Biomaterials and Biomechanics in Bioengineering, *Vol. 1, No. 2 (2014) 105-116*
(Formerly, Biomaterials and Biomedical Engineering)
DOI:http://dx.doi.org/10.12989/bme.2014.1.2.105

Preparation and characterization of a thermal responsive of poly(*N*-isopropylacrylamide)/chitosan/gelatin hydrogels

Shaghayegh Baghaei[1] and Mohammad T. Khorasani[*2]

[1]*Department of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Biomaterial Department of Iran Polymer and Petrochemical Institute, P.O. BOX 14965/115, Tehran, Iran*

(Received May 04, 2014, Revised June 30, 2014, Accepted June 30, 2014)

Abstract. Synthesis of interpenetrating polymer network (IPN) of chitosan-gelatin (Cs-Ge) (as a primary network) and *N*-isopropylacrylamide (NIPAAm) monomer (as the secondary network) was carried out with different ratio. Its structure was characterized by FT-IR, which indicated that the IPN was formed. The memberanes were studied by swelling, weight loss with time. The interior morphology of the IPN hydrogels was revealed by scanning electron microscopy (SEM); the IPN hydrogels showed a interpenetrated network of NIPAAm/chitosan has layers with more minute stoma and canals compared to interpenetrated network of NIPAAm/gelatin. Lower critical solution temperature (LCST), equilibrium swelling ratio (ESR) and de-swelling kinetics were measured. The DSC results noticed that LCST of IPN hydrogels with different ratio of Cs/Ge/PNIPAAm are around 33±2℃. The ESR obtained results showed that with a ratio of Cs/Ge/NIPAAm: 1/1/6, the swelling ratio increased drastically from room temperature to 36℃ but with a ratio of Cs/Ge/PNIPAAm: 1/3/6, decrease significantly at the same condition. Therefore the hydrogels have been changed from a hydrophilic structure to a hydrophobic structure. Furthermore with an increase in temperature from room to the LCST, the ESR of IPN with higher concentration of (PNIPAAm) and (Ge) decreases but de-swelling kinetics of them are faster. Due to the suitable and different kinetics of de-swelling and the equilibrium swelling ratio (ESR) in various proportions, and because of the morphology inside the mass which confirms other tests, these hydrogels are very appropriate as a smart thermosensitive hydrogels with rapid response.

Keywords: gelatin; chitosan; *N*-isopropylacrylamide; thermosensitive hydrogel

1. Introduction

Hydrogels are generally hydrophilic polymer networks which absorb sufficient water without being dissolved in water. Hydrogels, particularly smart hydrogels, are of particular interest to various industries due to their high capacity in responding and creating changes in shape and volume resulting from different external and environmental simulations (Mishra *et al.* 2011). Among polymers sensitive to the environment, those sensitive to temperature have been used extensively in various applications in biomedical fields such as drug delivery, optics and material science (Li *et al.* 2009, Carreira *et al.* 2010). Poly *N*-isoptopylacrylamide (PNIPAAm) is related to

*Corresponding author, Professor, E-mail: M.Khorasani@ippi.ac.ir

 ISSN: 2288-3738 (Print), 2288-3746 (Online)

polymers which have a hydrophobic and hydrophilic structure and which are of great importance in this group. The thermosensitive nature of these hydrogels has been studied because this factor can be easily controlled and is applicable in vitro and in vivo conditions. Based upon its conformational changes, various PNIPAAm hydrogels have been studied as controlled optical switches, limiters and modulators (Yu *et al.* 2012, Rejinold *et al.* 2011). PNIPAAm hydrogels are well-known for their discontinuous phase separation near their lower critical solution temperature (LCST) and exhibit a sudden shrinking in volume at a temperature above LCST (Jaiswal *et al.* 2010). Chitosan (CS) was chosen as a matrix gel forming material and CS-NIPAAm semi and full IPN hydrogels were prepared in previous works. Dusek 1993 demonstrated that adding Cs gel yielded a new smart hydrogel. They concluded that chemical combination of PNIPAAm in the Cs network has a great effect upon the properties of the gel. Chen *et al.* 2007 prepared a novel type of IPN hydrogel of NIPAAm-carbonymethyl chitosan (CMCS). Their results showed that the introduction of CMCS did not shift the LCST, and the LCST of IPN hydrolgel is similar to the pure PNIPAAM. They also showed that a combination of PH and temperature can be coupled to control the responsive behavior of PNIPAAM/CMCS hydrogels. PH and temperature responsive carboxymethyl chitosan/NIPAAM semi-IPN hydrogels were prepared for oral delivery of drugs by Mishra *et al.* 2011. Their results showed that increasing the carboxymethyl chitosan content can increase the release rate of co-enzyme A (CoA). This paper describes the preparation and characterization of a novel IPN hydrogel containing network of chitosan- gelatin and PNIPAAm. The synthesis of this intelligent hydrogel was carried out on the basis of chitosan-gelatin (as the primary network) and NIPAAm monomer (as the secondary network). The aim of the present study was to develop a smart thermosensitive hydrogel, with quick response, and LCST close to human body temperature so that it can be used in medical applications and medical instruments. These hydrogels can be useful in controlling the delivery of biomolecules with high molecular weight, genes, cell culture, lodging enzymes and similar cases (Bao *et al.* 2010, Liu 2012).

2. Experimental

2.1 Materials

N-isopropylacrylamide (NIPAAm) and Medium molecular weight chitosan were obtained from Aldrich Chemical (St. Louis, USA). Chitosan had a deacetylation percentage higher than 90%, and gelatin prepared from Fluka; and they were all used without further purification. *N*, *N*-methylenebisacrylamide (MBAAm), ammonium persulfate (APS) and *N*, *N*, *N*, *N*-tetramethylethylenediamine (TEMED) were purchased from Sigma Chemical (St. Louis, Missouri) and were used as supplied. Glutaraldehyde (25% solution) and acetic acid were obtained from Junsei Chemical Co., Ltd. and J.T. Baker Inc., respectively.

2.2 Preparation of the chitosan/gelatin hydrogel

Four % wt chitosan and gelatin solutions with various concentrations (Table 1) were prepared by dissolution in 1.5% wt acetic acid and were stirred for 24 h at room temperature then, degassed in vacuum for 2 h to form a homogenous solution. Next, 0.25 wt% glutaraldehyde solution was incorporated into the mixture drop by drop to cross-link the chitosan and gelatin and solutions were dried at room temperature. The prepared films were washed with distilled water and dried in

an oven at 37℃ for 24 h.

2.3 Synthesis of the IPN–NIPAAm/Cs/Ge hydrogel

Predetermined amounts of NIPAAm monomer and cross-linker MBAAm were dissolved in 8 cc of distilled water and were left at room temperature for 24 h (Table 1). The prepared films (section 2.2) were immersed into this monomer solution until almost all of the monomer solution was absorbed into the hydrogel network in about 72 h. The solution was thoroughly degassed via vacuum extraction. After degassing, APS and TEMED, as initiators, were added slowly to the polymer solution while being stirred. The reaction was carried out for 72 h at room temperature in a sealed glass vessel. Each hydrogel was kept in a freezer at -20℃ for 12 h. Then, the frozen hydrogels, placed in the Petri dish, was freeze dried (freeze-dryer FDU-830, Eyela Co.) for 72 h in order to dry the samples completely.

2.4 LCST measurement of the IPN–PNIPAAm/Cs/Ge hydrogels

The thermal behavior or LCST behavior of hydrogel was determined using a differential scanning calorimeter (DSC) (Mettler Toledo 822). All hydrogels were immersed in distilled water at room temperature and allowed to swell to equilibrium before the DSC measurement. About 10 mg of swollen sample was placed in a hermetic aluminum pan, and then sealed tightly by a hermetic aluminum lid. The thermal analyses were performed from 50℃ to 100℃ on the swollen hydrogel samples at a heating rate of 10℃/min under dry nitrogen. The onset point of the endothermal peak, determined by the intersecting point of two tangent lines from the baseline and slope of the endothermic peak, was used to determine LCST (Zhang and Chu 2003).

2.5 Measurement of equilibrium swelling ratio of hydrogels

The equilibrium swelling ratio (ESR) of hydrogels were measured by swollen in double distilled water over a temperature range of 22℃ to 52℃. The hydrogels were incubated in the medium for at least 24 h under each condition and then blotted with dry filter paper to remove excess water on the hydrogel surface, and were weighted. The average values among four measurements were taken for each sample and the equilibrium swelling rate was calculated by the following Eq. (1)

$$\mathrm{ESR} = \frac{W_s - W_d}{W_d} \times 100 \tag{1}$$

where, W_s is the weight of the swollen hydrogel at a particular condition and W_d is the weight of dry hydrogel.

2.6 Measurement of the de-swelling kinetics of hydrogels

To measure the de-swelling kinetics of the hydrogels, dried hydrogels were weighed, and then swollen hydrogels were taken in double distilled water at 22℃ to reach equilibrium. Then, the hydrogel samples were separately and quickly transferred into hot water at 52℃ for 5, 15, 35, 74, 125, 185, and 255 min. Wight of samples were measured after wiping off the water on the surfaces with dry filter paper. The temperature was selected to be above the LCST of the hydrogels. The

weight changes of the hydrogels were recorded at regular time intervals during the course of de-swelling. Water retention (WR) was calculated by the following Eq. (2)

$$Water\ \ retention = \frac{W_t - W_d}{W_s} \times 100 \tag{2}$$

where, W_t is the weight of the hydrogels at a given time interval during the course of de-swelling after the swollen hydrogels at 22℃ had been quickly transferred into hot water at 52℃.

2.7 Characterization

FTIR measurements were carried out using dried hydrogels with various compositions. FTIR spectra of freeze-dried samples were recorded on Nicolet Spectra 5700 spectrometer (Nicolet Instrument, Thermo Company, MD, USA) in a KBr flake (Jaiswal *et al.* 2010). The freeze-dried hydrogel samples were frozen in liquid nitrogen. The freeze-dried hydrogel was then fractured carefully and then fixed on stubs with sputter coated with gold before observation. Morphology of fractured surface of samples was observed by scanning electron microscope (SEM) (Hitachi S4500 SEM, Mountain View, CA).

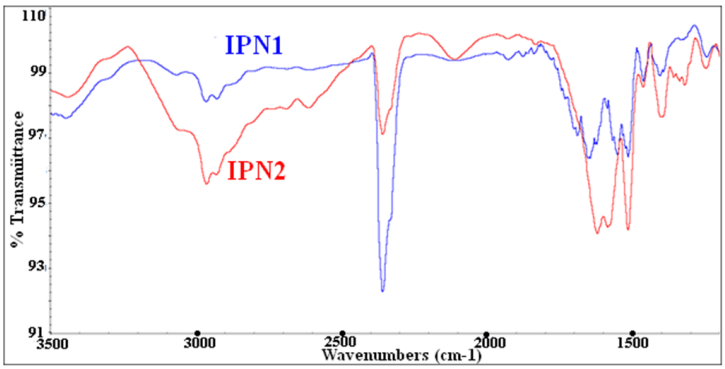
Table 1 Composition of interpenetrated network of chitosan/gelatin/*N*-isopropylacrylamide with different ratio

Sample	IPN 1	IPN 2	IPN 3	IPN 4	IPN 5	IPN 6
Chitosan (mg)	250	250	125	125	500	-
Gelatin (mg)	250	250	375	375	-	500
NIPAAM (mg)	250	1500	250	1500	1000	1000
MBAAM (mg)	5	30	5	30	20	20
H_2O (ml)	8	8	8	8	8	8
APS (mg)	0.1	0.1	0.1	0.1	0.1	0.1
TEMED (ml)	0.25	0.25	0.25	0.25	0.25	0.25

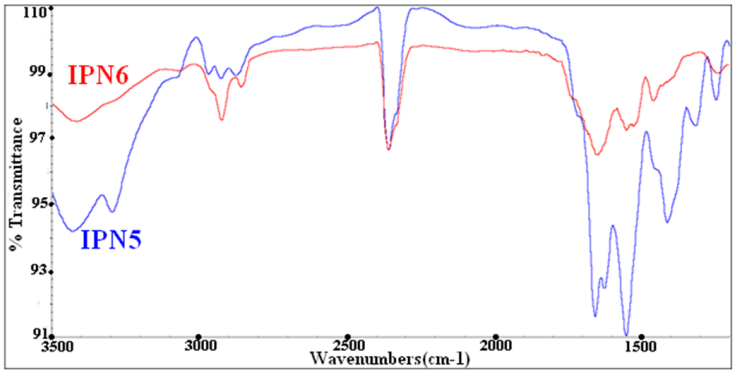
3. Result and discussion

3.1 FTIR spectroscopy

The chemical composition of IPN hydrogels were confirmed by FTIR measurements (Fig. 1(a)). The strong evidence to confirm that NIPAAm, chitosan and Gelatin were successfully incorporated into all hydrogels was that the specified bands of NIPAAm around 1547-1650 increased with an increase in feed ratio of NIPAAm into the chitosan-gelatin hydrogel (IPN2) has bigger absorption band than IPN1). According to chitosan structure and depending on the di-acetylation degree, the peak intensity corresponding to carbonyl-amid group is different. On the other hand, the peak at 1635 can be attributed to carbonyl-amid group in chitosan and gelatin. In addition, the NH-group exists in the polymer mixture both at tension and bending cases, which give this group a wide peak at 3432 and 1558 (IPN5), respectively. The NH- peak in tension state



(a)



(b)

Fig. 1 FTIR spectra of interpenetrated network of chitosan/gelatin/poly(*N*-isopropylacrylamide) hydrogels according to Table 1

overlaps with the OH- peak in this area revealing one peak. As shown in Fig. 1(b), the absorption bands at 1650 and 1547 were assigned to amid I and amid II bands, respectively and a methyl peak is observed at 2960. This peak reveals the entering of chitosan in interpenetrated network polymer of NIPAAm, chitosan and gelatin (Jinghong *et al.* 2007).

3.2 SEM study

SEM photomicrographs of cross section of samples according to table 1 are shown in Fig. 2. In this stage of microstructure, the shapes of pores, the geometry of the pores, the solidarity of pores, and the way pores are dispersed in cross-linked have been shown. With regard to the used magnification which is 500X, it is noticed that the samples have a structure full of conjunct pores. Generally, the process of freeze-drying can create a structure in the hydrogels that cause an increase in the swelling rate and the de-swelling kinetics of the hydrogel. In the images obtained from SEM test related to the hydrogel cross section, an uneven and porous structure is observed. This causes that the water to flow out more easily. As shown in Fig. 2, the interpenetrated network of NIPAAm/chitosan (IPN5) has layers with more minute stoma and canals compared to interpenetrated network of NIPAAm/gelatin (IPN6). Therefore, the interpenetrated network of

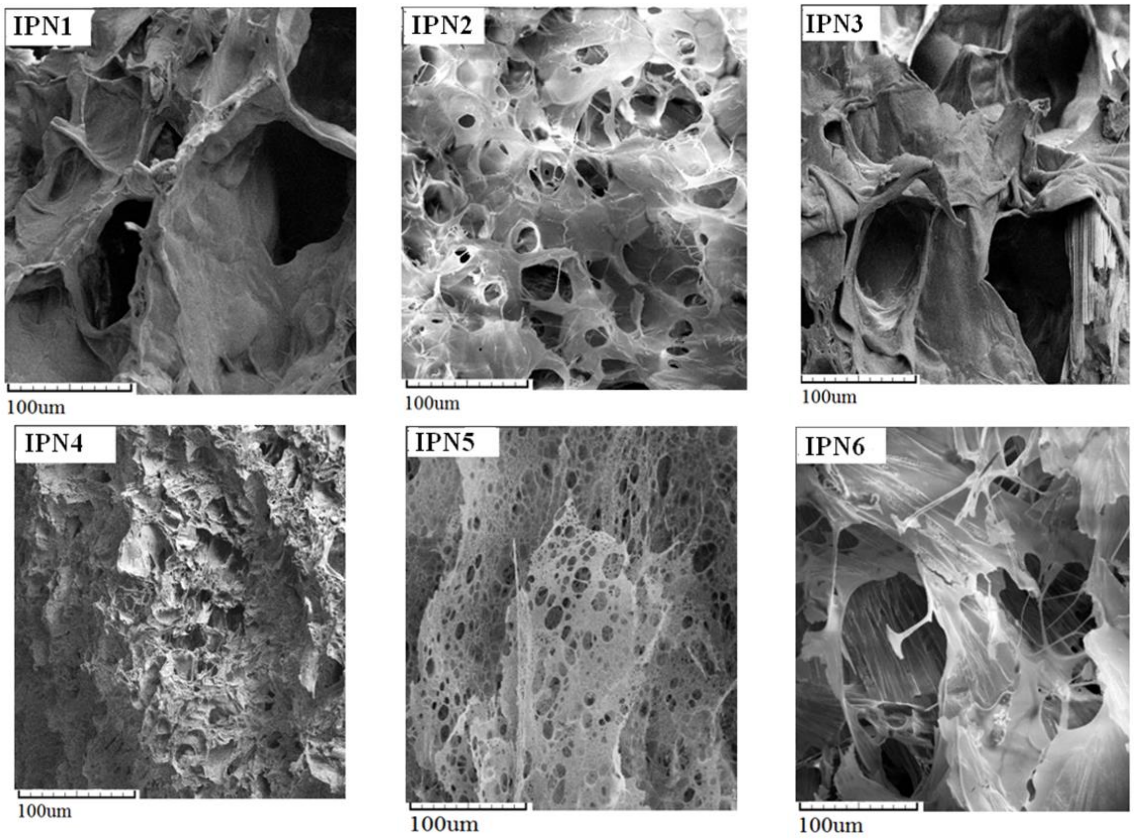


Fig. 2 SEM photomicrographs of freeze-dried cross section of chitosan/gelatin/poly(*N*-isopropylacrylamide) IPN hydrogels with different ratio according to tables 1 composition (magnification is 500X)

NIPAAm/chitosan is expected to have higher mechanical properties and show more power to absorb water. SEM photomicrographs of IPN1 to IPN2 and IPN3 to IPN4 are shown also in Fig. 2. It is observed that the IPN samples have a layered structure. These layers are seen with relatively similar intervals in some regions and with not so similar intervals in some other regions. The stoma and canals in these samples are connected to each other due to the existence of filaments; and with an increase in the NIPAAM monomer in the polymer, the size of pores has decreased. As a result of the effects of intermolecular interactions between hydrogel network parts, the whole matrix is pressurized causing a decrease in pore sizes.

3.3 Interpenetrated networks of chitosan/gelatin/NIPAAm

Low critical solution temperature (LCST) of PNIPAAm which is related to the fracture of hydrogen bonds between polymer and water causes a phase transition, and the DSC test measures the temperature resulting from the fracture in hydrogen bonds between water and polymer (Greever *et al.* 2006). At temperatures lower than LCST, there is a strong hydrogen bond between hydrophilic groups of polymer (amide groups) and water molecules which results in the solution of

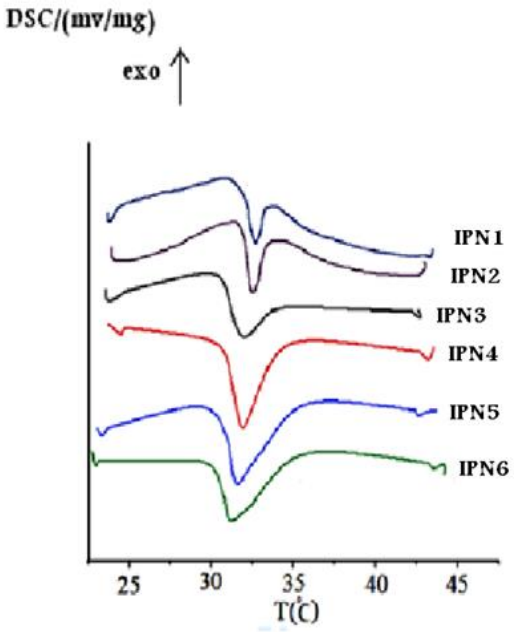


Fig. 3 DSC Thermograms of chitosan/gelatin/poly(*N*-isopropylacrylamide) hydrogels with different ratios according to table 1

polymer in the water. With an increase in temperature, the hydrogen bonds weaken and hydrophobic interactions are established between lateral groups. This shrinking-swelling of hydrogel as a result of temperature changes is revealed as phase transition in DSC spectrum. This phase transition of hydrogel is due to an increase in the system entropy as a result of separation between polymer chains and water molecules (Save *et al.* 2005). DSC spectra related to IPN1 toIPN6 have been shown in Fig. 3. On the whole, the LCST is the point of temperature onset of phase transition peak in DSC. Hence, on the basis of graphs obtained from DSC, it can be said that the amount of LCST of all hydrogels is around 33℃±3. Based on this result, it can be said that in the IPN system, NIPAAm network has retained its property. Therefore, since there is no chemical bond between chitosan-gelatin and PNIPAAm network and the temperature of the phase transition of hydrogel is independent of the type and content of the cross-linker, The LCST of all networked hydrogels has remained constant. The existence of LCST of PNIPAAm provides more witnesses for the formation of a thermosensitive interpenetrated network.

3.4 Swelling ratio of chitosan/ gelatin/NIPAAM hydrogels

To determine the temperature dependence of the swelling/collapse process, the equilibrium swelling ratio of hydrogels was studied. This means that the temperature dependence of swelling ratio is one of the most important parameters to evaluate the thermosensitive properties of hydrogels. As shown in Fig. 4, the swelling temperature data show that the IPN3 to IPN4 and IPN6 have similar swelling curves. In these curves the hydrogels swelling ratio decrease dramatically as the temperature increases to LCST that is about 33℃. This trend continues to approximately 36℃

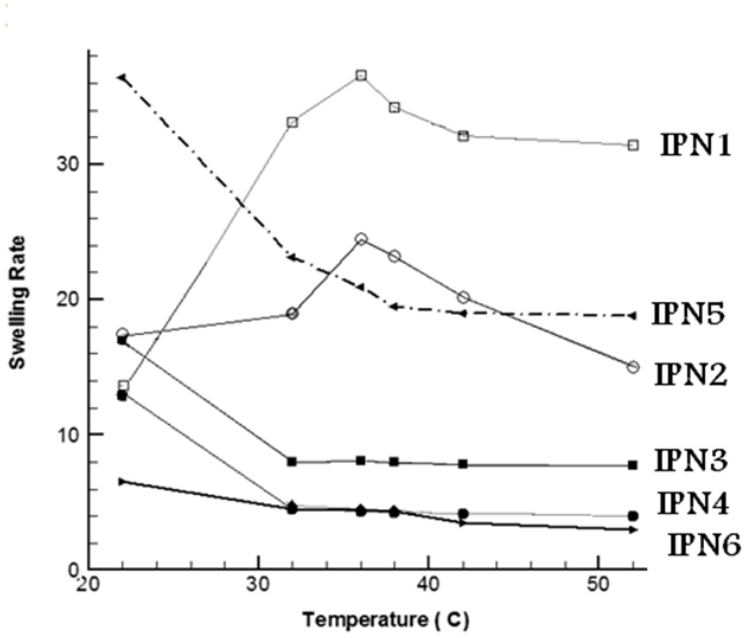


Fig. 4 ESRs of chitosan/gelatin/poly(*N*-isopropylacrylamide) IPN hydrogels with different ratio according to tables 1 composition in the temperature range from 20℃ to 52℃

and at temperatures above LCST the curve becomes horizontal and the slopes become zero. This indicates that the increase in temperature above the LCST will not affect the swelling ratio of hydrogels and also the hydrogels are turned from hydrophilic to hydrophobic structure. The phase transition temperatures or LCST of these hydrogels is in agreement with the thermal data from DSC study. Even though the LCSTs of the hydrogels were not affected by the IPN structure, the data in Fig. 4 shows that at temperatures below the phase transition temperature (e.g., room temperature 22℃), the equilibrium swelling rate of these hydrogels is reduced with an increase in NIPAAM monomer. It is also noticed that IPN6 shows a lower equilibrium swelling rate, which is depends on the hydrogel structure.

Based on the electron microscopic image, it is observed that the chitosan/NIPAAm (IPN5) structure has the stomata and canals that are to a large extent interconnected compared to gelatin-NIPAAm hydrogel (IPN6) and their sizes are also much smaller compared to gelatin-NIPAAm hydrogel. Therefore, from the comparison between IPN5 and IPN6, it can be concluded that some of the above differences are due to the presence of hydrophilic network in the networked sample of chitosan/NIPAAm so that the hydrophilic monomers of chitosan in the system act as leading canals for the faster and easier entry and exit of water molecules from the system in swelling/collapse conditions. Furthermore the amount of gelatin in IPN3 to IPN4 is more than that of chitosan, the curve is between chitosan/NIPAAm and gelatin/NIPAAm and closer to gelatin/NIPAAm. The decrease in ESR of IPN3 to IPN4 depends on the following reasons:

Firstly, the increase in matrix density of polymer in IPN's causes the accessible space for the penetration of water in the polymer network to decrease. Secondly, intermolecular interactions between two parts of network in IPN3 to IPN4 put the whole matrix under pressure causing a

decrease in the swelling proportion. As confirmed in the images obtained from SEM (Fig. 2), with an increase in polymer density, the structure becomes smaller. As a result, the canals can hold less water. This is a reason for a decrease in the ESR. The ESR of IPN1 to IPN2 is higher than the ESR of IPN3 to IPN4; but unexpectedly, these hydrogels show an opposite behavior in the range of 22℃ to 36℃, and the curve takes an ascending trend. From 22℃ to 32℃ in IPN1 to IPN2, the curve has a relatively sharp and positive slope and this trend continues to 36℃. As seen in SEM photomicrographs (Fig. 2), the morphology of cross section of hydrogels at a proportion of 75% gelatin/25% chitosan (IPN3 to IPN4) is different from cross section of hydrogels at a proportion of 50% gelatin/50% chitosan (IPN1 to IPN4). At the proportion of 75% gelatin/25% chitosan, we witness two matrix and dispersed phases while at the proportion of 50% gelatin-50% chitosan we witness a homogeneous morphology with regular large pores and it is not clear the effect of which phase is dominant. In this proportion, the collapse process has occurred at a higher temperature (about 36℃) while the LCST of IPN2 shows a temperature of about 32℃. This event is related to dynamics of chains. As a matter of fact, NIPAAm monomers have been struck within the network at a proportion of 50% gelatin-50% chitosan and cannot play their roles. With an increase in temperature, the mobility of chains increases and chitosan plays its role; and as seen in Fig. 4, with an increase in temperature, the hydrogel with a structure of 50% gelatin-50% chitosan and 0.25 gr of NIPAAm (IPN1) absorbs approximately the same amount of water as chitosan-NIPAAm (IPN5). When the hydrogel temperature reaches about 36℃, it is NIPAAm monomers that find the opportunity to play their roles. The more concentration of NIPAAm causes the more of density of the polymer and the smaller of porous structures. As a result, with the structures becoming smaller porosity, the canals can hold less water and this is a proof for a decrease in ESR. In fact, the less amount of NIPAAm in IPN causes the maximum water absorption capacity of chitosan but sensitivity to the hydrogel temperature decreases. In IPN3 to IPN4, actually gelatin does not allow chitosan to reach its maximum water absorption capacity. Therefore at IPN1 to IPN2, a different behavior is noticed.

3.5 De-swelling kinetics of Cs-Ge-PNIPAAm hydrogels

Fig. 5 illustrates the de-swelling kinetics of IPN-hydrogels after an equilibrated swollen specimen is transferred at 22℃ (below its LCST) to hot distilled water at 52℃ (above its LCST). It can be clearly seen that the shrinking rate of hydrogel with IPN2 and IPN4 was much higher than that of other IPNs, while IPN5 and IPN6 shrank at a slower rate than other IPNs. For instance, after 35 min in hot water, the IPN2 and IPN4 shrank and losing over 65% and 66% water, respectively. In contrast, almost 45% and 48% water was freed from IPN5 and IPN6 during the same time, respectively. However, in IPN1, and IPN3, only 53%, 51%, water was released during the same time, respectively. It is known that during the shrinkage of hydrogels at a temperature higher than LCST, the outermost surface of the hydrogel would be affected first. This hydrogel outermost surface layer, which has been collapsed, is denser than the interior matrix in the early stages of hydrogel shrinking process. Due to the hydrophobic interaction between polymer chains, this thick and dense layer restricts the outward permeation of trapped water from the hydrogel interior, greatly slowing down the outflow rate of water (Kaneko *et al.* 1995). At this stage of shrinking process, internal pressure starts to build up in the hydrogel. With an increase in the pressure, the hydrogel overcomes the barrier from the outermost thick and dense skin layer, and bubbles containing water appear on the surface. As time passes, these bubbles swell, and finally allow water in the hydrogel to permeate through the bubbles (convection). Internal pressure is thus

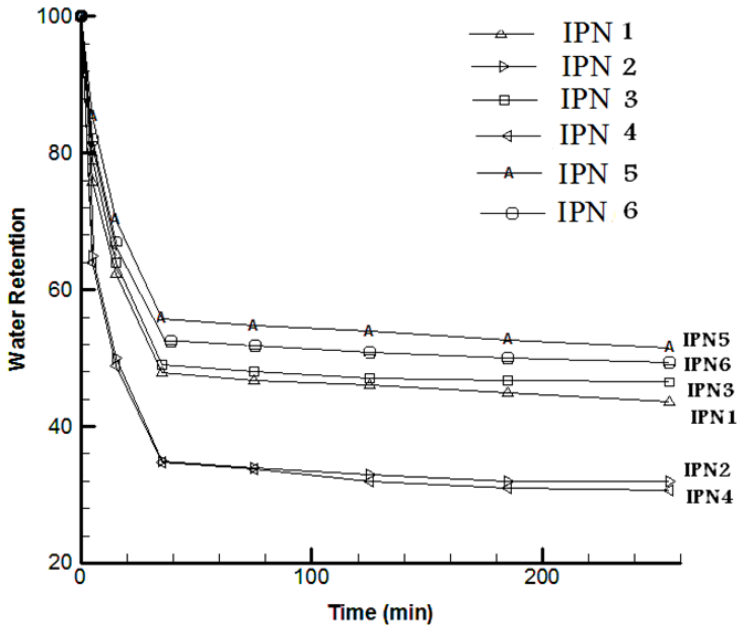


Fig. 5 De-swelling kinetics of chitosan/gelatin/poly(*N*-isopropylacrylamide) hydrogels with different ratios according to tables 1 at 52℃

decreased, the bubbles become smaller, and ultimately the hydrogel reaches a stable shrunk state. The porous network which has a higher distribution of secondary network (NIPPm) similar to hydrogels with a ratio of 75% gelatin/25% chitosan and 1 and 1.5 gr of NIPAAm (IPN4) may prevent the formation of the dense surface layer in the process of de-swelling; consequently leading to an increase in water penetration with the highest de-swelling kinetics forming no bubbles during the de-swelling process. From the de-swelling kinetic data, it was understood that the response rate of hydrogels can also be controlled through IPN formation and the composition ratio of the two network structures within hydrogel. This controllable response rate in hydrogels is very useful as far as their various applications in the future are concerned. For instance, the much faster response rates of IPN4 are useful in cases where fast response rates are required such as in artificial organs and in on-off switches (Osada *et al.* 1992). As a matter of fact, many studies reported recently focused on the improvement of the response rate of thermosensitive hydrogels (Temtem *et al.* 2012, Chen *et al.* 2012, Zhao *et al.* 2011).

4. Conclusions

Hydrogels were synthesized with interpenetrated network structure of chitosan-gelatin–NIPAAm with different ratios with the help of MBAAM cross-linker and APS and TEMED initiators. In this way, a new hydrogel was formed with a secondary network of NIPAAm inside chitosan-gelatin hydrogel. There are strong evidences to confirm that NIPAAm, chitosan and gelatin have successfully entered all hydrogels. It was clarified that the LCST of networked

hydrogels is around 32℃ and that the IPN of NIPAAm/chitosan has more water absorption power. Regarding to the amount of swelling ratio of the IPNs of chitosan/NIPAAm and gelatin/NIPAAm and IPN3 to IPN4, the hydrogels have been changed from a hydrophilic structure to a hydrophobic structure. The de-swelling kinetics of IPN4 and IPN2 is much faster than other hydrogels whereas the IPNs of chitosan/NIPAAm and gelatin/NIPAAm have been de-swelled at a lower rate compared to IPN2 and IPN4. The data obtained from the de-swelling kinetics reveal that the response rate of hydrogels is controllable through the ratio of combination in the network structures of the hydrogel. This controllable response rate will be very useful for their various applications in the future.

Acknowledgements

The authors would like to thank of Dr A. Karkhaneh. For usefull suggestion and wish to acknowledge the Biomaterial Department of Iran polymer and Petrochemical Institute and Department of Biomedical Engineering of Islamic Azad University of Iran for making the instrumental facility in their laboratories.

References

Bao, H., Li, L., Leong, W.C. and Gan, L.H. (2010), "Thermo-responsive association of chitosan-graft-poly(*N*-isopropylacrylamide) in aqueous solutions", *J. Phys. Chem.* B, **114**(32), 10666-10673.

Carreira, A.S., Gonçalves, F.A.M.M., Mendonça, P.V., Gil, M.H. and Coelho, J.F.J. (2010), "Temperature and pH responsive polymers based on chitosan: applications and new graft copolymerization strategies based on living radical polymerization", *Carbohyd. Polym.*, **80**(3), 618-630.

Chen, J., Sun, J., Yang, L., Zhang, Q., Zhu, H., Wu, H., Hoffman, A.S. and Kaetsu, I. (2007), "Preparation and characterization of a novel IPN hydrogel memberane of poly(*N*-isopropylacrylamide)/carboxymethyl chitosan (PNIPAAM/CMCS)", *Radiat. Phys. Chem.*, **76**(8-9), 1425-1429.

Chen, J., Kuo, C. and Lee, W. (2012), "Thermo-responsive wound dressings by grafting chitosan and poly(*N*-isopropylacrylamide) to plasma-induced graft polymerization modified non-woven fabrics", *Appl. Surf. Sci.*, **262**, 95-101.

Dusek, K. (1993), Advances in Polymer Science, Responsive Gels, Volume Transitions II, Springer, Berlin.

Greever, L.M., Devine, D.M., Nugent, J.D., Kennedy, J.E., Lyons, J., A. Hanley. and Higginbotham, C.L. (2006), "Lower critical solution temperature control and swelling behavior of physically crosslinked thermosensitive copolymers based on *N*-isopropylacrylamide", *Eur. Polym. J.*, **42**(10), 2540-2548.

Jaiswal, M.K., Banerjee, R., Pradhan, P. and Bahadur, D. (2010), "Thermal behavior of magnetically modalized poly(N-isopropylacrylamide)-chitosan based nanohydrogel", *Colloids Surf. B: Biointerfaces*, **81**(1), 185-194.

Jinghong, M., Xu, Y., Zhang, Q., Zha, L. and Liang, B. (2007), "Preparation and characterization of pH- and temperature responsive semi-IPN hydrogels of carboxymethyl chitosan with poly(*N*-isopropyl acrylamide) crosslinked by clay", *Colloid Polym. Sci.*, **285**(4), 479-484.

Kaneko, Y., Sakai, K., Kikuchi, A., Yoshida, R., Sakurai, Y. and Okano, T. (1995), "Influence of freely mobile grafted chains length on dynamic properties of comb-type grafted poly(*N*-isopropylacrylamide) hydrogels", *Macromolecules*, **28**(23),7717-7723.

Li, F., Wu, H., Zhang, H., Li, F., Gu, C.H. and Yang, Q. (2009), "Antitumor drug Paclitaxel-loaded pH-sensitive nanoparticles targeting tumor extracellular pH", *Carbohyd. Polym.*, **77**(4), 773-778.

Liu, M., Su, H. and Tan, T. (2012) "Synthesis and properties of thermo- and pH-sensitive poly(*N*-

isopropylacrylamide)/polyaspartic acid IPN hydrogels", *Carbohyd. Polym.*, **87**(4), 2425-2431.
Mishra, D., Bhunia, B., Banerjee, I., Datta, P., Dhara, S. and Maiti, T.K. (2011), "Enzymatically crosslinked carboxymethyl–chitosan/gelatin/nano-hydroxyapatite injectable gels for in situ bone tissue engineering application", *Mater. Sci. Eng., C*, **31**(7), 1295-1304.
Osada, Y., Okuzaki, H. and Hori, H. (1992), "A polymer gel with electrically driven motility", *Nature*, **355**, 242-244.
Rejinold, N.S., Sreerekha, P.RR., Chennazhi, K.P., Nair, S.V. and Jayakumar, R. (2011), "Biocompatible, biodegradable and thermo-sensitive chitosan-g-poly(N-isopropylacrylamide) nanocarrier for curcumin drug delivery", *Int. J. Biol. Macromol.*, **49**(2), 161-172.
Save, N.S., jassal, M. and Agrawal, A.K. (2005), "Stimuli sensitive copolymer poly(*N*-tertbutylacrylamideran-acrylamide): synthesis and characterization", *J. Appl. Polym. Sci.*, **95**(3), 7979-7987.
Temtem, M., Barroso, T., Casimiro, T., Mano, J.F.A. and Ricardo, A.A. (2012), "Dual stimuli responsive poly(N-isopropylacrylamide) coated chitosan scaffolds for controlled release prepared from a non residue technology", *J. Supercrit. Fluids*, **66**, 398-404.
Yu, Y., Liu, Y., Kong, Y., Zhang, E., Jia, F. and Li, S. (2012), "Synthesis and characterization of temperature-sensitive poly(*N*-isopropylacryamide) hydrogel with comonomer and semi-IPN material", *Polym. Plastics Tech, Eng.*, **51**(8), 854-860.
Zhang, X.Z. and Chu, C.C. (2003), "Synthesis and characterization ofthe semi-IPN-like PNIPAAm gel with improved response rate", *J. Appl. Polym. Sci.*, **89**(3), 1935-1941.
Zhao, S., Li, L., Cao, M., Xu, W. (2011), "pH- and thermo-sensitive semi-IPN hydrogels composed of chitosan, N-isopropylacrylamide, and poly(ethylene glycol)-co-poly(ε-caprolactone) macromer for drug delivery", *Polym. Bull.*, **66**(8), 1075-1087.

CC