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International Journal of Pharmaceutics 278 (2004) 343–351
international journal of pharmaceutics
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Preparation of poly(*N*-isopropylacrylamide/itaconic acid) copolymeric hydrogels and their drug release behavior

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Received 26 April 2003; received in revised form 8 March 2004; accepted 18 March 2004

Available online 28 May 2004

Abstract

N-isopropylacrylamide/itaconic acid copolymeric hydrogels were prepared by irradiation of the ternary mixtures of *N*-isopropylacrylamide/itaconic acid/water by γ-rays at ambient temperature. The effect of comonomer concentration, irradiation dose and pH on the swelling equilibria were studied. Lidocaine was used as a model drug for the investigation of drug release behaviour of hydrogels. Lidocaine adsorption capacity of the hydrogels were found to increase from 3.6 to 862.1 (mg lidocaine/g dry gel) with increasing amount of itaconic acid in the gel structure. Adsorption and release processes were followed at 4 and 37 °C, respectively. The release studies showed that the basic parameters affecting the drug release behaviour of the hydrogels were pH and temperature of the solution and cross-link density of the gels.

Keywords: Hydrogel; N-isopropylacrylamide; Itaconic acid; Lidocaine; Drug release

1. Introduction

Hydrogels are cross-linked polymer networks swollen with water. Poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels are attracting more and more interest in biomedical applications because they exhibit a well-defined lower critical solution temperature (LCST) in water around 31–34 °C which is close to the body temperature. PNIPAAm hydrogels swell when cooled below LCST, and they collapse when heated above the LCST. Mechanical properties, as well as the swelling and shrinking behavior of the gels, change in response to physical or chemical stimuli, such as temperature, pH, ionic strength, solvent composition and electric fields. Hence the gels can be expected to act as intelligent materials in drug release (Lim et al., 1997; Safrany, 1997; Kaetsu, 1996), immobilization of enzymes and cells (Dong and Hoffman, 1986; Tümtürk et al., 1999) and in separation of aqueous solution of proteins (Kayaman et al., 1998).

There is renewed interest in radiation induced polymerization and cross-linking in polymeric hydrogels. The degree of cross-linking, which strongly deter-

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doi:10.1016/j.ijpharm.2004.03.017

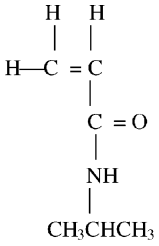
mines the extent of swelling in hydrogels, can be controlled easily by varying the absorbed doses and dose rates. Nagaoka et al. (1993) have reported the synthesis of temperature sensitive PNIPAAM hydrogel by γ-radiation technique. In more recent years, a series of papers has been published by Güven and co-workers who synthesized pH-sensitive hydrogels from the copolymers of acrylamide and diprotic itaconic and maleic acid and showed that the use of even very small quantities of diprotic acid proved to impart remarkable properties to the hydrogels of starting monomers and/or homopolymers (Saraydın et al., 1995; Karadağ et al., 1996; Güven and Şen, 1999; Şen et al., 2000). Double ionization of itaconic acid at different pH values provides stepwise release behavior of specifically adsorbed drugs or other adsorbates by control of pH of the medium.

In principle, it is possible to combine the two effects (temperature and pH) in one single material by copolymerizing the diprotic acid groups containing monomers into a polymer network of a thermosensitive polymer. The purpose of this study is to develop a temperature and pH-responsive hydrogel. In this respect, *N*-isopropylacrylamide/itaconic acid copolymeric hydrogels were prepared by irradiating the ternary mixtures of *N*-isopropylacrylamide/itaconic acid/water by γ-rays at ambient temperature. The influence of comonomer concentrations (1, 2 and 3 mol% of itaconic acid) and irradiation dose on equilibrium swelling behavior of these novel hydrogels was investigated. Lidocaine was used as a model drug for the investigation of release behavior of hydrogels. Adsorption and release characteristics of these systems were analyzed by UV-Vis spectroscopy.

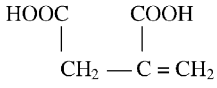
2. Materials and methods

2.1. Materials

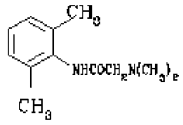
N-isopropylacrylamide (NIPAAm) was obtained from Aldrich Chemical Company. Itaconic acid (IA) was purchased from Fluka Chemical Company. Lidocaine was provided from Department of Pharmacy of İstanbul University as a gift. The chemical formulae of these chemicals are shown in Scheme 1.



N-isopropylacrylamide (NIPAAm)



Itaconic acid (IA)



Lidocaine (LD)

Scheme 1.

2.2. Preparation of hydrogels

The hydrophilic NIPAAm monomer was used as the base monomer in the synthesis of hydrogels. The comonomer was itaconic acid (IA) carrying diprotic acid groups. Aqueous solutions of NIPAAm (10%, w/w) were prepared in distilled water. Different amounts of IA were added to 1 ml of NIPAAm solution (NIPAAm/IA mole ratios, 100:0, 99:1, 98:2, 97:3). Monomer solutions thus prepared were placed in a glass tube with 5 mm inner diameter and each glass tube was stoppered. All irradiations were carried out under air at 25 °C with a PX-30 Issladovatelj type gamma irradiator in Ankara Nuclear Research and Training Centre. The absorbed dose was between 48 and 104 kGy at a dose rate of 3 kGy/h. No attempts has been made to remove dissolved O_2 from the solutions prior to irradiation. Water was chosen as the extraction solvent for the crude hydrogels and employed at room temperature. After polymerization, cross-linked copolymers were removed from tubes and the hydrogels obtained in long cylindrical shapes were cut into pieces of approximately 1 cm length. Then, they are dried in vacuum at 30 °C to constant

weight and subjected to Soxhlet extraction with water as solvent. Each sample was placed in an excess of water and the solvent was replaced every other day over a period of at least 1 week. Uncross-linked polymer and/or residual monomer were removed with this extraction from the gel structure. Extracted gels were dried again in vacuum and the gel fraction was calculated. The amount of uncross-linked IA was determined by titration of extract against NaOH to phenolphthalein end point (Şen et al., 1999). The percentage gelation, W_g (%), was calculated as

$$W_g\,(\%) = \left(\frac{m_{ae}}{m_{be}}\right) \times 100 \tag{1}$$

where m_{ae} and m_{be} are the weights of dry gels after and before extraction, respectively.

2.3. Swelling measurements

Dried hydrogels (1 cm length, 5 mm diameter) were immersed in vials (100 mL) filled with distilled deionized water. The vials were set in a temperature-controlled bath at 25 ± 0.1 °C. In order to reach the equilibrium degree of swelling, the gels were immersed in distilled water for at least 1 week. Each swelling ratio reported in this work is an average of three separate measurements.

The mass swelling and equilibrium mass swelling percentages were calculated from the following equations:

$$\text{Mass swelling}\,(\%) = \left[\frac{m_t - m_0}{m_0}\right] \times 100 \tag{2}$$

$$\text{Equilibrium mass swelling}\,(\%) = \left[\frac{m_\infty - m_0}{m_0}\right] \times 100 \tag{3}$$

where m_0 is the mass of the dry gel and m_t and m_∞ the mass of swollen gel at time t and equilibrium, respectively.

2.4. Determination of M_c and v_e values

In this study, it was assumed that the fraction of charged structural units, i.e., weakly ionized IA in the networks is sufficiently low to have a negligible effect on the mixing term. The Flory–Huggins theory with a Flory χ parameter fitted to network swelling data was used in order to obtain a reasonable value of χ (Flory, 1953). The χ values were calculated by using the following equation:

$$\chi = \frac{\ln(1 - v_{2m}) + v_{2m}}{v_{2m}^2} \tag{4}$$

where v_{2m} is the volume fraction of the swollen gel in the equilibrium state. By applying the Flory–Rehner equation to PNIPAAm gels, χ was calculated as a constant 0.53 in water (Erbil et al., 1999). In this work, χ values of homopolymer and copolymer gels of NIPAAm were found to be 0.53 and 0.51, respectively; χ was held constant at 0.52.

Equilibrium swelling values were used to calculate the effective cross-linking densities of polymer networks (v_e) by the following equation (Bae et al., 1990):

$$v_e = \frac{\ln(1 - v_{2m}) + v_{2m} + \chi v_{2m}^2}{V_1 v_2^0[(v_{2m}/v_2^0)^{1/3} - 0.5(v_{2m}/v_2^0)]} \tag{5}$$

where v_{2m} is the volume fraction of the swollen gel in the equilibrium state and V_1 the molar volume of the solvent.

2.5. Drug loading and release experiments

The dry hydrogels were equilibrated in 5000 ppm (mg/L) of lidocaine (LD) prepared in phosphate buffer at pH 7.4 at 4 °C for 1 week. After incubation the polymer rods were removed from the solution and rinsed in cold buffer. The LD release experiments were carried out by transferring previously incubated drug gels in a vessel containing 50 mL of phosphate buffer at pH 7.4 at 37 °C at a constant shaking rate. At various times aliquots of 3 mL were drawn from medium to follow LD release and placed again into the same vessel so that the liquid volume was kept constant. LD release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophotometer at 262 nm. The release of non-specifically adsorbed LD was followed at pH 7.4. The amount of the percentage release of LD at pH 7.4 was calculated from the following equation:

$$\text{The release \% of non-specific adsorbed LD} = \left(\frac{w_t}{w_{\text{total}}}\right) \times 100 \tag{6}$$

where w_t is the weight of released LD at time t and w_{total} the total weight of specific and non-specific adsorbed LD in the gel structure.

For the release of specifically bonded LD from the gels, buffers at pH 5.5, 4.0 and 2.0 were used. The percentage release of specific adsorbed LD were calculated from the following equation:

$$\text{The release \% of specific adsorbed LD} = \left(\frac{w_t}{w_{sp}}\right) \times 100 \quad (7)$$

where w_t is the weight of released LD at time t and w_{sp} the total weight of specific adsorbed LD in the gel system. After release at pH 2, the gels were immersed in a 0.1 mol/L HCl for 2 days to remove any remaining LD in the gel structure.

3. Results and discussion

3.1. Swelling properties

When *N*-isopropylacrylamide (NIPAAm)/water and *N*-isopropylacrylamide/itaconic acid/water mixtures have been γ-irradiated with gamma rays, polymerization and cross-linking reactions take place simultaneously. The equilibrium percentage mass swelling for each sample is presented in Table 1. All swelling studies were performed at 25 °C and the water uptake was monitored gravimetrically. The same table presents also the hydrogel composition and percentage gelation. The results showed that the mass swelling percentage of an ionic network strongly depends on the concentration of ionizable groups in the network. Increase in the itaconic acid content from 0 to 3 mol% causes a large increase in water uptake in distilled water. The equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogel increased from 1260 to 11,280 as the mole per cent of itaconic acid content increased from 0 to 3. The incorporation of IA into the polymer network with higher IA content will lead to an increase in electrostatic repulsive force between charge sites on carboxylate ions upon their complete dissociation and enhance a more extended configuration. The extended structure with high IA content might cause a higher swelling ratio of the hydrogels in water.

Table 1 also illustrates the effect of irradiation dose on the equilibrium swelling behavior of the hydrogels. The equilibrium percentage mass swelling of PNIPAAm/IA copolymeric hydrogels (at fixed comonomer concentration) decrease as the irradiation dose increase because of increasing cross-linking percentage in the hydrogels. This is explained to be due to higher cross-link density at high irradiation dose results in smaller pore structures and lower equilibrium swelling ratio.

3.2. pH sensitivity of hydrogels

Fig. 1 represents pH dependence of the equilibrium mass swelling percentage for NIPAAm/IA hydrogels at 25 °C in phosphate buffer solution from pH 2 to 8. Consistent with poly-electrolyte behavior, swelling of hydrogels was found to increase with pH. In all

Table 1
The characterization of P(NIPAAm/IA) hydrogels

Gel name	Irradiation dose (kGy)	IA (mol%)		W_g (%)	Equilibrium % (mass swelling)
		In feed	In gel		
PNIPAAm(1)	48	0	0	98	1260
P(NIPAAm/IA)-1	48	1	0.98	97	3390
P(NIPAAm/IA)-2	48	2	1.97	96	5890
P(NIPAAm/IA)-3	48	3	2.98	95	11200
P(NIPAAm/IA)-4	84	1	0.98	94	2550
P(NIPAAm/IA)-5	84	2	1.98	93	4280
P(NIPAAm/IA)-6	84	3	2.99	93	6830
P(NIPAAm/IA)-7	104	1	0.99	97	2700
P(NIPAAm/IA)-8	104	2	1.99	95	3980
P(NIPAAm/IA)-9	104	3	2.99	94	1090

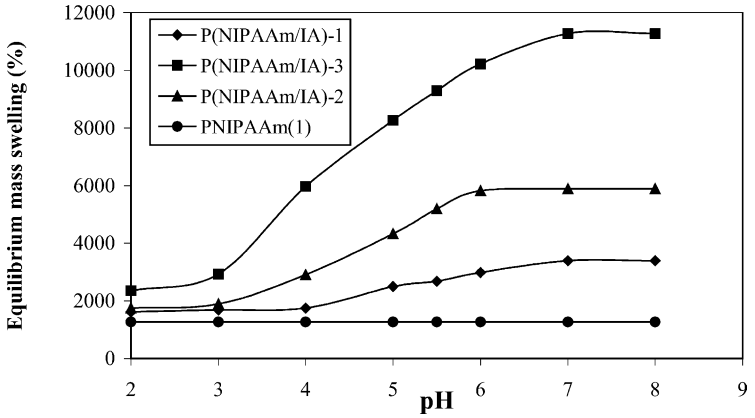


Fig. 1. Effect of pH on the equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogels.

compositions maximum extents of swelling were reached at pH 7, this being due to the complete dissociation of acidic groups of itaconic acid at this pH value. The first and second dissociation constants of IA are $pK_{a1} = 3.85$, $pK_{a2} = 5.44$, respectively (Weast, 1972). The results indicate that under acidic conditions, anionic carboxylate groups are protonated, and the copolymeric network shrinks significantly. At high pH values, the concentration of anionic groups in the polymer network increases. This occurrence makes the percentage mass swelling of the hydrogels increase with an increase in ionizable constituent.

The equilibrium mass swelling percentage for pure PNIPAAm is not affected by varying the pH of the swelling medium since PNIPAAm is non-ionic hydrogel and does not have any group that could be ionized in aqueous solution. With the introduction of the diprotic acid groups into the main chain, pH of the solution becomes an even more important factor determining swelling kinetics and equilibrium swelling value.

3.3. Determination of M_c and ν_e values

One of the basic parameters that describe the structure of an electrolyte and non-electrolyte hydrogels is the number average molecular weight between cross-links ($\overline{M_c}$). This describes the average molecular weight of polymer chains between two consecutive junctions. These junctions may be chemical cross-links, physical entanglements, crystalline regions, or even polymer complexes. Several theories have been proposed to calculate the molecular weight between cross-links in polymeric networks. The earliest theory to describe the equilibrium swelling characteristics of networks was developed by Flory and Rehner for a cross-linked polymer system where the polymer chains are reacted in the solid state, and the macromolecular chains exhibit a Gaussian distribution. The Flory–Rehner theory is used to determine $\overline{M_c}$, effective cross-linking densities of polymer networks (ν_e) and polymer–solvent interaction parameter (χ). The Flory–Rehner theory consists of the elastic, mixing, and ion contributions. The analysis of the terms in the Flory–Rehner equation shows that the influence of χ becomes minor for charged hydrogels at high degrees of swelling (Flory, 1953).

The effect of presence of itaconic acid on the network properties of polymer–solvent (water) interaction parameter is obvious from Table 2. With increasing amount of ionizable constituent (IA) in

Table 2
$\overline{M_c}$ and ν_e values of P(NIPAAm/IA) hydrogels

Gel name	$\overline{M_c}$ ($\times 10^{-3}$ g/mol)	ν_e ($\times 10^5$ mol/cm^3)
PNIPAAm(1)	37	3.0
P(NIPAAm/IA)-1	123	0.9
P(NIPAAm/IA)-2	245	0.5
P(NIPAAm/IA)-3	652	0.2
P(NIPAAm/IA)-4	99	1.1
P(NIPAAm/IA)-5	160	0.7
P(NIPAAm/IA)-6	303	0.4
P(NIPAAm/IA)-7	102	1.1
P(NIPAAm/IA)-8	104	1.1
P(NIPAAm/IA)-9	132	0.8

Table 3
Variation of total lidocaine (LD) uptake with itaconic acid (IA) content in the gel structure

	IA (%)			
	0	1	2	3
Total LD uptake (mg/g dry gel)	192	437	669	863

Total dose given was 48 kGy.

copolymer structure the average $\overline{M_c}$ values increase, whereas the effective cross-linking densities of polymer networks (ν_e) decrease.

3.4. Release of LD from the hydrogels

Lidocaine (LD) was used as a model drug for the investigation of drug release behavior of PNIPAAm and P(NIPAAm/IA) hydrogels. Lidocaine (LD) is a local anesthetic drug and widely used for the short-term management of life-threatening ventricular arrhythmias (Dollo et al., 2001).

The interactions between cationic groups of the drug and carboxyl groups of itaconic acid lead to specific drug adsorption. On the other hand, non-specific drug adsorption is due to physical inclusion, pore filling action of drugs into completely ionized gel at pH 7.4 or any pH above 5.44 which corresponds to second p*K*a of itaconic acid. The amounts of total (specific and non-specific) LD uptake into 1 g of dry PNIPAAm and P(NIPAAm/IA) hydrogels are given in Table 3. As can be seen from this table, the LD uptake into the hydrogels increases with increase in IA content due to ionic interactions. LD is a water soluble, slightly basic structure, and it has specific interaction with P(NIPAAm/IA) hydrogels through electrostatic interactions. Table 4 illustrates that the LD uptake into the hydrogels decreases with increase in the irradiation dose. The cross-linking density gets higher as the irradiation dose increases, so the pores (molecular mesh) in this hydrogel become smaller and LD could not penetrate inside the network sufficiently.

Table 4
Variation of total lidocaine (LD) uptake with irradiation dose (mole % of IA = 3)

	Irradiation dose (kGy)		
	48	82	104
Total LD uptake (mg/g dry gel)	862	580	388

Table 5
The equilibrium LD release amount of non-specific adsorbed LD from P(NIPAAm/IA) hydrogels in phosphate buffer solution of pH 7.4 at 37 °C

Gel name	$m_{\text{non-specific}}$ (mg/g dry gel)	m_{total} (mg/g dry gel)
PNIPAAm(1)	113	192
P(NIPAAm/IA)-1	137	437
P(NIPAAm/IA)-2	146	669
P(NIPAAm/IA)-3	191	862

The release profiles of LD in NIPAAm/IA copolymeric gels in phosphate buffer solution of pH 7.4 at 37 °C are shown in Fig. 2. For all gels, the LD release increases rapidly at first and then gradually reaches the equilibrium value in approximately 24 h. Fig. 2 also shows that the release percent for non-specific adsorbed LD was higher for pure PNIPAAm hydrogel than those for P(NIPAAm/IA) hydrogels. The release percent decreases with the increase of IA content in the gel structure. This can be explained by the increase in the diffusional path due to the high swelling of P(NIPAAm/IA) hydrogels. While 76% of LD was released from PNIPAAm hydrogels, this value decreased to 13% with increasing IA content to 3% in the gel structure. However, as illustrated in Table 5, the equilibrium amount of the released drug per unit mass of dry P(NIPAAm/IA) hydrogels was higher than from the PNIPAAm hydrogel. At the constant temperature of 37 °C, the amount of the equilibrium drug released increased with increase in IA content, because the equilibrium swelling ratio of NIPAAm/IA copolymeric hydrogels and drug loading increased with increasing IA content in the gel structure.

The incomplete release of LD from P(NIPAAm/IA) hydrogels at pH 7.4 was expected to be due to binding of the cationic LD to the polymer. The difference between the total and non-specific LD uptake is therefore taken to be equal to the amount of specific adsorbed LD in the hydrogel. The release of specific adsorbed drug from PNIPAAm/PIA microspheres was investigated primarily at pH 5.5 since the second dissociation constant (pK_{a2}) of IA is around 5.44 (Weast, 1972). The drug release was followed until equilibrium and then the hydrogel was transferred into drug free buffer at pH 4 ($pK_{a1} = 3.85$). In the release of

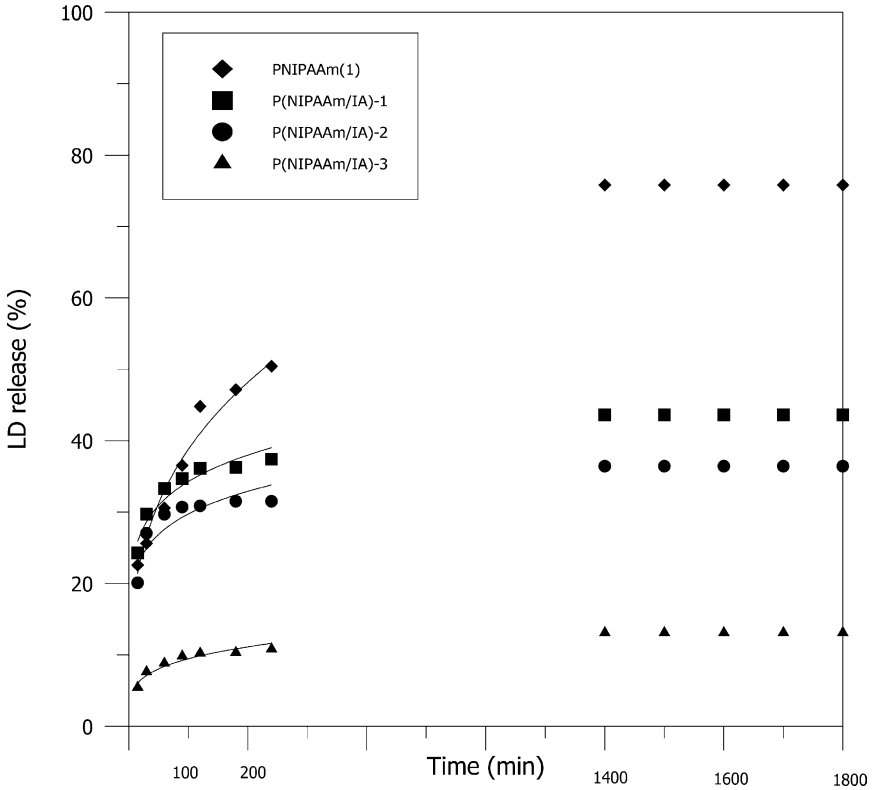


Fig. 2. Release percent of non-specific adsorbed LD from P(NIPAAm/IA) hydrogels in phosphate buffer solution of pH 7.4 at 37 °C.

specific adsorbed drug from the hydrogel, one of the anionic carboxylate groups was protonated at pH 5.5. Then the first protonation was completed by keeping the spheres at pH 4. Finally, the microspheres were placed into pH 2 buffer for the complete protonation of acid groups since the second p*K*a is 3.85. The percentage release of LD with time at each hydrogel system is given in Fig. 3.

Fig. 4 shows the variation of the total, non-specific and specific LD uptake with itaconic acid content in the gel structure. The results obtained from Figs. 3 and 4 illustrate that the release amount of the specific adsorbed drug from the hydrogels increases with the increasing IA content in the gel structure in all buffer solutions and the maximum equilibrium release amount was realized at pH 2. Even at pH 2, the LD is not completely released and some portions are entrapped within the gel. Since the drugs were initially loaded at a temperature well below the LCST of base material PNIPAAm, in fully swollen state and release

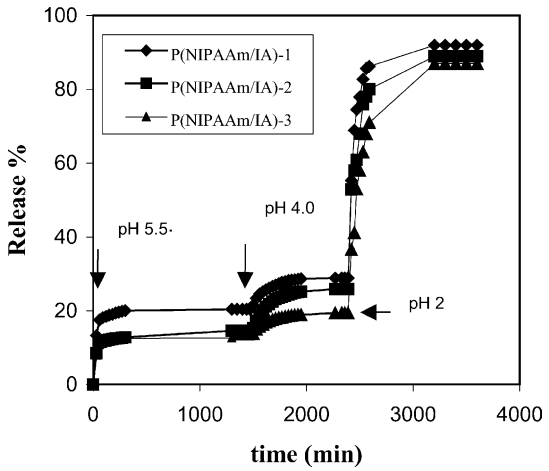


Fig. 3. Release % of specific adsorbed LD from P(NIPAAm/IA) hydrogels prepared at different IA concentrations.

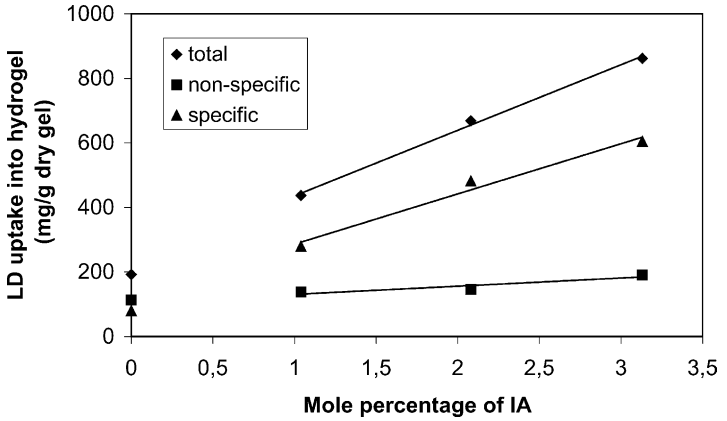


Fig. 4. Variation of total, non-specific and specific adsorbed LD with itaconic acid content in the gel structure prepared at the irradiation dose of 48 kGy.

studies were performed at 37 °C where the PNIPAAm chains were in collapsed state, some portion of drugs might well have been entrapped in the shrunken part of the spheres collapsed state (Savaş and Güven, 2001).

4. Conclusion

In this study, the preparation of the novel P(NIPAAm/IA) hydrogels and their drug release behaviors have been investigated. For the characterization of these hydrogels, molecular weight between cross-links and cross-linking densities were studied. It has been found that the specific and non-specific adsorption capacity of hydrogels both increase with increasing IA content in the gel structure. This has been explained due to the incorporation of more specific acidic groups into the network and consequent higher swelling capacity of the gels. Since the hydrogels prepared in this study can be considered as potential carriers for the drug delivery systems, their drug release behaviors were investigated at body temperature (37 °C) and the physiological pH. The release studies show that some of the basic parameters affecting the drug release behavior of P(NIPAAm/IA) hydrogels are pH and temperature of the solution. To conclude, the hydrogels prepared in this study may be used as especially local therapeutic application of cationic drugs such as lidocaine under controlled pH and temperature conditions.

Acknowledgements

This work was supported by Turkish–Macedonian Science and Technology Program for 2001–2003 and Çekmece Nuclear Research and Training Center. The authors thank Ayhan Mesci for their technical assistance. BMB and OG acknowledge the support of TUBA, Turkish Academy of Sciences.

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