Investigation of drug release from thermo- and pH-sensitive poly(N-isopropylacrylamide/itaconic acid) copolymeric hydrogels

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Received 26 February 2004; Accepted 4 June 2004

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 dependence of swelling properties and phase transitions on the comonomer concentration and temperature were investigated. The hydrogels showed both temperature and pH responses. The effect of comonomer concentration on the uptake and release behavior of the hydrogels was studied. Methylene blue (MB) was used as a model drug for the investigation of drug uptake and release behavior of the hydrogels. The release studies showed that the basic parameters affecting the drug release behavior of the hydrogels were pH and temperature of the solution. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: hydrogels; poly(N-isopropylacrylamide/itaconic acid); methylene blue; drug delivery systems; swelling

INTRODUCTION

Hydrogels are crosslinked hydrophilic polymers capable of imbibing large volumes of water, but swellable when immersed. The water retaining capacity of these materials is due to the presence of hydrophilic functional groups such as –OH, –COOH, –CONH₂, –CONH, –SO₃H along the polymer chains.¹,²

Hydrogels are known as good candidates for the controlled release formulations for pharmaceutical applications mostly due to their high biocompatibility. In recent years, these polymeric carriers have been extensively considered in sustained and controlled release devices for the delivery of water-soluble drugs.³,⁴

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 body temperature. In more recent years, a series of papers have been published by Güven and coworkers who synthesized new 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.⁵–⁷

The use of radiation in the preparation of hydrogels has recently been reviewed by Rosiak and Olejniczak, who have investigated the medical applications of radiation formed hydrogels.⁸ Nagaoka et al. have reported for the first time the synthesis of PNIPAAm hydrogel by γ-radiation technique.⁹

The purpose of this study is to develop a temperature- and pH-reversible 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), irradiation dose and pH on equilibrium swelling behavior of the hydrogels was investigated. Another aim of this work is to study drug adsorption capacity and drug release behavior of these novel hydrogels.

MATERIALS AND METHODS

Materials

N-Isopropylacrylamide (NIPAAm) was obtained from Aldrich Chemical Company. Itaconic acid (IA) was purchased from Fluka Chemical Company. Methylene blue (MB) was purchased from Merck AG. The chemical formulae of these chemicals are shown in Scheme 1.

Preparation of hydrogels

The hydrophilic NIPAAm monomer was used as the base monomer in the synthesis of the hydrogels. The comonomer was IA carrying diprotic acid groups. Aqueous solutions of NIPAAm (%10 w/w) were prepared in distilled water.
The mass swelling and equilibrium mass swelling percentages were calculated from the following equations:

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

(2)

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

(3)

where \( m_0 \) is the mass of the dry gel and \( m_t \) and \( m_{\infty} \) is the mass of swollen gel at time \( t \) and at equilibrium, respectively.

### Drug loading and release experiments

The dry hydrogels were equilibrated in 5000 ppm (mg/l) of MB 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 MB 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 the medium to follow MB release and placed again into the same vessel so that the liquid volume was kept constant. MB release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophotometer at 664 nm. The release of non-specifically adsorbed MB was followed at pH 7.4. The amount of the percentage release of MB at pH 7.4 was calculated from the following equation:

\[ \text{The release percentage of non-specific absorbed MB} = \left( \frac{w_t}{w_{\text{total}}} \right) \times 100 \]  

(4)

where \( w_t \) is the weight of released MB at time \( t \) and \( w_{\text{total}} \) is the total weight of specific and non-specific adsorbed MB in the gel structure.

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

\[ \text{The release percentage of specific absorbed MB} = \left( \frac{w_t}{w_{\text{sp}}} \right) \times 100 \]  

(5)

where \( w_{sp} \) is the total weight of specific adsorbed MB 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 MB in the gel structure.

### RESULTS AND DISCUSSION

#### Composition of hydrogels

When NIPAAm/water and NIPAAm/IA/water mixtures have been irradiated with gamma rays, polymerization and crosslinking reactions take place simultaneously. Mole percentages of IA in the feed and in the copolymeric gels and percentage gelation are summarized in Table 1. These results show that increasing mole percentage of IA causes a decrease in the extent of gelation from monomer to gel.

#### pH Sensitivity of hydrogels

The percentage mass swelling, as a function of time for NIPAAm/IA copolymeric hydrogels in several pH buffer solutions are shown in Fig. 1. Consistent with polyelectrolyte behavior, swelling of hydrogels was found to increase with

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**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.

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**Scheme 1.**

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 a 5 mm inner diameter. All irradiations were carried out under air at 25°C with a PX-30 Issladovatelj type gamma irradiator at the Ankara Nuclear Research and Training Center. The absorbed dose was 48–104 kGy at a dose rate of 3 kGy/hr. After polymerization, crosslinked copolymers were removed from 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 MB in the gel structure.
pH. In all compositions maximum extents of swelling were reached at pH 7, this being due to the complete dissociation of acidic groups of IA at this pH value. The first and second dissociation constants of IA are $pK_{a1} = 3.85$, $pK_{a2} = 5.45$, respectively. 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.

### Effect of comonomer concentration

As shown in Table 1, equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogels (at fixed irradiation dose) increase as the comonomer concentration increases because of increasing the electrostatic interactions of the neighboring carboxylate groups in IA in the hydrogels. It can be seen that the percentage mass swelling of an ionic network very much depends on the concentration of ionizable groups in the network. Increase in the IA content from 0 to 3 mol% causes immense increases in water uptake in deionized water. Figure 2 illustrates the temperature dependence of the equilibrium swelling ratio for a series of NIPAAm/IA copolymeric gels at different comonomer concentration at the same irradiation dose. The results clearly show that as the comonomer concentration increases, the swelling ratio increases. The higher IA content leads to the broader phase transition and a shift of the LCST to a higher temperature (Table 1). It has been shown that the LCST of PNIPAAm

<table>
<thead>
<tr>
<th>Gel name</th>
<th>Irradiation dose (kGy)</th>
<th>Mol% of IA</th>
<th>Equilibrium mass swelling (%)</th>
<th>LCST</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(NIPAAm)</td>
<td>48</td>
<td>0</td>
<td>98</td>
<td>1260</td>
</tr>
<tr>
<td>P(NIPAAm/IA)</td>
<td>48</td>
<td>1</td>
<td>97</td>
<td>390</td>
</tr>
<tr>
<td>P(NIPAAm/IA)</td>
<td>48</td>
<td>2</td>
<td>96</td>
<td>5890</td>
</tr>
<tr>
<td>P(NIPAAm/IA)</td>
<td>48</td>
<td>3</td>
<td>95</td>
<td>11200</td>
</tr>
</tbody>
</table>

*Table 1. The characterization of P(NIPAAm/IA) hydrogels*
copolymers is strongly influenced by the nature of the comonomer. Hydrophobic compounds lower the LCST and hydrophilic compounds raise it.

Drug uptake and release of MB from the hydrogels

MB was used as the model drug for the investigation of drug uptake and release behavior of PNIPAAm and P(NIPAAm/IA) hydrogels. The amounts of total (specific and non-specific) MB uptake into 1 g of dry PNIPAAm and P(NIPAAm/IA) hydrogels are given in Table 2. As can be seen from Table 2, the MB uptake into the hydrogels increases with increase in IA content due to ionic interactions. Drug loading efficiency increases for the drug, due to some specific interactions of carboxyl groups of IA with the drug. Different binding features are responsible for the drug loading contents. MB is a water soluble, positively charged dye molecule and has specific interaction with P(NIPAAm/IA) hydrogels through electrostatic interactions.

The release profiles of MB in NIPAAm/IA copolymeric gels in phosphate buffer solution of pH 7.4 at 37°C are shown in Fig. 3. For all gels, the MB release increases rapidly at first and then gradually reaches an equilibrium value in approximately 24 hr. Figure 4 also shows that the release percent for non-specific adsorbed MB 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 indicates that specific binding of the drug is totally due to the presence of IA.

The incomplete release of MB from P(NIPAAm/IA) hydrogels at pH 7.4 was expected to be due to binding of the cationic MB to the polymer. The difference between the total and non-specific MB uptake is therefore taken to be equal to the amount of specific adsorbed MB in the hydrogel. The release of specific adsorbed drug from P(NIPAAm/IA) hydrogel was investigated primarily at pH 5.5 since the second dissociation constant (pK_a2) of IA is around 5.44. 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 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 hydrogels were placed into pH 2 buffer for the complete protonation of acid groups since the second pK_a is 3.85. The percentage release of MB with time at each hydrogel system is given in Fig. 4.

One of the most attractive features of PNIPAAm-based hydrogels as drug carriers is their intelligent property to external temperature changes. It is important and practical to examine the drug release data from those P(NIPAAm/IA) hydrogels at a temperature >LCST like body temperature (37°C). Figure 5 shows the MB release behavior from pure

Table 2. Variation of total MB uptake with IA content in the gel structure. Total dose given was 48 kGy

<table>
<thead>
<tr>
<th>IA%</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MB uptake (mg/g dry gel)</td>
<td>5.0</td>
<td>27.3</td>
<td>49.0</td>
<td>74.6</td>
</tr>
</tbody>
</table>

Figure 3. Release percentage of non-specific adsorbed MB from P(NIPAAm/IA) hydrogels in phosphate buffer solution of pH 7.4 at 37°C.

Figure 4. Release percentage of specific adsorbed MB from P(NIPAAm/IA) hydrogels prepared at different IA concentrations.

Figure 5. Release of non-specific adsorbed drug from pure PNIPAAm-1 and P(NIPAAm/IA)-3 hydrogels in phosphate buffer solution of pH 7.4 at two different temperatures (20 and 37°C).

Investigation of drug release from copolymeric hydrogels
PNIPAAm-1 and P(NIPAAm/IA)-3 hydrogels in buffer at pH 7.4 at two different temperatures (20 and 37°C). Release of non-specific adsorbed MB at 37°C were lower than those at 20°C because of the collapse nature of PNIPAAm structure at a temperature greater than its LCST.\(^{12}\)

Table 3 shows the variation of the total, non-specific and specific MB uptake with IA content in the gel structure. The results 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 MB 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 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.

**CONCLUSION**

In this study, the preparation of the novel P(NIPAAm/IA) hydrogels and their drug release behaviors have been investigated. The influence of external stimuli such as pH and temperature of the swelling media on the equilibrium swelling properties were also investigated. The equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogel increased from 1260 to 11 200 as the mol% of IA content increased from 0 to 3. This has been explained due to the incorporation of more specific acidic groups into the network and consequent higher swelling capacity of the gels. 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 MB under controlled pH and temperature conditions.

**REFERENCES**