Synthesis and Swelling Properties of pH- and Temperature-Sensitive Interpenetrating Polymer Networks Composed of Polyacrylamide and Poly(γ-glutamic acid)

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ABSTRACT: Novel hydrogels of interpenetrating polymer networks (IPNs) composed of polyacrylamide and poly(γ-glutamic acid) were synthesized. In these systems, both polymers were crosslinked independently; this reduced the potential loss of a polymer during the washing process, as often occurs in semi-IPN systems. Interpolymer interactions were investigated with Fourier transform infrared spectroscopy and differential scanning calorimetry. These studies suggested possible interactions between both polymers by the formation of hydrogen bonds. The swelling behavior of these hydrogels was analyzed by immersion of the hydrogel samples in deionized water at 25 and 37°C and in buffer solutions with pHs of 3, 7, and 10. The kinetics of swelling showed increases in the values of the swelling ratio with increasing immersion time in the swelling medium, molar proportion of the biopolymer in the hydrogel, temperature, and pH of the swelling medium. All of the hydrogels swelled rapidly and reached equilibrium in an average time of 40 min. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 3531–3537, 2011

Key words: biopolymers; hydrogels; interpenetrating networks (IPN)

INTRODUCTION

Hydrogels are crosslinked, three-dimensional hydrophilic polymer networks that can absorb large amounts of water while maintaining their three-dimensional structure.[1,2]

Actually, stimuli-responsive hydrogels have attracted much attention because these materials are soft and respond to a wide variety of external stimuli,[3] such as temperature,[4,5] pH,[4,6] solvent composition,[7] ionic concentration,[8] and electric fields.[9]

Interpenetrating polymer networks (IPNs), which are composed of two or more polymer networks that are not chemically crosslinked with one another but are interpenetrating or physically entangled within one another such that they cannot be separated,[10,11] have been used to improve the properties of polymer blends and composites.[2,12]

Recently, thermosensitive and pH-sensitive IPN hydrogels have attracted great interest because the temperature and pH are important environmental factors in biomedicine and other systems.[2] This class of hydrogels has been used in a variety of applications, such as tissue engineering[13] and controlled drug delivery.[14]

Important information related to the interaction between the constituent polymers of an IPN is revealed by studies of differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy. If two immiscible polymers are formed into an IPN, and the glass-transition temperatures (T_g's) of the two polymers are shifted inward, the IPN obtained is a compatible material. On the other hand, the interpolymer associations in IPN systems have been studied by means of displacements in the absorption bands of functional groups within the IR spectrum.[15,16]

Synthetic polymers with natural basic polymeric components have been used to form smart hydrogels.[17] Polyacrylamide (PAAm) hydrogels are widely used within these systems of IPNs because of their interesting swelling properties.[6,12,18–20] Moreover, the addition to these systems of a biopolymer, such as poly(γ-glutamic acid) (γ-PGA), which possesses important features of biodegradability, biocompatibility, and nontoxicity, provides the material with

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interesting properties, which makes this material suitable for a great variety of applications, such as tissue engineering and controlled drug release.[21,22] This biopolymer is an anionic peptide, is water-soluble, and is unique in that it is composed of naturally occurring L-glutamic acid linked through amide bonds.[23] In a previous article, we reported the synthesis of semi-IPN hydrogels formed by a polymeric network of PAAm and linear γ-PGA (Scheme 1).[24] Also, some extraction studies have shown that in semi-IPN systems, the linear polymer acts as a porogen and a small amount of this polymer is removed during the washing process.[25] With the aim of reducing the potential loss of this polymer during the washing process and in this way maintaining the properties of biodegradability and biocompatibility attributed to this biopolymer, we proposed the synthesis of IPN systems because, in these materials, γ-PGA is too crosslinked.

The main aim of this study was to synthesize novel IPN systems composed of PAAm and γ-PGA and to evaluate the swelling properties of these materials.

In this article, we report the synthesis of novel IPN hydrogels composed with different molar proportions of PAAm and γ-PGA. The interaction between both polymers of the IPNs was studied, along with their swelling properties and sensitivity to temperature and pH changes.

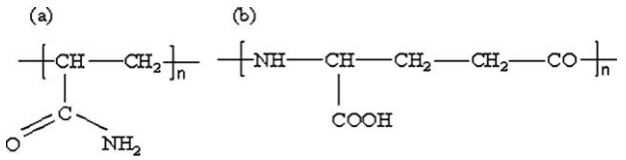
EXPERIMENTAL

Materials

Bacillus licheniformis (ATCC 9945a). Acrylamide (AAm), *N′N′*-methylene bisacrylamide (MBAAm), and *N,N,N′,N′*-tetramethylenediamine (TEMED) were purchased from Sigma (U.S.A.). 1,5-Dibromepentane was obtained from Fluka (Steinheim, Germany), dimethyl sulfoxide (DMSO) and ammonium persulfate (APS) were obtained from J. T. Baker (U.S.A.), and acetone was obtained from Meyer (México). All reagents were used as received. The water was previously deionized.

Production and purification of γ-PGA

γ-PGA was produced on the basis of our previously published method.[24] *B. licheniformis* (ATCC 9945a) was grown in the nutrient broth proposed by González et al.[26] Cells were cultured at 37°C in a shaking incubator at 300 rpm for 96 h. After fermentation, the cells were removed by centrifugation at 15,000 rpm for 30 min at 12°C. The supernatant containing γ-PGA was poured into an equal volume of acetone to precipitate the polymer, and then, it was dissolved in deionized water and dried through a freeze-drying method. The purification of γ-PGA was carried out by a dialysis method with deionized water. The average molecular mass of the polymer was determined by gel permeation chromatography (GPC) with poly(-styrene sulfonic sodium salts) standards.



Scheme 1 Chemical structures of (a) PAAm and (b) γ-PGA.

Synthesis of the PAAm/γ-PGA interpenetrating networks

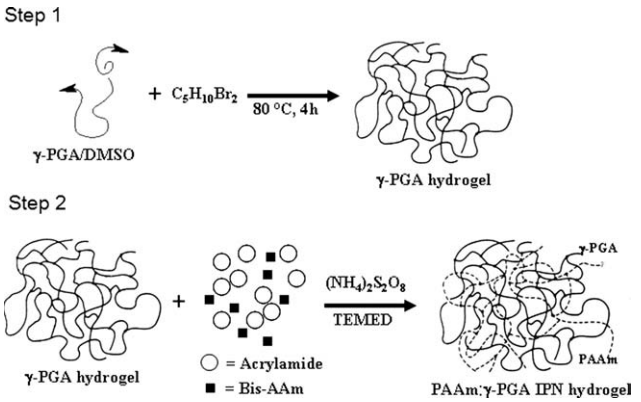
The IPNs were synthesized with a sequential method. First, a hydrogel of γ-PGA was synthesized on the basis of the method proposed by González et al.[26] γ-PGA (0.4 g, weight-average molecular weight = 150,000 g/mol) was dissolved in 1.6 mL of DMSO by means of magnetic stirring, and then, 0.05 mL of 1,5-dibromepentane was added to carry out the crosslinking of polymer. The resulting solution was then maintained at 80°C for 4 h. The hydrogels were washed first with phosphate buffer at pH 7.2 for 48 h and then with deionized water for 24 h. The γ-PGA hydrogels were dried with a freeze-drying method with a Labconco Freezone 4.5 freeze dryer (Kansas City, MO) (0.5-mBar vacuum and −46°C in the collector). We synthesized the second polymeric network by swelling the dry hydrogel (Xerogel) of γ-PGA on the AAm solution (29 g of AAm and 1 g of MBAAm in 100 mL of deionized water). MBAAm was used as a crosslinker; then, we added APS (2.5 mg/mL, initiator) and TEMED (3.75 μL/mL, catalyst). The reaction was carried out in an ice bath to allow the complete absorption of the solution in the hydrogel of γ-PGA; this was completed in about 10 min to allow the formation of IPNs. The hydrogels of the IPNs composed of PAAm/γ-PGA were prepared at molar ratios of 95 : 5, 90 : 10, 85 : 15, 80 : 20, and 75 : 25. These molar ratios were obtained with different volumes of AAm solution.

FTIR spectroscopy

FTIR spectra were obtained by a Nicolet 550 FTIR spectrophotometer (Minnesota, U.S.A.). The dry sample was directly embedded into a KBr pellet and measured in transmittance mode. This study was carried out to identify possible polymer–polymer interactions by means of displacements in the characteristic absorption bands of both polymers.

DSC

DSC measurements were obtained with a 2920 MDSC instrument (New Castle, DE) with a heating rate of 5°C/min from 25 to 230°C under a nitrogen atmosphere. The samples were dried previously. This study



Scheme 2 Schematic representation of the synthesis of the IPN hydrogels of PAAm and γ-PGA. *N′N′*-methylene bisacrylamide (Bis-AAm)

was realized to also identify possible interpolymer associations on the basis of inward displacements of the T_g's of both polymers.

Thermogravimetric analysis

The thermal stability of the dry hydrogels was investigated by means of thermogravimetric analysis performed by a Q500 thermogravimetric analysis instrument (New Castle, DE) at a heating rate of 10°C/min from 25 to 650°C under a nitrogen atmosphere.

Swelling measurements

The effect of the temperature on the swelling properties of the hydrogels was measured in deionized water at 25 and 37°C, whereas the effect of pH was measured with a swelling medium phosphate/citrate buffer solution with pH values of 3 and 7 (ionic strength = 0.15) and a carbonate buffer solution with a pH of 10 (ionic strength = 0.1) at 25°C. In this study, we used hydrogel disks 16 mm in diameter and 3 mm in thickness; these were obtained by means of the realization of cuts on the synthesized hydrogels with a circular mold. Preweighed hydrogel samples were placed into beakers containing swelling medium, and then, the beakers were immersed in a water bath that was maintained at the desired temperature. Afterward, at definite intervals of time, the hydrogel samples were removed from the swelling medium and tapped with filter paper to remove excess water on the hydrogel surface. The hydrogel samples were repeatedly weighed and re-immersed in the swelling medium until the swollen weight reached a constant value. The swelling ratio (δ) of the samples was calculated from the following expression:

$$\delta = \frac{W_s - W_d}{W_d}$$

where W_s is the weight in the swollen state and W_d is the weight of the dry hydrogel.

RESULTS AND DISCUSSION

IPN hydrogels with molar proportions of PAAm to γ-PGA of 95 : 5, 90 : 10, 85 : 15, 80 : 20, and 75 : 25 were synthesized with a sequential method (Scheme 2).

FTIR studies have been reported that identify interpolymer associations on IPN systems, and they have been attributed to hydrogen-bond formation.[11,16,27] We used FTIR spectroscopy to investigate possible interpolymer associations in IPN systems composed of PAAm/γ-PGA. Figure 1 shows the FTIR spectra corresponding to the hydrogels of PAAm, γ-PGA, and IPNs. Figure 1(a,c) shows a broad band in the PAAm and γ-PGA hydrogels at 3437 cm^{-1}, which was attributed to the N—H stretching, whereas the spectrum of IPN with a molar ratio of 80 : 20 [Fig. 1(b)] showed this broad band at 3422 cm^{-1} and showed a considerable displacement toward a minor wave number, which corresponded to 15 cm^{-1}. Moreover, the C—O stretching bands in PAAm and γ-PGA were observed at 1663 and 1637 cm^{-1}, respectively. In the hydrogels of IPNs, this band presented a displacement toward an intermediate value of wave number; this displacement corresponded to 6 and 20 cm^{-1} in relation to the bands observed in the pristine sample hydrogels of PAAm and γ-PGA, respectively. These

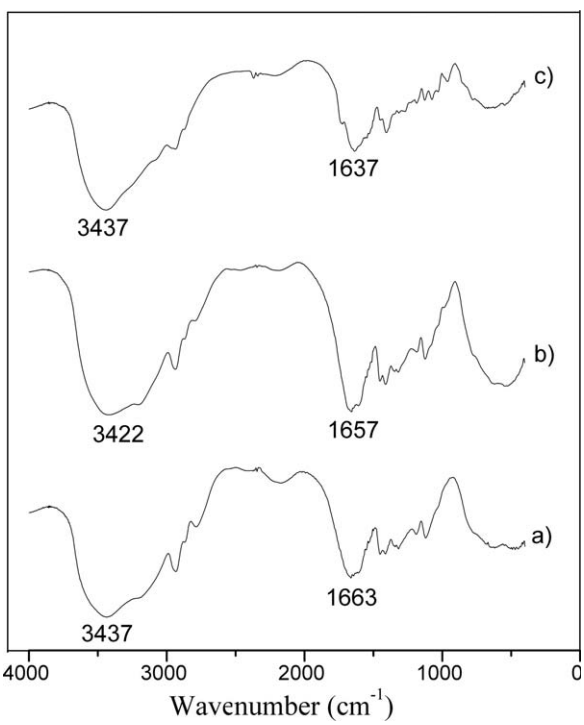


Figure 1 Amide and carbonyl stretching bands obtained via FTIR spectroscopy from the hydrogel samples: (a) PAAm, (b) IPN system, and (c) γ-PGA.

TABLE I
T_g Values by DSC in Hydrogels of IPNs with Different PAAm/γ-PGA Molar Ratios

Molar ratio	T_g (°C)
100 : 0	196
95 : 5	190
90 : 10	188
85 : 15	186
80 : 20	185
75 : 25	182
0 : 100	144

displacements in the absorption bands of the N—H and C—O groups in the IPN hydrogels with regard to individual polymers were attributed to polymer–polymer interactions, in which we propose the existence of hydrogen bonds between the N[bond]H and C—O groups present in PAAm and γ-PGA. To supplement these results, the displacements in the T_g values of the IPNs in comparison with the individual polymers were analyzed by the DSC method.

DSC measurements were carried out to identify possible associations between PAAm and γ-PGA in the IPN systems on the basis of the analysis of the obtained T_g values and to reinforce studies realized by FTIR. It is known from the literature that the two T_g values were indicative of a phase-separated structure, with the T_g of individual components often shifted toward each other, which indicated a partial mixing of the networks, and the one value intermediate of T_g indicated a complete mixing of the networks.[28] The T_g values corresponding to each of the hydrogels elaborated in this study were measured by DSC reversible analyses; to eliminate the effect of moisture, we realized two cycles of heating and cooling, considering that the temperature region would not bring about the thermal degradation during the first heating run. The results obtained in the second run are shown in Table I. The T_g value in the PAAm hydrogel was shown at 194°C, and the T_g value of the γ-PGA hydrogel was shown at 144°C, whereas all of the IPN hydrogels showed one T_g value intermediate between the values of the individual polymers. These results suggest that the IPNs of the PAAm/γ-PGA were compatible. The compatibility between PAAm and γ-PGA could be explained on the basis of intermolecular hydrogen bonding.[16] Similarly, as observed in the studies by FTIR spectroscopy, the decrease in the T_g values of the IPN hydrogels compared with the T_g value of the PAAm hydrogel suggested an increased flexibility of the material, which was attributed to the addition of γ-PGA, which is a flexible polymer. This manner favored the movement of polymer segments. This behavior also had an effect on the swelling properties of the IPN hydrogels (the results are discussed in detail later), where the addition of γ-PGA favored the swelling degree of hydrogel. This was attributed, in part, to an increase in the flexibility of the material.

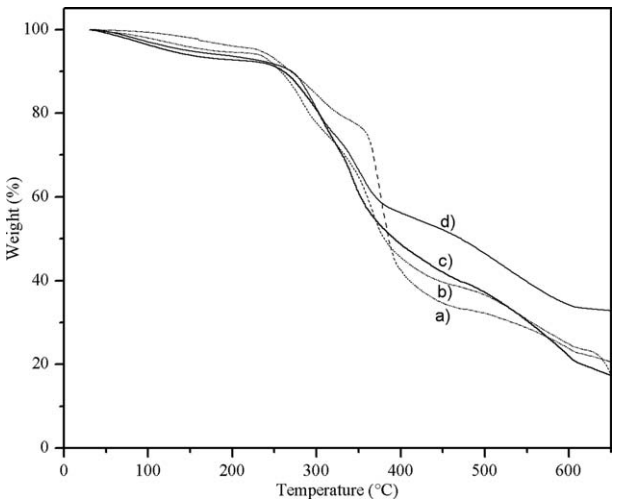


Figure 2 Thermogravimetric curves of (a) PAAm, (b) IPN with a molar ratio of 90 : 10, (c) IPN with a molar ratio of 80 : 20, and (d) γ-PGA.

The thermal stability of the hydrogels was investigated by means of thermogravimetric analysis; a first thermal event was observed in the temperature range 25–260°C, where all samples presented a mass loss ranging from 6 to 10%, which was attributed to the evaporation of water (Fig. 2). The chemical degradation of the samples was initiated between 250 and 260°C. In the region from 380 to 570°C, the IPN hydrogels showed a clear intermediate thermal stability in relation to individual polymers.

The swelling kinetics of the IPN hydrogels carried out in deionized water at 25 and 37°C are plotted in Figures 3 and 4, respectively. In both studies, all of the hydrogels swelled rapidly and reached equilibrium in an average time of 40 min. The values of the

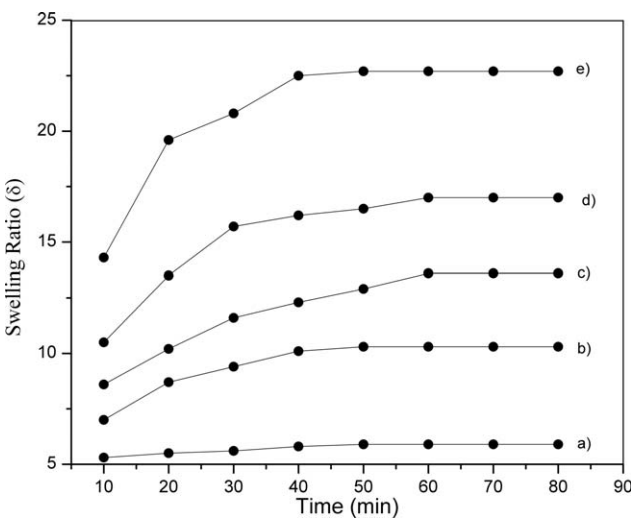


Figure 3 Swelling kinetics of IPN samples with different PAAm/γ-PGA molar ratios in deionized water at 25°C: (a) 95 : 5, (b) 90 : 10, (c) 85 : 15, (d) 80 : 20, and (e) 75 : 25.

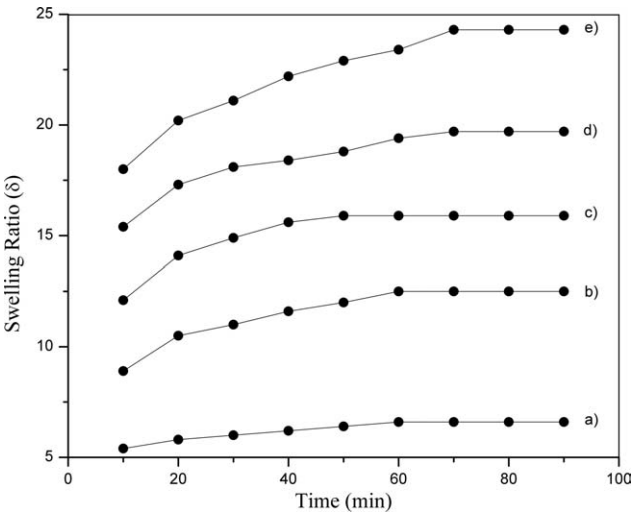


Figure 4 Swelling kinetics of the IPN samples with different PAAm/γ-PGA molar ratios in deionized water at 37°C: (a) 95 : 5, (b) 90 : 10, (c) 85 : 15, (d) 80 : 20, and (e) 75 : 25.

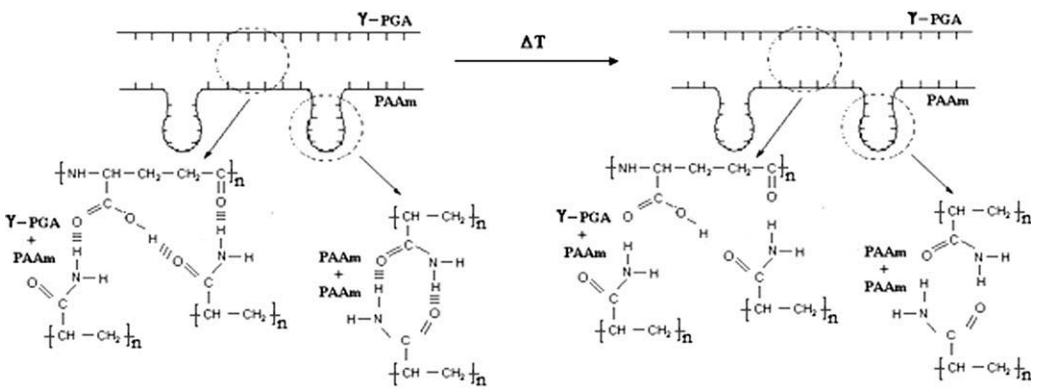
swelling ratio plotted in these figures are the average of three trials. The γ-PGA hydrogels showed the highest values of swelling ratio; however, they were highly flexible materials, and to strengthen their mechanical properties, they should be mixed with a polymer with a high rigidity and good swelling properties, such as PAAm. Both swelling kinetics showed an increase in the swelling ratio values dependent on increases in the γ-PGA content and time. In this manner, the IPN hydrogels with the highest content of γ-PGA (75 : 25) presented equilibrium swelling ratios of 22.5 and 24.5 at 25°C and 37°C, respectively (Table II). This behavior was attributed to an increase in the flexibility of the hydrogel, which allowed the movement of polymeric chains and, thus, favored the diffusion of water into the material,[11] and to a decrease in the polymeric density due to a decrease in the PAAm content in the hydrogel; this allowed more free space volume in

TABLE II
Equilibrium Swelling Ratios at 25 and 37°C for Hydrogels of IPNs with Different PAAm/γ-PGA Molar Ratios

	Equilibrium swelling ratio	
Molar ratio	25°C	37°C
100 : 0	5.8 ± 0.05	5.9 ± 0.25
95 : 5	5.9 ± 0.2	6.6 ± 0.05
90 : 10	10.3 ± 0.3	12.5 ± 0.6
85 : 15	13.6 ± 0.1	15.9 ± 0.5
80 : 20	17.0 ± 0.6	19.7 ± 0.8
75 : 25	22.5 ± 1.6	24.5 ± 0.7
0 : 100	35.8 ± 0.9	43.5 ± 1.05

the polymeric network, and thus, the volume available for swelling increased. The high hydrophilic character of this biopolymer, which was due to free carboxyl groups in its structure, which encouraged polymer–water interaction and allowed a wider diffusion of this solvent into the material, increased the swelling ability of these materials.[14] On the other hand, a general increase in the capacity of swelling in relation to an increase in temperature was also observed. This behavior was attributed to hydrogen-bond dissociations of intrapolymer and interpolymer complexes caused by the increase in the temperature; this increased the number of available hydrophilic sites than could interact with water molecules and also decreased the crosslinking density of the hydrogel, which increased the separation of the polymeric chains, which favored the diffusion of water into the hydrogel (Scheme 3).

The swelling kinetics of the IPN hydrogels carried out in buffer solutions with pH values of 3, 7, and 10 at 25°C are shown in Figures 5, 6, and 7, respectively. All of the hydrogels showed an increase in the swelling ratio values that was dependent on the increase in the γ-PGA content and time. The hydrogels with a higher



Scheme 3 Schematic representation of the formation and dissociation of hydrogen bonds in the PAAm/γ-PGA IPNs with respect to increases in temperature. Temperature increase (ΔT).

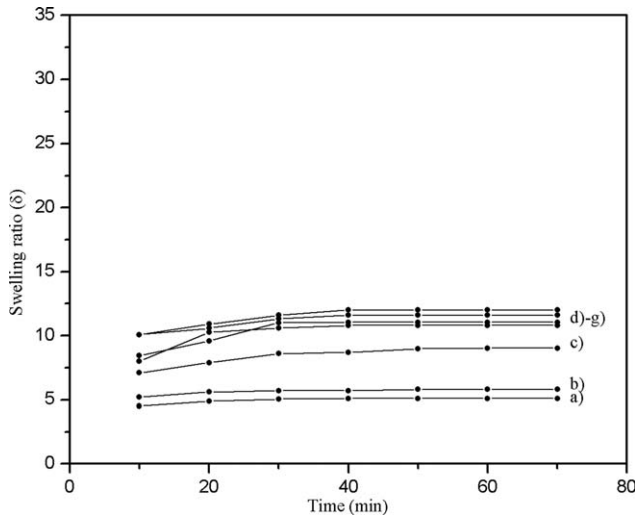


Figure 5 Swelling kinetics in a pH 3 buffer solution at 25°C in IPN samples with different PAAm/γ-PGA molar ratios: (a) 100 : 0, (b) 95 : 5, (c) 90 : 10, (d) 85 : 15, (e) 80 : 20, (f) 75 : 25, and (g) 0 : 100.

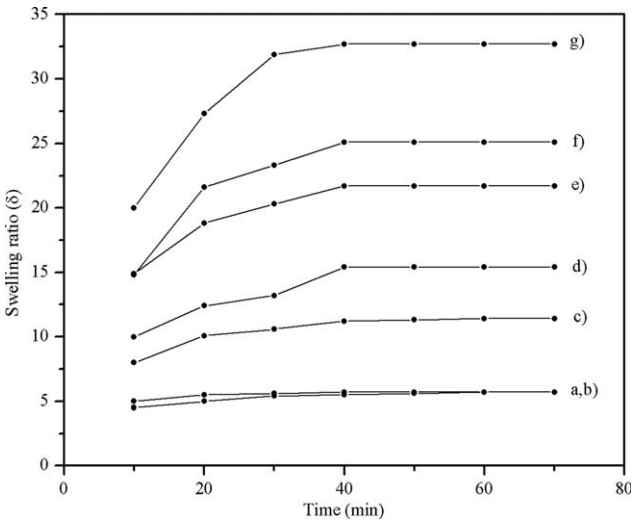


Figure 7 Swelling kinetics in a pH 10 buffer solution at 25°C in IPN samples with different PAAm/γ-PGA molar ratios: (a) 100 : 0, (b) 95 : 5, (c) 90 : 10, (d) 85 : 15, (e) 80 : 20, (f) 75 : 25, and (g) 0 : 100.

content of γ-PGA showed the highest values of swelling ratio, whereas hydrogels with a lower content of this biopolymer showed the lowest values of swelling ratio. In this way, whereas hydrogels with a molar ratio of 95 : 5 showed swelling ratios in equilibrium of 5.1, 5.5, and 5.7 in the kinetics realized in the buffer solutions with pH values of 3, 7, and 10, respectively. In the hydrogels with a molar ratio of 75 : 25, the values of the equilibrium swelling ratio were of 11, 19.3, and 25.1, respectively (Fig. 8). This increase in the capacity of swelling was attributed to the high hydrophilic character of γ-PGA due to free carboxyl groups in its structure and to a decrease in the polymeric density due to the decrease of the PAAm content in the IPN in addition to the increased flexibility of the hydrogel. On the other hand, the hydrogels of the IPNs studied were sensitive to external changes of pH. An increase in the values of the swelling ratio with increasing pH was observed. The hydrogels studied at pH 3 showed the lowest values of swelling (Fig. 8); this behavior could be explained on the basis of the existence of carboxyl free groups attached to the chains of the biopolymer. At pH 3, these carboxyl groups were not ionized, and they formed hydrogen bonds with amino and carbonyl groups in PAAm. This increased the density of crosslinking and simultaneously decreased the diffusion of the solvent into the hydrogel. This was reflected in the low absorption capacity of the material.[11] The kinetics studied with pH values above the pK_a of the γ-PGA (4–

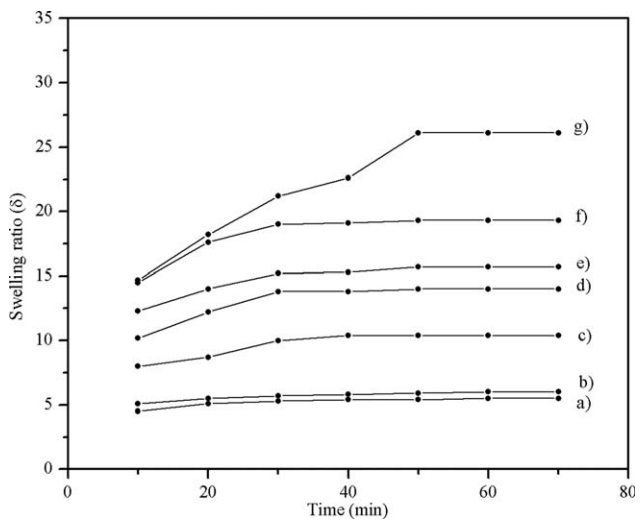


Figure 6 Swelling kinetics in a pH 7 buffer solution at 25°C in IPN samples with different PAAm/γ-PGA molar ratios: (a) 100 : 0, (b) 95 : 5, (c) 90 : 10, (d) 85 : 15, (e) 80 : 20, (f) 75 : 25, and (g) 0 : 100.

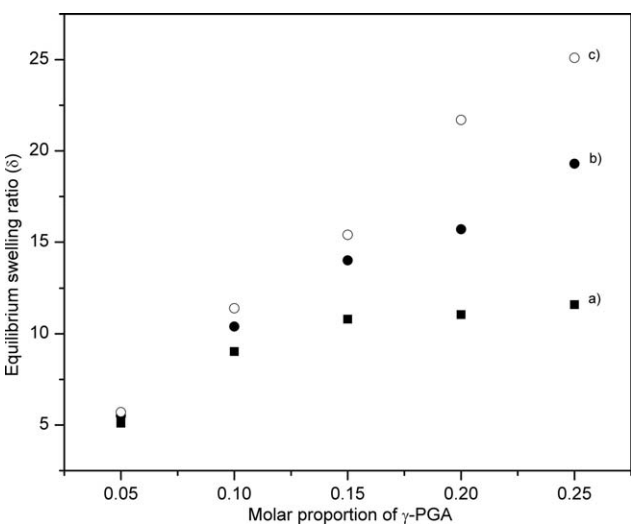


Figure 8 Swelling behavior of the IPN hydrogels with different γ-PGA molar ratios in buffer solutions at 25°C: (a) pH 3, (b) pH 7, and (c) pH 10.

4.8)[29] showed values of swelling ratio higher in comparison to values obtained in the kinetics realized at pH values lower than the pK_a of this biopolymer. In this manner, the hydrogels studied at pH 7 showed a greater capacity for swelling than the hydrogels analyzed at pH 3. These results were attributable to the dissociation of hydrogen bonds; this reduced the density of crosslinking of the hydrogel and increased the absorption capacity of water and the electrostatic repulsions between the polymeric chains due to the formation of carboxylate groups. Finally, studies realized at pH 10 revealed the highest values of the swelling ratio. At this pH, the full ionization of carboxyl groups in γ-PGA was considered; this created a greater electrostatic repulsion between the polymeric chains and low crosslinked density and, in this manner, was conducive to the swelling of the material. In general, these results assume pH sensitivity of the IPN hydrogels composed of PAAm/γ-PGA. Similar but less marked behavior was reported for poly(*N*-vinylpyrrolidone) and poly(acrylic acid) semi-IPNs.[11] All of the hydrogels swelled rapidly and reached equilibrium in an average time of 40 min.

The ionic strengths of the buffer solutions at pH 3 and pH 7 were equal to 0.15, whereas that in the buffer solution at pH 10 was equal to 0.1. Therefore, the increasing swelling ratio values in relation to the increase in pH from 3 to 7 was not influenced by the ionic strength because the value was the same. However, the ionic strength in the buffer solution at pH 10 was smaller than those in buffer solutions at pH 3 and pH 7; this contributed, in part, to the increase in the values of the swelling ratio obtained in kinetics carried out at pH 10.

CONCLUSIONS

Novel IPN hydrogels composed of PAAm/γ-PGA with different molar ratios were obtained. Studies by IR spectroscopy and DSC suggested possible interactions between PAAm and γ-PGA in the IPNs by means of hydrogen bonds. The swelling kinetics showed an increase in the values of swelling ratio in relation to the contact time in the swelling medium, the content of γ-PGA in the IPNs, and increases in the temperature and pH. Moreover, all of the hydrogels quickly reached swelling equilibrium in average time of 40 min.

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