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# New Interpenetrating Polymer Networks of N-isopropylacrylamide/ N-acryloxysuccinimide: Synthesis and Characterization

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## Summary

Interpenetrating polymer networks (IPNs) based on poly (N-isopropylacrylamide), PNIPAAm, and poly (N-acryloxysuccinimide), PNAS, were prepared by a sequential method; the PNIPAAm which was polymerized and crosslinked by gamma irradiation, was swelled in a solution of PNAS/polylysine, which function is as crosslinking agent for this monomer and as anchoring element of vesicles.
The thermosensitivity properties (limit swelling time, lower critical solution temperature (LCST) and water retention), chemical composition (FTIR and elemental analysis), thermal properties (DSC and TGA) and morphology (SEM) were studied to characterize the IPNs.

## Introduction

Many hydrogels, which usually show good compatibility in contact with blood, body fluids and tissues, are promising classes of materials for biomedical application. Stimuli responsive hydrogels are very attractive due to their changes in shape and volume under environmental stimuli like temperature, pH, electric field, etc. [1-7]. The hydrogel of cross-linked poly (N-isopropylacrylamide) (PNIPAAm) is a well-known thermal-responsive hydrogel, which swells below and deswells above its LCST (around 33°C) [8-12]. Many studies of this polymer have been reported because of the reversible thermal-responsive behavior which can be utilized in biomedical applications such as controlled drug release [13-16], protein concentration and separation [17,18], immobilization of enzymes and lipids [19-22]. PNIPAAm hydrogels have been used in devices to control the release from vesicles or liposomes immobilized on the network, poly (N-acryloxysuccinimide) (PNAS) provides a functional group which is readily displaced by the amino groups of lysyne, and these groups, are the anchoring element of vesicles, and also acts as a crosslinking agent of PNAS. NAS has been used as an active ester to bind proteins through bond formation with lysyne residues [23-26], and have interesting applications in water purification and biology [27].

However all these polymeric systems studied have a slow response rate to external changes of temperature which limit their applications in high performance devices or systems such as rapid actuators and artificial organs. In addition, the reinforcement of conventional PNIPAAm hydrogel is a major problem in the expansion of its applications because the hydrogel has poor mechanical properties when swelling in water. Polymers with microphase separation, such as copolymers, seem to possess improved mechanical properties, this morphology of separated microphase can also be achieved with interpenetrating polymer networks (IPNs) [28, 29], or semi-interpenetrating networks [30-31], and they have the advantage to enhance of response rates at the same time.

An interpenetrating polymer network (IPN) can be defined as a mix of polymers in network form, without any substantial quantities of crosslinks, graft or block junctions among the several polymer chains, where at least one polymer is polymerized and/or crosslinked in the immediate presence of the others [32-34].

Radiation processes have many advantages over other conventional methods of polymerization and crosslinking when preparing IPNs, because catalysts or additives are not necessary to initiate the reaction [35]. The radiation method is relatively simple and the degree of crosslinking can be controlled easily by varying the absorbed dose.

In this work, sequential interpenetrating networks made of PNIPAAm hydrogel obtained by gamma radiation, and PNAS crosslinked within PNIPAAm hydrogel by reaction with polylysine, were synthesized and characterized. This system could be employed to immobilize liposomes through amino groups, and the immobilization is reversible due to the thermosensitivity of PNIPAAm.

## Experimental

All the reagents were obtained from Aldrich Chemical USA. N-isopropylacrylamide (NIPAAm) was purified by recrystallization in toluene/n-hexane (50/50); N,N-methylenebisacrylamide (BIS), N-hydroxysuccinimide (NHS), morpholino-ethanesulfonic acid (MES) and polylysine (MW 400-2500 and 4000) were used as received. Acryloyl chloride was distilled to remove any trace of inhibitor; chloroform was distilled from $P_2O_5$ after refluxing for 12 h with $MgSO_4$, and triethylamine ($NEt_3$) was distilled and dried with KOH. Tetrahydrofuran (THF) and dimethylformamide (DMF) were used as received.

### *Synthesis of the PNIPAAm hydrogels*

Aqueous solutions of NIPAAm (10% wt) with and without crosslinking agent (BIS) at 0.0024 M were charged into glass ampoules, bubbling with argon to remove the oxygen and sealed. Then they were irradiated from 50 to 70 kGy, at room temperature, with a $^{60}Co$ source (Gamma Beam 651PT of Nordion Co.) at a dose rate of 3.85 kGy/h. After polymerization and crosslinking, the synthesized hydrogels were immersed in water at room temperature for 48 h to eliminate the non-crosslinked polymer [36, 37].

The PNIPAAm hydrogel fraction was calculated from the weight ratio of the insoluble fraction and the weight of the monomer.

$$\text{Gel (\%)} = (W/Wo) \times 100 \qquad (1)$$

Where W and Wo are the weights of crosslinked dry samples and initial monomer, respectively.

*Polymerization of NAS*

The N-acryloxysuccinimide (NAS) was synthesized by the Pollak method [23] and polymerized with gamma radiation. Solutions of NAS in THF (7%) were placed in ampoules, bubbled with argon and sealed. The samples were gamma irradiated at a dose of 3.4 kGy/h, and radiation dose of 10 kGy. The non-polymerized NAS was removed by extracting from the polymer with THF for 24 h. The molecular weight of linear PNAS was determined by size exclusion chromatography (SEC) using DMF as the mobile phase and polystyrene as standards, in a Varian 9002 with a RI-4 model (refraction index) detector, resulting the PNAS with molecular weight of 8,000.

*Formation of IPNs*

PNAS (200 mg) was dissolved in DMF (1 mL) and subsequently the PNIPAAm hydrogel (200 mg) synthesized with or without BIS, was immersed in this solution until maximum swelling was reached. Polylysine (100 mg) in 2-N-morpholinoethanesulfonic acid (10 mL, 0.05 M, pH 7.4) was added to the PNIPAAm hydrogel/PNAS mixture and incubated at 10°C during 48 h with constant agitation (59 rpm) [38]. The resulting interpenetrating networks were washed with DMF and then with water for 24 h. The composition of IPNs were confirmed by Fourier-transform infrared (FTIR) spectroscopy with a Perking Elmer model Paragon 50, ATR mode, and evaluated by elemental analysis, Desert Analytics Tucson, Arizona.

*Swelling Measurements*

The equilibrium swelling time of the samples was measured gravimetrically. The samples were swollen in distilled water at room temperature and after immersion, they were removed and blotted with filter paper to remove the excess of water on the surface, and weighed. The immersion time and drying procedure was repeated until the weight of swollen samples was constant. The swelling percent was defined as follows:

$$\text{Swelling } \% = [(W_s - W_d) / W_d] \times 100 \qquad (2)$$

Where $W_s$ and $W_d$ are the swollen and dry weights, respectively.

*Determination of the Phase-Transition Temperature (LCST)*

The experiment was carried out in a thermostatic water bath equipped with systems of heating and cooling. The samples, hydrogels of PNIPAAm and IPNs of PNIPAAm/ PNAS, were placed in distilled water at several temperatures, ranging from 10 to 50°C until the equilibrium swelling of the samples was reached (200 and 100 min for the PNIPAAm and IPNs, respectively). Then, the water excess on the samples surface was removed as stated above, and the samples were weighed. The phase transition temperature associated with the LCST, was determined as the inflexion point in the plot of swelling as a function of the temperature, and confirmed by differential scanning calorimetry (DSC), using a TA instruments model 2010. All samples were

immersed in distilled water at room temperature and allowed to swell to reach equilibrium before DSC measurements. These swollen samples were placed in hermetically sealed pans and the thermal analysis was performed at a heating rate of 3°C/min under a nitrogen flow (60 ml/min). The onset point of the endothermic peak was used to estimate the LCST [39-41].

*Glass transition temperature (Tg)*

The glass transition temperature of samples was determined by DSC, in the range of 20 to 200°C at a heating rate of 10°C$min^{-1}$ (Table 1).

*Morphology*

Scanning Electron Microscopy, SEM, (Jeol model 5600) was employed to study the morphology of PNIPAAm and IPN hydrogels under swollen conditions. The samples were frozen in liquid nitrogen, the frozen hydrogels were fractured and vacuum dried in order to study the cross section morphology of the samples, then they were fixed on aluminum stubs and coated with gold.

**Results and Discussions**

The influence of the molecular weight of the polylysine in the formation of IPNs was studied. Results of two series of IPNs are reported. In the first series, polylysine have a molecular weight range of 500-2000 and the second one a molecular weight of 4000, both of them were used with the PNIPAAm network with and without BIS. The effect of the percentage of crosslinking in the PNIPAAm network on the properties of IPNs was also studied. It is important to point out that similar results were obtained from swelling measurements and LCST, using the two series with polylysine of different molecular weight; thus we only present some results to make the interpretation and understanding easier.

FTIR spectra confirm the incorporation of PNAS in the interpenetrating network because of the appearance of three bands (1800, 1772 and 1740 $cm^{-1}$) characteristics of the succinimide group (Figure 1C). These bands are observed in PNAS spectrum at 1808, 1777 and 1727 $cm^{-1}$, (Figure 1B); PNIPAAm homopolymer (Figure 1A), shows the characteristic bands of C=O and N-H stretching of the amide group around 1656 and 1543 $cm^{-1}$, respectively. Another stretching vibration of the N-H amine group at 3276 $cm^{-1}$ and the vibration of the isopropyl group at 1385 $cm^{-1}$ are also observed. The spectra of the IPNs contain the characteristic succinimide absorptions as well as bands at 3417 and 1660 $cm^{-1}$ corresponding to the groups of PNIPAAm mentioned above but shifted to higher wavelength.

The molar ratios of the IPNs calculated by elemental analysis were PNAS/PNIPAAm 1:3 for both series A and B, under different conditions of synthesis.

The limit swelling time was determined and it was smaller for the interpenetrating networks (about 100 min) than for the PNIPAAm hydrogel that requires 200 min (Figure 2). This is probably a consequence of the new arrangement formed by the two polymeric networks and the separation of intramolecular hydrogen bonds between NIPAAm groups.

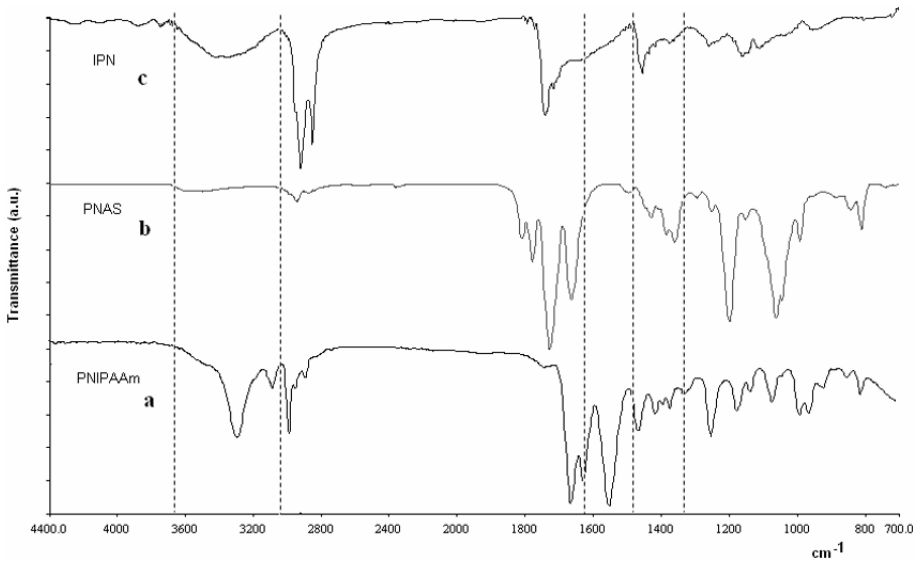


Figure 1. FTIR spectrum of (a) PNIPAAm hydrogel, (b) PNAS and (c) IPN irradiated at 60 kGy without BIS and with polylysine molecular weight of 500 to 2000.

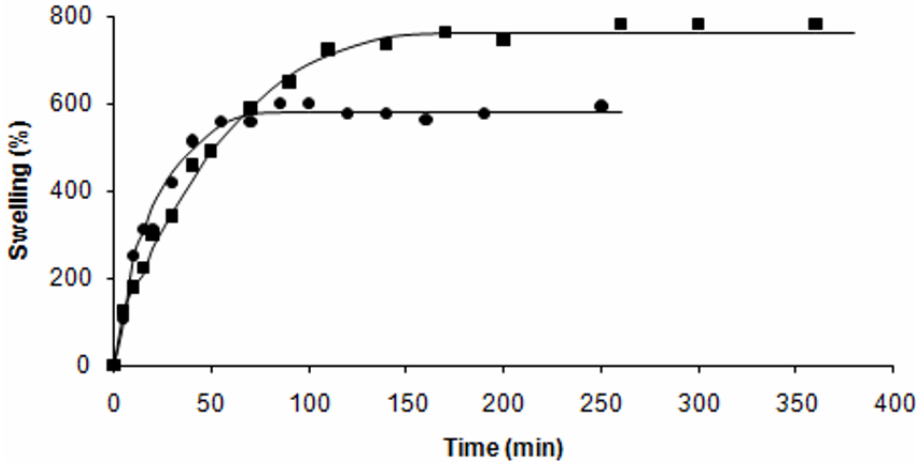


Figure 2. Limit swelling time (■) PNIPAAm hydrogel 60 kGy without BIS, (●) its IPN with low molecular weight polylysine.

However the phase transition or LCST seems not to have been affected with the introduction of PNAS in the structure (Figure 3) because the system consists of two more or less independent networks. The thermosensitivity (relation between the maximum and minimum swelling, before and after LCST) is more pronounced in the interpenetrating network, but this thermosensitivity decrease with an increase in radiation dose due to higher crosslinking density.

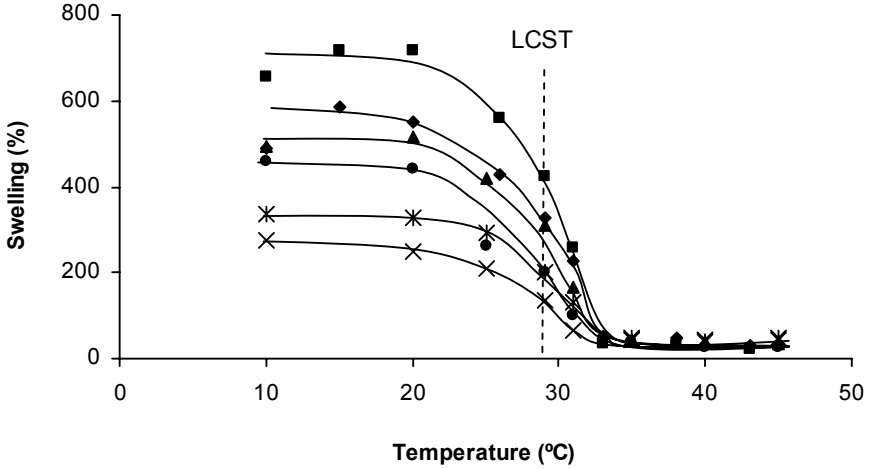


Figure 3. LCST of different IPNs: (♦) IPN 50kGy; (■) IPN 50kGy + BIS; (▲) IPN 60kGy; (●) IPN 60kGy + BIS; (∗) IPN 70kGy; (×) IPN 70 kGy + BIS. PNAS crosslinked with low molecular weight polylysine (500-2000).

The LCST of different IPN samples was confirmed by DSC (Figure 4 and Table 1). It is concluded that the LCST is not modified significantly in the IPNs, and this effect is important for potential applications of biomedical devices or in reversible systems.

Table 1. Glass transition and lower critical solution temperature of different IPNs

| ***PNIPAAm Dose kGy*** | ***Polylysine Molecular weight*** | ***LCST (°C)*** | ***Tg1 (°C)*** | ***Tg2 (°C)*** |
|---|---|---|---|---|
| 50 | 500-2000 | 29.0 | 52.6 | 120.4 |
| 50 | 4000 | 29.0 | 50.5 | 149.4 |
| 50 + BIS | 500-2000 | 30.5 | 54.3 | 136.8 |
| 50 + BIS | 4000 | 30.5 | 52.4 | 137.4 |
| 60 | 500-2000 | 29.0 | 53.7 | 139.5 |
| 60 | 4000 | 30.0 | 48.0 | 130.1 |
| 60+ BIS | 500-2000 | 30.0 | 56.4 | 143.1 |
| 60 + BIS | 4000 | 31.0 | 50.0 | 134.2 |
| 70 | 500-2000 | 30.7 | 55.3 | 127.3 |
| 70 | 4000 | 29.4 | 51.4 | 132.2 |
| 70 + BIS | 500-2000 | 28.9 | 55.3 | 149.8 |
| 70 + BIS | 4000 | 29.0 | 50.3 | 125.9 |

The DSC curves of the homopolymers and the IPN are shown in Figure 5; the Tg values for PNAS and PNIPAAm were observed at 59 and 140°C, respectively; while the IPN showed two Tg values at 61 and 160°C. We can see a shift in the Tg corresponding to the PNIPAAm because of the interaction among polymer chains, a higher temperature should be necessary to promote the chain mobility since the polymer-polymer interactions could act as promoters of physical crosslinks; but the presence of two Tg values indicates that the system contains two independent networks with some interactions between them. Similar behavior is observed in all IPN´s synthesized (Table 1).

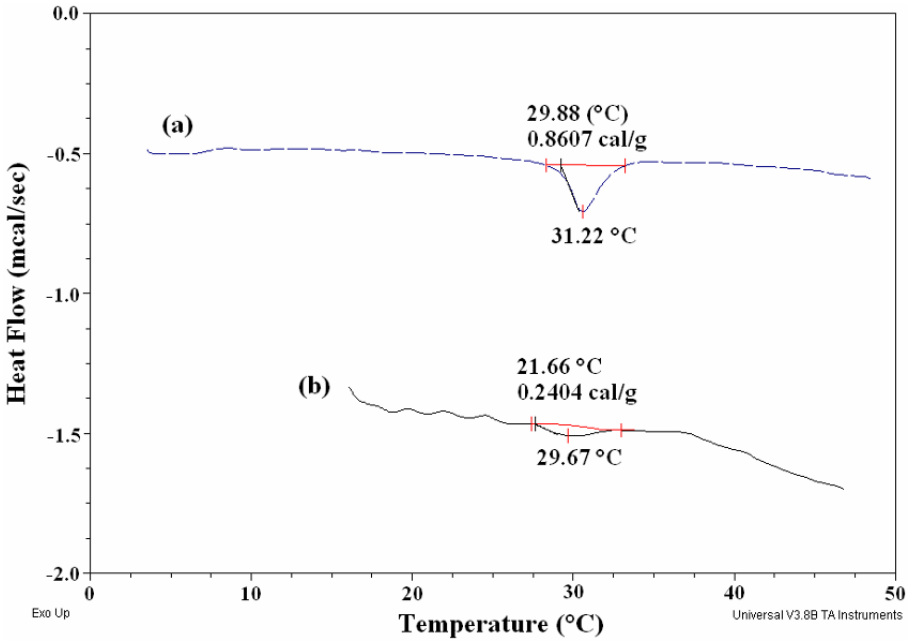


Figure 4. LCST determinated by DSC: (a) IPN 60 kGy, low molecular weight polylysine; (b) PNIPAAm hydrogel 60 kGy, without BIS.

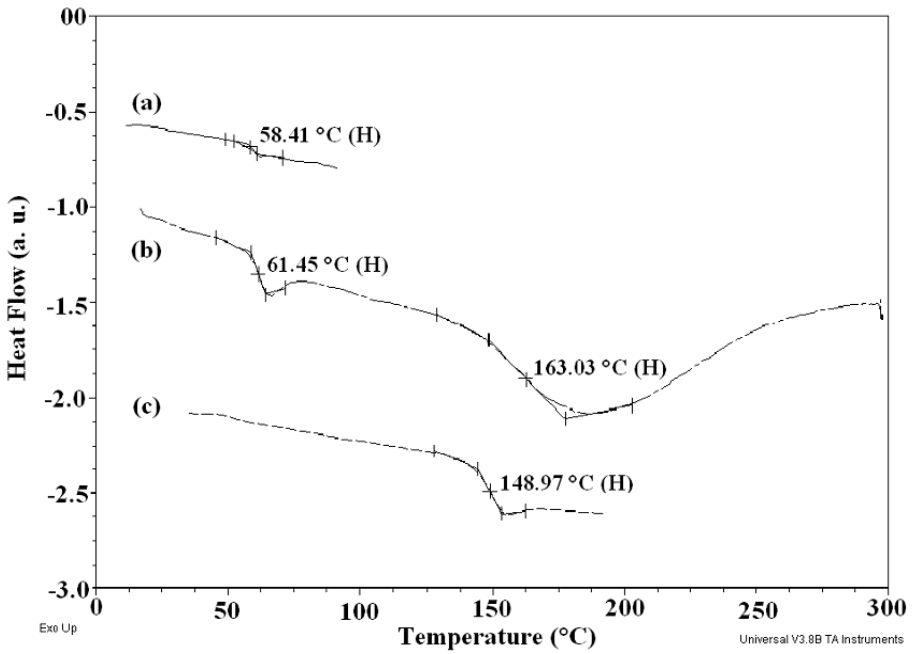


Figure 5. Glass transition: (a) PNAS 10 kGy, (b) IPN 60 kGy low molecular weight polylysine, (c) PNIPAAm 60 kGy.

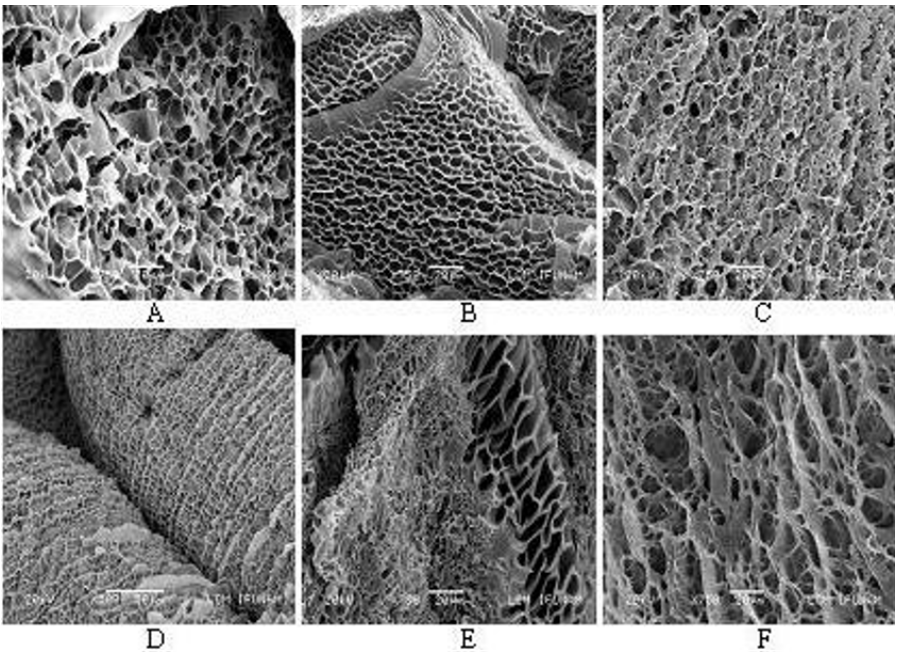


Figure 6. SEM micrographs of the swollen PNIPAAm hydrogels without BIS: (A) 50 kGy, (B) 60 kGy, (C) 70 kGy; with BIS (D) 50 kGy, (E) 60 kGy, (F) 70 kGy. X 750 magnification, 20 kV, 20μm.

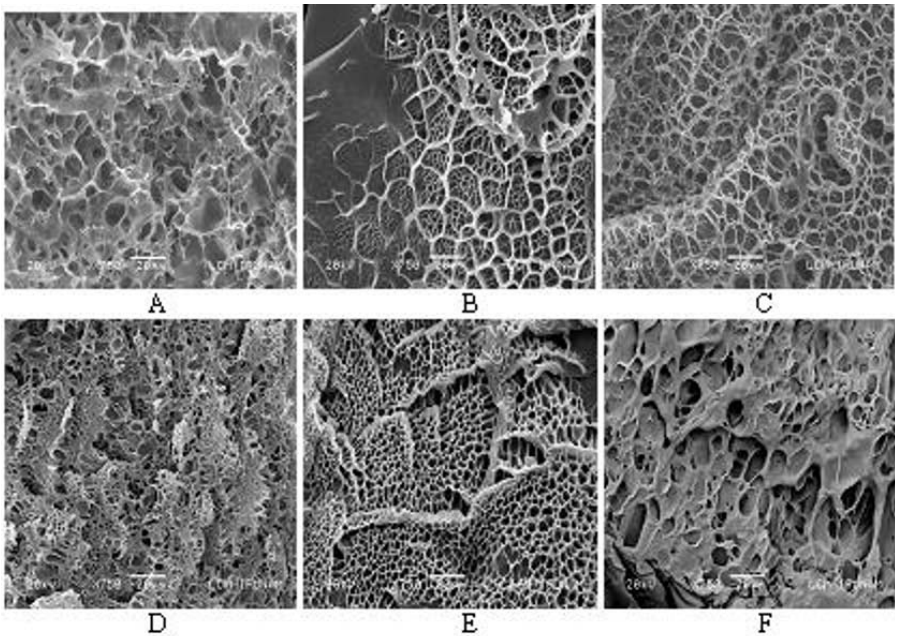


Figure 7. SEM images of the IPN PNIPAAm/PNAS. Without BIS (A) IPN 50kGy, (B) IPN 60kGy, (C) IPN70kGy; with BIS (D) IPN 50kGy, (E) IPN60kGy, (F) IPN70kGy. Low molecular weight polylysine.

The morphology of the PNIPAAm hydrogels under swollen condition is shown in Figure 6. The micrographs display cross-sectional SEM images and show that the voids of the cells of PNIPAAm hydrogels decrease with an increase in radiation dose. This result is consistent with higher crosslinking density and lower swelling percentages (Figures 6A, 6B, 6C). The decrease is greater when NIPAAm hydrogels were synthesized in the presence of the crosslinking agent BIS (Figure 6D, 6E, 6F) because of the increase in the crosslinking density; however these networks are less homogeneous than those crosslinked through radiation without the addition of BIS. When the IPNs are synthesized, the PNAS filled the voids of PNIPAAm hydrogels with smaller networks, and it is possible to see the second network (PNAS) inside the first one of (PNIPAAm), (figure 7).

## Conclusions

A new interpenetrating network of PNIPAAm and PNAS has been prepared. The temperature phase transition LCST is conserved in this IPN, and it is possible to join it with biomolecules due to IPN arrangement, the thermosensitivity and velocity of response were increased. The characterization by FTIR and DSC confirmed the IPNs formation. Additionally, diverse factors that can affect the formation of the IPN were explored and the optimum conditions for synthesis of the IPN were found to involve crosslinking without BIS and a radiation dose of 60 kGy. A higher radiation dose increases the crosslinking density of PNIPAAm and decrease PNAS and polylysine diffusion in the first network. The molecular weight of polylysine was an important factor to be considered.

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