dye through the membrane of IPN hydrogels decreases if the temperature is raised up to 35 °C. The shrinking results in a decrease of the average pores size that makes more difficult the diffusion of Orange II. The average pore size was evaluated in several stages by analysis of SEM micrographs of freeze dried samples: 102.0 ± 14.3 μm at 25 °C, 15.7 ± 5.4 μm at 33 °C and 0.4 ± 0.3 μm at 40 °C. Below the LCST of PNIPAAm, the IPN hydrogels exhibit a morphology characterized by open pores but above the LCST their surface becomes more regular and compact. As a consequence, an increase of the apparent activation energy for permeability, $E_p^{\#}$, of Orange II is measured.

Keywords: Alginate-Ca^{2+}/PNIPAAm networks; Thermo-responsive hydrogel; Membrane

1. Introduction

Hydrogels have been extensively applied in the biomedical field due to their biocompatibility, good morphological and mechanical properties [1–9]. Specifically, hydrogels composed of an alginate matrix have significantly contributed for the progress of biotechnology [10–12]. Shapiro and Cohen [13] have described the formation of sites suitable for cell growth in alginate-Ca^{2+}. Due to their mechanical stability alginate-Ca^{2+} hydrogels are also often used to prevent endovascular embolization [14,15]. The alginates are obtained from alginic acid [16–18] and it is found in brown marine algae [18]. It is a material of relatively low cost that presents a remarkable gelation in aqueous solution in the presence of some divalent cations such as Ca^{2+}, Mg^{2+}, etc [19,20]. Ionic bonding between carboxylate groups of the sodium alginate with Ca^{2+} ions results in the formation of mechanically stable networks [15,21]. There are several possible arrangements of chains segments of alginic acid because it contains two different kinds of monomers having carboxylic groups. These segments are designated as L-mannuronic and L-guluronic acid [19].

On the other hand, it is well known that poly(*N*-isopropylacrylamide), PNIPAAm, is a thermosentive polymer with remarkable interest in biotechnology [22,23] that has been widely used in hydrogel synthesis [24,25]. The main characteristic of PNIPAAm is the transition from a hydrophilic to a hydrophobic macromolecule when the temperature is raised above 32–35 °C, the lower critical

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solution temperature (LCST) of PNIPAAm in water [22,26–28]. When a swollen PNIPAAm hydrogel is heated above the LCST, PNIPAAm chains collapse and this is accompanied by a drastic contraction of the gel [22,23]. However, PNIPAAm hydrogels are not conveniently used as membrane because of their poor mechanical properties [29,30]. To overcome this problem, membranes composed of an alginate-Ca^{2+} network having PNIPAAm chains included (semi-IPN) were developed in our lab [31]. However, it was verified that the PNIPAAm chains diffuse out of the hydrogel after immersion in water for some time so that the hydrogel looses its thermal response [31]. The main goal of this work is to develop membranes of thermo-sensitive hydrogels based on fully interpenetrated networks of alginate-Ca^{2+} and PNIPAAm. Such membranes have not yet been reported in the literature. In the present investigation the alginate-Ca^{2+} network is formed inside the PNIPAAm network by interaction of alginate-Na^{+} with Ca^{2+} ions resulting in an interpenetrated network system IPN.

2. Experimental

2.1. Materials

Sodium alginate, *N*-isopropylacrylamide, NIPAAm, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, TEMED, and sodium persulfate were purchased from Aldrich. *N*,*N*′-methylene-bis-acrylamide, MBAAm and Orange II were purchased from Plusone and Across organics, respectively. The ratio of mannuronic acid to guluronic acid (M/G) of the alginate is 1.56, according to the manufacturer. It has already been reported [32] that the values of the average-number (M_n) and average-weight (M_w) molecular weights for this alginate are 339,000 g mol^{-1} and 1,073,000 g mol^{-1}, respectively.

2.2. Preparation of IPN hydrogels

Aqueous solutions containing different amounts of *N*-isopropylacrylamide, *N*,*N*′-methylene-bis-acrylamide, as cross-linking agent, tetramethylethylenediamine, as accelerator and sodium alginate were prepared. In all cases after 18 ml of the solution was deoxygenated by bubbling N_2 for 15 min, 2 ml of aqueous solution of sodium persulfate as initiator at a concentration of 42 mMol l^{-1} was added. The resultant solution was injected between two square glass plates 0.12 m in size separated by a rubber gasket 1.5 mm thick. This system was kept at room temperature for 24 h. After the gelation of NIPAAm monomer has occurred a semi-IPN is obtained. The top plate is then carefully removed and the other plate, supporting the hydrogel, was immersed in a $CaCl_2$ aqueous solution (1 or 5 wt% in concentration) for 48 h. After the gelation of alginate chains has occurred the IPN hydrogel is released and immersed for 24 h in distilled water to remove the unreacted Ca^{2+} ions. The extent of the Ca^{2+} ions that has been coordinated to the carboxylate anions in alginate was assessed by atomic absorption at 422.7 nm using C_2H_2 as gas flame. The absorbance data were taken on a Varian model Spectra 10Plus equipment. Calibration curve was performed using a $CaCl_2$ standard solution. Before the measures, IPN hydrogels were treated with aqueous disodium ethylenediaminetetraacetic acid (EDTA) at 0.1 Mol l^{-1} for withdrawing the calcium ions and dissolving the alginate-Ca^{2+} networks. Afterwards, a lanthanum solution at 0.1 wt% was used to avoid the formation of byproducts, like calcium oxide (CaO). The notation (A-C-P), where A, C and P are the initial concentrations, in wt%, of sodium alginate, calcium chloride and *N*-isopropylacrylamide, respectively, was used to identify the different formulations.

2.3. Water uptake measurements

In this work the water uptake (WU) was defined and measured as the ratio of the weight of hydrogel swollen to equilibrium to the weight of dried hydrogel.

2.4. Permeability measurements

The permeability to Orange II was determined by measuring the concentration of dye diffused through the membrane as a function of time. A circular piece of membrane was put between two compartments of a permeability cell, one of them was filled with 50 ml of distilled water (downstream) and the other (upstream) filled with an aqueous solution of Orange II (1×10^{-4} Mol l^{1}) the concentration of which changes by less than 5% during the experiment. The concentration of the dye in the downstream compartment was determined as a function of time by UV–Vis spectroscopy at 486 nm for diffusion at temperatures from 25 to 40 °C.

2.5. Scanning electron microscopy of the freeze dried hydrogels

The (1-1-5) IPN hydrogel was swollen to equilibrium in water at 25 °C, 33 °C and 40 °C, respectively. Afterwards the samples were removed and quickly frozen in liquid nitrogen before being lyophilized for 72 h in a freeze dryer (Christ gefriertrocknungsanlagen) the temperature of which was kept at −55 °C. The micrographs of freeze dried samples of the (1-1-5) IPN hydrogel were obtained with a Shimadzu scanning electron microscope, model SS-550 Superscan. The average pore size was determined by analysis of micrographs obtained for hydrogels initially swollen at the three different temperatures.

2.6. Visualization of the shrinking of IPN hydrogels

The (1-1-5) IPN hydrogel cut in circular form was kept in

distilled water at temperatures controlled with a precision of ±0.1 °C in a thermostat programmed to rise at temperature from 25 to 45 °C at a rate of 2 °C/h. Pictures of the swollen hydrogels were taken from 30 to 45 °C using a Sony digital camera. To obtain good contrast the hydrogels were dyed with a trace of Methylene Blue.

3. Results and discussion

3.1. Water uptake

In Fig. 1 it is observed that the WU of IPN hydrogels (3-1-P) shows a considerable decrease at temperatures above 32–33 °C, the LCST of the PNIPAAm in aqueous solution. That decrease of WU is accompanied by a drastic shrinking of the IPN hydrogel when it is heated from 25 to 40 °C. By collapsing, the PNIPAAm network pulls back the flexible network [19] of alginate-Ca^{2+}. It is interesting to notice that the presence of a large amount of PNIPAAm in the (3-1-10) IPN also contributes for a reduction of WU at 25 °C. This is because even at that temperature the PNIPAAm matrix is less hydrophilic than the alginate-Ca^{2+} matrix. Though the pure alginate-Ca^{2+} hydrogel (3-1-0) exhibits only a slight increase in WU with temperature a remarkable shrinking occurs when the PNIPAAm network is interpenetrated. That effect is more pronounced when the concentration of PNIPAAm inside the alginate-Ca^{2+} network is larger.

Curves of WU as a function of the temperature in (3-C-P) hydrogels are shown in Fig. 2. The presence of PNIPAAm always leads to a decrease in WU as the temperature is raised above LCST but the amount of Ca^{2+} added for gelation of the alginate network also affects the water absorption. Table 1 shows the amount of carboxylate groups (COO^-) from alginate (A) and calcium ions (Ca^{2+}) (B) added to 35 ml of feed solution to prepare the hydrogel, Ca^{2+} coordinated to carboxylate anions (C) and the ratio of coordinated COO^- to the total COO^- for (3-C-P) hydrogels. Higher content of Ca^{2+} results in a more densely crosslinked alginate network and thus in a lower WU. As can be seen, the decrease of WU is less important on (3-5-5)

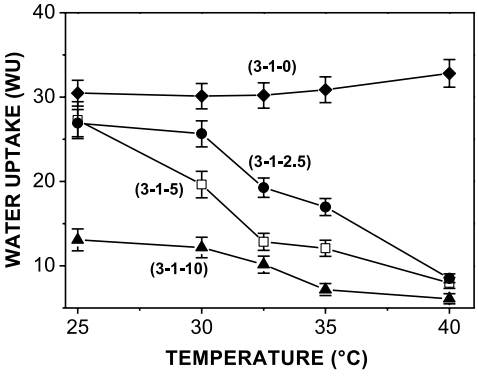

Fig. 1. Water uptake as a function of temperature for (3-1-P) IPN hydrogels composed of alginate-Ca^{2+} and PNIPAAm networks.

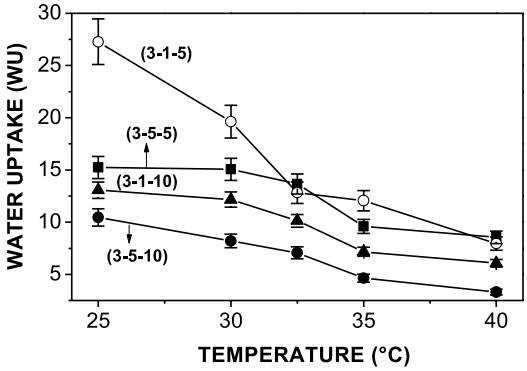

Fig. 2. Water uptake as a function of temperature for (3-C-P) IPN hydrogels composed of alginate-Ca^{2+} and PNIPAAm networks.

hydrogel than (3-1-5) one, see Fig. 2. The same is true for the (3-5-10) compared to the (3-1-10) hydrogels. When the alginate content in the IPN hydrogel is only 1 wt% the decrease of WU is very drastic near the LCST. This is especially obvious for the (1-1-5) hydrogel (Fig. 3). Low concentrations of both crosslinking agents (Ca^{2+} for alginate and *N*,*N*′-methylenebisacrylamide for NIPAAm) give rise to the most a prominent shrinking of the hydrogels. In those conditions, the PNIPAAm network collapses more easily, pulling back the more flexible alginate-Ca^{2+} matrix.

3.2. Shrinking and re-swelling

The shrinking and re-swelling of IPN hydrogels were measured by weighing the hydrogels as a function of time and normalizing their WU to the initial value showed before shrinking and re-swelling. The results are presented in Fig. 4a and b. In the shrinking measurements (Fig. 4a) the IPN hydrogels were initially in equilibrium at 25 °C and the temperature was quickly raised to 40 °C. At 40 °C the IPN hydrogel containing the lowest amount of alginate (1-1-5) shows the fastest shrinking. In this case, higher flexibility and mobility of alginate-Ca^{2+} chains and also of PNIPAAm chains are the consequence of the low concentration of polymers inside that hydrogel. The amount of water decreases to ca. 15% of the initial value after 60 min but

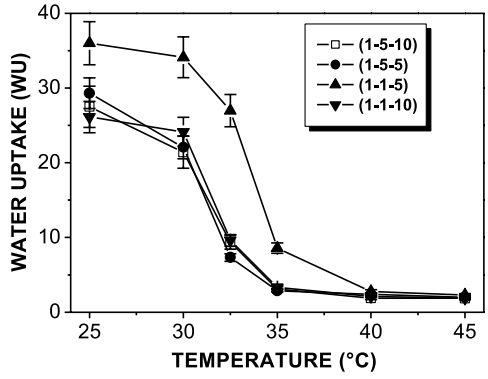

Fig. 3. Water uptake as a function of temperature for (1-C-P) IPN hydrogels composed of alginate-Ca^{2+} and PNIPAAm networks.

Table 1
Amount of carboxylate groups (COO^-) from alginate (A) and calcium ions (Ca^{2+}) (B) added to 35 ml of feed solution to prepare the hydrogel

Hydrogel	*A*	*B*	*C*[a]	$\frac{COO^-_{coordinated}}{COO^-_{total}}$ ratio
(3-1-5)	5.99	15.0	1.06	0.35
(3-5-5)	5.99	74.8	1.69	0.56
(3-1-10)	5.99	15.0	0.60	0.20
(3-5-10)	5.99	74.8	0.78	0.26

Ca^{2+} coordinated to carboxylate anions (C) and the ratio of coordinated COO^- to the total COO^-. Values are given in mMol.
[a] Values determined by atomic absorption measurement.

continues to decrease even after 100 min. On the other hand, the (3-1-5) hydrogel presents a lower level of shrinking but the loss of water is finished after 50–60 min. The higher the amount of alginate in the IPN hydrogel the more the difficult is the shrinking. The pure (3-1-0) hydrogel (i.e. the alginate-Ca^{2+} matrix without PNIPAAm) does not shrink in the range of temperatures between 25 and 40 °C. Actually, the opposite occurs: the amount of water amount inside the hydrogel is slightly higher at 40 °C than at lower temperatures.

The curves of normalized WU as a function of time, shown in Fig. 4b, represents the swelling of IPN hydrogels. In these experiments, the IPN hydrogels in equilibrium at 40 °C, were quickly transferred to water at 25 °C. It was observed that in IPN hydrogels the swelling is slower than the shrinking, comparing the same kinds of hydrogels. The swelling takes more than 100 min and it is important to notice that this is not enough to reach the initial state

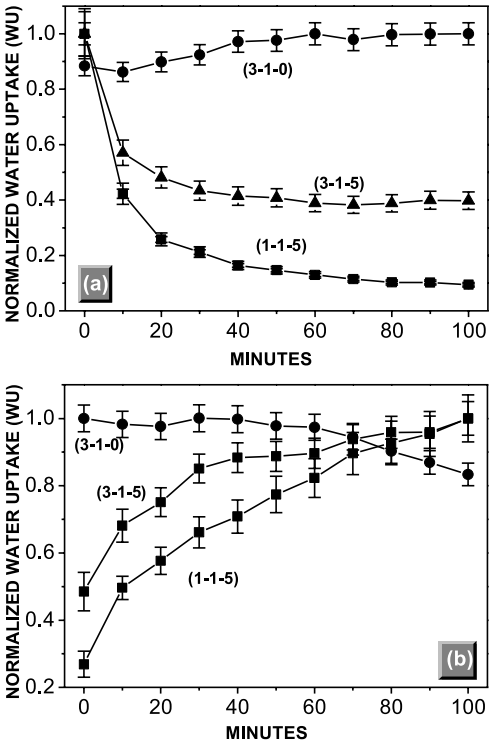

Fig. 4. Normalized WU as a function of time for IPN hydrogels composed of alginate-Ca^{2+} and PNIPAAm networks and for pure alginate-Ca^{2+} hydrogel; (a) Kinetic of shrinking and (b) Kinetic of swelling.

whereas the pure (3-1-5) hydrogel swell completely in 60 min. The difference of shrinking and swelling processes in IPN hydrogels can be explained by the water penetration within IPN hydrogel in shrunken state. In this condition it is not fully dried due to the presence of the alginate that is a hydrophilic polymer. When a dried hydrogel is brought in aqueous media, the water molecules that penetrate within hydrogel hydrate primarily the polar groups to form the primary bounded water. In this initial hydrating hydrophobic groups are exposed to the water molecules creating secondary bounded water [33]. The additional absorbed water is driven by the osmotic force of the network and leads to an enhancement of swelling. For these reasons, it is reasonable to suggest that the re-hydration of an IPN hydrogel in shrunken state begins absorbing additional water because it presents primary and secondary bounded-water. On the other hand, shrunken hydrogel has a tighter structure that decreases the mobility of polymer chains. Furthermore, when the hydrogel shrank forms denser external layer (or skin) and, therefore, the re-hydration process is hindered in the beginning. In view of these statements, higher time for re-hydration and expansion of the shrunken hydrogel is required, compared to the shrinking of swollen ones.

3.3. Permeability to orange II through membranes

From the diffusion of the Orange II through the IPN membranes their permeability was determined at several temperatures. As pointed out in the experimental part, the dye concentration in the upstream compartment is practically constant (it decreases by less than 5%) during the diffusion experiments. On the other hand, it was verified that in all diffusion experiments the concentration of Orange II in the downstream compartment increases linearly as a function of time. Eq. (1), based on the first Fick's law, may thus be used and the permeability values for IPN hydrogels calculated from it [31,34].

$$P = (\mathrm{d}C/\mathrm{d}t)V\mathrm{d}/(CA) \quad (1)$$

where $(\mathrm{d}C/\mathrm{d}t)$ is the slope of straight line given by the concentration of diffused dye vs. time, V and C are the volume and initial concentration of Orange II in the upstream compartment, respectively, and A is the membrane area in contact with the upstream solution. The

parameter d is the thickness of the membrane swollen to equilibrium in water at desired temperature. It was carefully measured using a micrometer.

Permeability curves of (A-1-P) membranes of IPN hydrogels to Orange II as a function of temperature are presented in Fig. 5. The permeability decreases as the temperature increases and an inflection point may be seen in the curves of permeability vs. temperature close to 32–33 °C, the LCST of PNIPAAm in water. For the (1-5-5) IPN hydrogel the permeability changes from 6×10^{-10} m^2 s^{-1} at 25 °C to 1×10^{-10} m^2 s^{-1} at 40 °C. According to the earlier discussion, above the LCST the collapse of the PNIPAAm chains leads to a shrinking of IPN hydrogels resulting in a significant reduction of the average pores size that makes more difficult the diffusion of Orange II through of the membrane. The pure (3-1-0) hydrogel (without PNIPAAm), used in this work as a 'blank', exhibits an increase in permeability to Orange II, because there is no shrinking of the alginate-Ca^{2+} hydrogel.

3.4. Apparent activation energy for permeability

Curves of logarithm of the permeability to Orange II through the pure alginate-Ca^{2+} hydrogel and (1-1-10) IPN hydrogel as a function of inverse of temperature (log P vs. $1/T$) are presented in Fig. 6. For the IPN hydrogel two straight lines are observed: one above LCST and the other below LCST. However, for the (3-1-0) alginate-Ca^{2+} hydrogel only one straight line is observed. The apparent activation energy for permeability to Orange II, $E_p^{\#}$, was obtained from the slope of the log P vs. $1/T$ curves.

The values of $E_p^{\#}$, calculated for IPN and alginate-Ca^{2+} hydrogels are presented in Table 1. The interpretation of the data shown in Table 2 should be done carefully. It is required to consider the variation on $E_p^{\#}$ values ($\Delta E_p^{\#}$), occurring when the hydrogels are heated from 25 to 40 °C. This approach has been reported in the literature [31]. It can be observed that $E_p^{\#}$ in IPN hydrogels is always higher at temperatures above LCST and only the increment of energy,

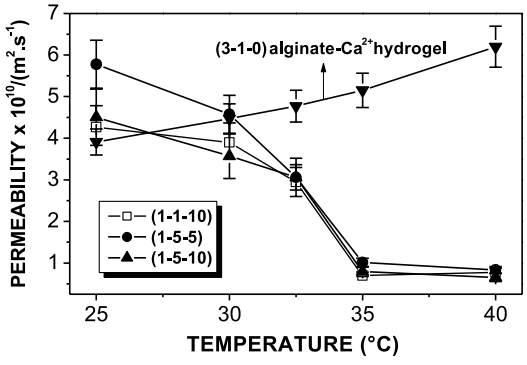

Fig. 5. Permeability to Orange II as a function of temperature of IPN hydrogels composed of alginate-Ca^{2+} and PNIPAAm networks and of pure alginate-Ca^{2+} hydrogel.

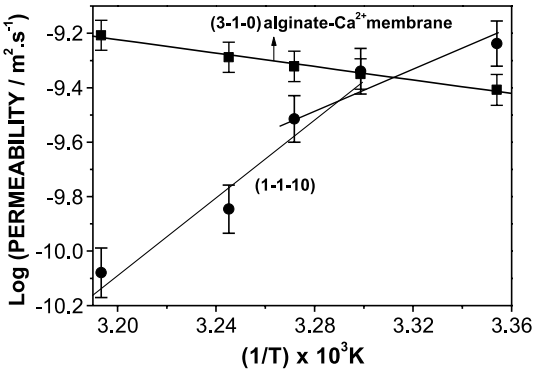

Fig. 6. Arrhenius plot for the permeability of the (1-1-10) IPN hydrogel composed of alginate-Ca^{2+} and PNIPAAm networks and pure (1-1-0) alginate-Ca^{2+} hydrogel.

represented by $\Delta E_p^{\#}$, for the diffusion of Orange II above the LCST PNIPAAm is significant. That increment is the consequence of a decrease of the average pore size that makes the diffusion of Orange II more difficult. This is confirmed by analysis of micrographs obtained by scanning electronic microscopy (SEM) of the (1-1-5) freeze dried IPN hydrogels. For that purpose, the hydrogels swollen to equilibrium at 25, 33 and 40 °C were first frozen in liquid nitrogen then freeze dried at −55 °C. In those conditions it can be assumed that the morphologies of the swollen samples were preserved. The micrographs presented in Fig. 7a, b and c concerning the (1-1-5) hydrogel, initially swollen at 25, 33 and 40 °C, are representative of the shrinking process. From these micrographs the average pore size in the (1-1-5) hydrogel was evaluated at the three temperatures. At 25 °C, the average pore size is approximately 102.0 ± 14.3 μm (Fig. 7a). However, when the (1-1-5) hydrogel is warmed to 33 °C (Fig. 7b), the average pore size is reduced approximately to 15.7 ± 5.4 μm. This confirms that the collapse of the PNIPAAm network results in a reduction in average pore size of the of IPN hydrogels. At 40 °C, a magnification of 10,000 was required to visualize pores at the surface of the IPN hydrogel (Fig. 7c). At 40 °C the measured value of the average pore size is approximately 0.4 ± 0.3 μm. A drastic change of the surface of the (1-1-5) IPN hydrogel thus occurs when it is heated from 25 to 40 °C. The results obtained by SEM clearly confirm the results obtained from the water uptake and permeability to Orange II measurements.

3.5. Visualization of the shrinking of IPN hydrogels

The shrinking process of the IPN hydrogel in the presence of water was also visually monitored at temperatures from 30 up to 40 °C. It is important to report that close to the LCST the shrinking is a gradual process, i.e. it does not occur immediately. This is visible in pictures of Fig. 8, taken at several temperatures where we can observe different stages of shrinking of the (1-1-5) IPN hydrogel.

Table 2
Apparent activation energy, $E_p^{\#}$, for permeability to orange II of IPN hydrogels of different compositions and pure alginate-Ca^{2+} hydrogel

Hydrogel	$E_p^{\#}$ (kJ Mol^{-1})		$\Delta E_p^{\#}$ (kJ Mol^{-1})
	a	*b*	*c*
(1-1-5)	−91.5	−44.3	47.2
(1-1-10)	−132.1	−63.1	69.0
(1-5-10)	−143.0	−82.9	60.1
(1-5-5)	−148.7	−95.8	52.9
(3-1-0)	–	–	24.0

(a)—Values of $E_p^{\#}$ below LCST of PNIPAAm; (b)—Values of $E_p^{\#}$ above LCST of PNIPAAm; (c)—Difference between $E_p^{\#}$ values obtained at temperatures above and below LCST of PNIPAAm.

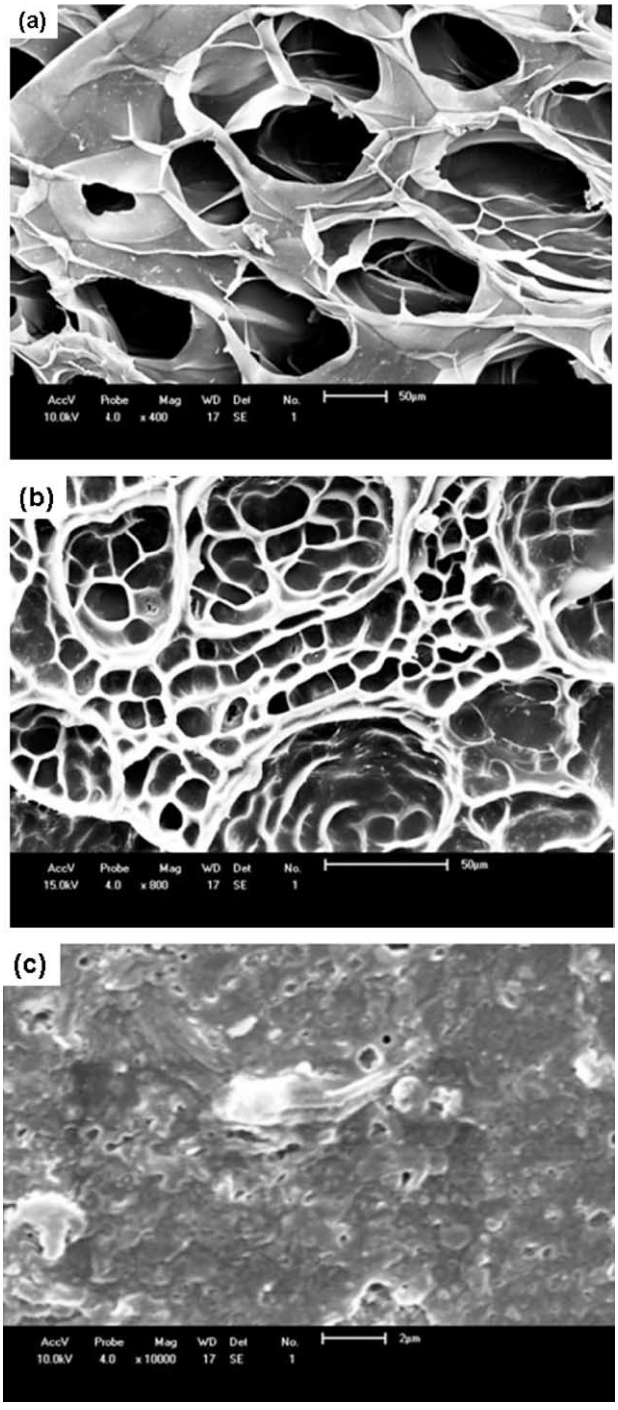

Fig. 7. Scanning electron microscopy of the (1-1-5) IPN hydrogel composed of alginate-Ca^{2+} and PNIPAAm networks. Micrographs of the hydrogel freeze dried after swelling at temperatures (a) 25 °C, (b) 33 °C and (c) 40 °C.

The rate of shrinking can be controlled by the rate of temperature rising. As shown in Fig. 5, the permeability of IPN hydrogel to Orange II decreases gradually as temperature is raised from 25 to 35 °C. From 35 to 40 °C, the value of permeability tends to be constant. This means that the shrinking process practically finishes at ca. 35 °C. This may be also observed in pictures of Fig. 8.

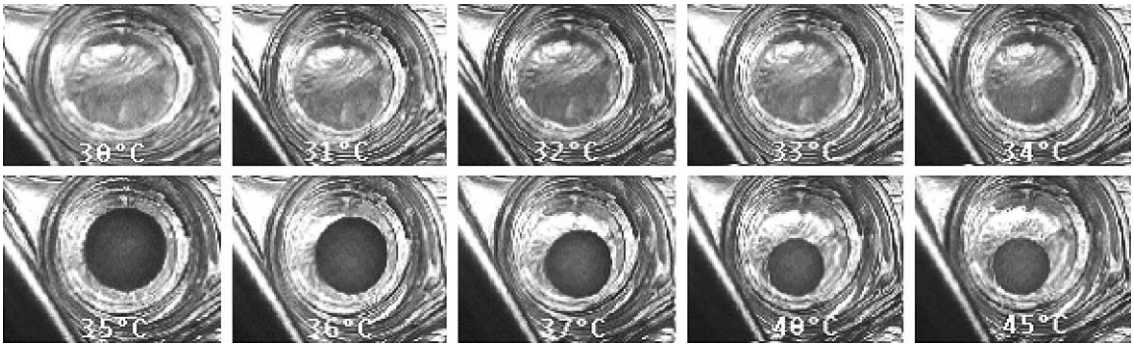

Fig. 8. Pictures of the (1-1-5) IPN hydrogel immersed in water at temperatures from 30 up to 40 °C.

4. Conclusions

Hydrogels composed of alginate-Ca^{2+} and PNIPAAm interpenetrated networks exhibit a sharp decrease of WU values when heated from 25 to 40 °C. This is associated to a drastic shrinking. At temperatures above 32 °C the chains of PNIPAAm collapse contracting and pulling back the alginate-Ca^{2+} matrix. The rate of shrinking is dependent on heating rate. The phenomenon is more pronounced and faster in IPN hydrogels containing low amounts of alginate-Ca^{2+}. The shrunken IPN hydrogel can be re-hydrated but the re-swelling is slower than the shrinking. When the IPN hydrogel is warmed up to 35 °C the diffusion of Orange II through the membrane decreases. The shrinking results in a decrease of the average pores size that makes more difficult the diffusion of Orange II. This is reflected in an increase in the apparent activation energy for permeability $E_p^{\#}$. The average pore size was evaluated in several stages from the SEM micrographs: 102 ± 14.3 μm at 25 °C, 15.7 ± 5.4 μm at 33 °C and 0.4 ± 0.3 μm at 40 °C. Below the LCST of PNIPAAm the IPN hydrogels have open pore morphology but above the LCST their surface becomes more regular and compact.

The novelty of IPN-hydrogel synthesized in this work is that its porous morphology may be tailored by the temperature adjusting. A potential use for it would be in culture of cells, e.g. once grown-up in these hydrogels, adhered cells might be detached by thermal stimuli. This would provide a rise on efficiency of transfer-cultured cells. Therefore, these hydrogels are convenient for further tests as scaffold for adherent cells growth and detachment. Applications as membranes for separation process are in progress.

Acknowledgements

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References

[1] Liu X, Qian L, Shu T, Tong Z. Polymer 2003;44:407.
[2] Holte O, Onsoyen E, Myrvold R, Karlsen J. Eur J Pharm Sci 2003;20:403.
[3] Fuchs B, Koelwel C, Goepferich A, Lohmann CP. Invest Ophthalmol Vis Sci 2002;43:4196.
[4] Kim JH, Lee SB, Kim SJ, Lee YM. Polymer 2002;43:7549.
[5] Kaufman MR, Tobias GW. Clin Plast Surg 2003;30:539.
[6] Doria-Serrano MC, Ruiz-Trevino FA, Rios-Arciga C, Hernandez-Esparza M, Santiago P. Biomacromolecules 2001;2:568.
[7] Kong H-J, Lee KY, Mooney DJ. Polymer 2002;43:6239.
[8] Yokoyam EF, Achife C, Matsuoka M, Shimamura K, Yamashita Y, Monobe K. Polymer 1991;32:2911.
[9] Wang X, Spencer HG. Polymer 1998;39:2759.
[10] Raj NKK, Sharma CPJ. J Biomater Appl 2003;17:183.
[11] Webber RE, Shull KR. Macromolecules 2004;37:6153.
[12] Coradin T, Nassif N, Livage J. Appl Microbiol Biot 2003;61:429.
[13] Shapiro L, Cohen S. Biomaterials 1997;18:583.
[14] Becker TA, Kipke DR. J Biomed Mater Res 2002;6:533.
[15] Lee KY, Rowley JA, Eiselt P, Moy EM, Bouhadir KH, Mooney DJ. Macromolecules 2000;33:4291.
[16] Zheng H, Zhang H, Zhang Q, Konno M, Yang S, Wang J. Carbohydr Polym 1998;35:215.
[17] Pillay V, Fassihi R. J Control Release 1999;55:243.
[18] Kim SR, Yuk SH, Jhon MS. Eur Polym J 1997;33:1009.
[19] Ouwerx C, Velings N, Mestdagh MM, Axelos MAV. Polym Gels Netw 1998;6:393.
[20] Montero P, Pérez-Mateos M. Food Hydrocol 2002;16:375.
[21] Melzoch K, Rychtera M, Hábová V. J Biotech 1994;32:59.
[22] Schild HG. Prog Polym Sci 1992;17:163.
[23] Bell CL, Peppas NA. Adv Polym Sci 1995;22:125.
[24] Guilherme MR, Da Silva R, Girotto EM, Rubira AF, Muniz EC. Polymer 2003;44:4213.
[25] Kuckling D, Hoffmann J, Plötner M, Ferse D, Kretschmer K, Adler H-JP. Polymer 2003;44:4455.
[26] Lin S-Y, Chen K-S, Run-Chu L. Polymer 1999;40:2619.
[27] Tanaka T, Filmore DJ. J Chem Phys 1979;70:1214.
[28] Grinberg VY, Dubovik AS, Kuznetsov DV, Grinberg NV, Grosberg AY, Tanaka T. Macromolecules 2000;33:8685.
[29] Gutowska A, Bae YH, Jacobs H, Feijen J, Kim SW. Macromolecules 1994;27:4167.
[30] Muniz EC, Geuskens G. Macromolecules 2001;34:4480.
[31] Guilherme MR, Toledo EA, Rubira AF, Muniz EC. J Membr Sci 2002;210:129.
[32] Kim JH, Lee SB, Kim SJ, Lee YM. Polymer 2002;43:7549.
[33] Hoffman AS. Adv Drug Deliv Rev 2002;43:3.
[34] Muniz EC, Geuskens G. J Membr Sci 2000;172:287.