Anticancer drug release from poly(N-isopropylacrylamide/itaconic acid) copolymeric hydrogels

B. Taşdelen\textsuperscript{a,}\textsuperscript{*}, N. Kayaman-Apohan\textsuperscript{b}, O. Güven\textsuperscript{c}, B.M. Baysal\textsuperscript{d,e}

\textsuperscript{a}Chemistry Department, Çekmece Nuclear Research and Training Center, P.O. Box 1, 34831 Istanbul, Turkey
\textsuperscript{b}Department of Chemistry, Marmara University, 81040 Göztepe/Istanbul, Turkey
\textsuperscript{c}Department of Chemistry, Hacettepe University, 06532 Beytepe/Ankara, Turkey
\textsuperscript{d}TUBITAK Marmara Research Center, Research Institute of Materials and Chemical Technologies, 41470 Gebze, Kocaeli, Turkey
\textsuperscript{e}Department of Chemical Engineering, Boğaziçi University, 80815 Istanbul, Turkey

Received 8 September 2004; accepted 18 October 2004

Abstract

The drug uptake and release of anticancer drug from N-isopropylacrylamide/itaconic acid copolymeric hydrogels containing 0–3\% of itaconic acid irradiated at 48 kGy have been investigated. 5-Fluorouracil (5-FU) is used as a model anticancer drug. The effect of 5-FU solution on swelling characteristics of PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels have also been studied. The percent swelling, equilibrium swelling, equilibrium water/5-FU content and diffusion constant values are evaluated for poly(N-isopropylacrylamide) (PNIPAAm) and poly(N-isopropylacrylamide/itaconic) (P(NIPAAm/IA)) hydrogels at 130 ppm of 5-FU solution at room temperature. Diffusion of 5-FU solution into the hydrogels has been found to be the non-Fickian type. Finally, the kinetics of drug release from the hydrogels are examined.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Hydrogel; N-isopropylacrylamide; Itaconic acid; 5-Fluorouracil; Drug release

1. Introduction

Biomaterials play a key role in most approaches for engineering tissues as substitutes for functional replacement, for components of devices related to therapy and diagnosis, for drug delivery systems and supportive scaffolds for guided tissue growth. Modern biomaterials could be composed of various components, e.g. metals, ceramics, natural tissues, polymers (Rosiak and Yoshii, 1999). In this last group, the hydrogels, hydrophilic polymeric gels with requested biocompatibility and designed interaction with living surrounding seem to be one of the most promising group of biomaterials. Especially, if they are formed by means of ionising radiation. A radiation technique is more preferable than a chemical one, because of the advantage to control gently the level of cross-linking by variation of the absorbed dose. This method offers unique advantages for the synthesis of new and modification of existing materials: it is simple, additive-free process at all temperatures, reactions such as polymerisation, cross-linking and grafting can easily be controlled.

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

\textsuperscript{*}Corresponding author. Tel.: +90 212 548 40 50; fax: +90 212 548 22 30.
\textsuperscript{E-mail address: btasdelen2002@yahoo.com (B. Taşdelen).}

0969-806X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
31–34 °C which is close to the body temperature. The use of radiation in the preparation of hydrogels has been recently reviewed by Rosiak and Olejnizak, who have investigated the medical applications of radiation-formed hydrogels (Rosiak and Olejnizak, 1993). Nagaoaka et al. have reported for the first time the synthesis of PNIPAAm hydrogel by γ-radiation technique (Nagaoaka et al., 1993).

Recently, we have studied radiation-induced synthesis of poly(N-isopropylacrylamide/maleic acid) P(NIPAAm/MA) and poly(N-isopropylacrylamide/itaconic acid) P(NIPAAm/IA) copolymeric hydrogels (Taşdelen et al., 2004a,b). The hydrogels thus prepared were characterized with respect to their swelling properties and network structures. The dependence of swelling properties and phase transitions on the comonomer concentration and temperature were investigated.

In this study, 5-Fluorouracil (5-FU) was chosen as a model drug for the investigation of drug uptake and release behavior of the P(NIPAAm/IA) copolymeric hydrogels. 5-FU is one of the antitumor agents most frequently used for treating solid tumors, such as breast, colorectal, and gastric cancers (Zhang et al., 2002). It is an appropriate candidate for this type of system of drug administration due to the large number of secondary effects that accompany its conventional administration. 5-FU release was studied as a function of temperature, disc thickness, disc load and degree of crosslinking of the poly(2-hydroxyethylmethacrylate) hydrogels (Garcia et al., 2000) and poly(acrylamide-co-monomethyl itaconate) hydrogels (Blanco et al., 1996). The 5-FU was trapped in the gels by its inclusion in the polymerization mixture. To incorporate the 5-FU to the feed mixture of polymerization, water solutions of 5-FU were used. However, in our work, we have studied drug uptake and release behavior of PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels after the radiation-induced formation of the hydrogels. The effect of 5-FU solution on swelling characteristics of PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels have also been investigated. Finally, the kinetics of drug release from the hydrogels are examined. These measurements are made with the purpose of characterizing these hydrogels as drug delivery systems.

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. 5-FU (C₄H₃N₂O₂F, of molecular weight 130.1 Da) was supplied by Roche Laboratories as a crystalline powder.

2.2. Preparation of hydrogels

The hydrophilic NIPAAm monomer was used as the base monomer in the synthesis of hydrogels. The comonomer was 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 Issldovatetj type gamma irradiator in Ankara Nuclear Research and Training Centre. The absorbed dose was 48 kGy at a dose rate of 3 kGy/h. Water was chosen as the extraction solvent for the crude hydrogels and employed at room temperature. After polymerization, crosslinked 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 day over a period of at least 1 week. Uncrosslinked polymer and/or residual monomer were removed with this extraction from the gel structure. Extracted gels were dried again in vacuum and stored for further studies.

2.3. Swelling measurements

Dried hydrogels (1 cm length, 5 mm diameter) were immersed in vials (100 ml) filled with 5-FU solutions at room temperature. The vials were set in a temperature-controlled bath at 25 ± 0.1 °C. After a certain time, they are weighed and placed in the same solutions. The percentage swelling in drug solution (S) was determined gravimetrically by the following equation (Güven and Şen, 1990):

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

where \( m_0 \) is the initial dry weight and \( m_t \) is the weight of a swollen gel at time, \( t \).

2.4. Drug loading and release experiments

The dry hydrogels were equilibrated in 130 ppm (mg/l) of 5-FU prepared in distilled water at 4 °C for 1 week. After incubation the polymer rods were removed from the solution and rinsed in cold buffer. The 5-FU release experiments were carried out by transferring previously incubated drug gels in a vessel containing 10 ml of distilled water at 37 °C at a constant shaking rate. At various times, aliquots of 3 ml were drawn from medium to follow 5-FU release and placed again into the same vessel so that the liquid volume was kept constant.
release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophotometer at 266 nm. For examining the release of 5-FU from P(NIPAAm/IA) hydrogels, percentage release of the drug was calculated from the following equation:

$$\% \text{Release} = \frac{W_t}{W_{\text{total}}} \times 100$$

where $W_t$ is the weight of released drug in water at any time and $W_{\text{total}}$ is the initial total weight of the drug taken by the gel system.

3. Results and discussion

3.1. Swelling properties

A fundamental relationship exists between the swelling of a polymer in a solvent and the nature of the polymer and the solvent. The 5-FU solution intake of initially dry hydrogels was followed for a period of time, gravimetrically. Swelling curves of the PNIPAAm and P(NIPAAm/IA) hydrogels were constructed and representative swelling curves at 130 ppm of 5-FU solutions are shown in Fig. 1. As can be seen from the figure, the swelling capabilities of the hydrogels are increased by time, reaching constant swelling (equilibrium swelling) after a certain period of time. In the previous work, it is indicated that the equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogels in water increased from 1260 to 11,200 as the mol% of IA content increased from 0 to 3 (Taşdelen et al., 2004b). In the presence of 5-FU, these percentages (474–7577%) are lower than in water. The molecular size of the 5-FU is larger than the size of water; hence, molecules of water can diffuse into gel pores more easily than molecules of 5-FU. The equilibrium swelling percentages of PNIPAAm and P(NIPAAm/IA) hydrogels in distilled water and 5-FU solutions, are given in Table 1, respectively. 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 hydrogel in the drug solutions.

3.2. Drug diffusion into hydrogels

The swelling curves of PNIPAAm and P(NIPAAm/IA) hydrogels in aqueous 5-FU solutions were used for the calculation of a certain diffusion characteristics. The following equation was used to determine the nature of diffusion of 5-FU solution into hydrogels (Crank, 1970):

$$F = \frac{M_t}{M_\infty} = kt^n$$

Here $F$ is the fractional uptake, $M_t/M_\infty$, where $M_t$ is the amount of diffusant absorbed at time $t$, $M_\infty$ is the maximum amount absorbed, $k$ is a constant incorporating characteristics of macromolecular network system and the penetrant, $n$ is the diffusion exponent, which is indicative of the transport mechanism. Eq. (3) is valid for the first 60% of the normalized solvent uptake. For Fickian kinetics in which the rate of penetrant diffusion is rate limiting, $n = 0.5$, whereas values of $n$ between 0.5 and 1 indicate the contribution of non-Fickian processes such as polymer relaxation.

The plots of $\ln F$ versus $\ln t$ for the series of pure PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels in aqueous 5-FU solutions were evaluated and the

![Graph](image1.png)

**Fig. 1.** Percentage mass swelling as a function of time for the series of NIPAAm/IA copolymeric hydrogels in 130 ppm of 5-FU solutions at 25°C.

![Graph](image2.png)

**Table 1**

<table>
<thead>
<tr>
<th>Gel name</th>
<th>Mol% of IA</th>
<th>Equilibrium mass swelling (%) in distilled water</th>
<th>Equilibrium mass swelling (%) in 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAAm(1)</td>
<td>—</td>
<td>1260</td>
<td>470</td>
</tr>
<tr>
<td>P(NIPAAm/IA)-1</td>
<td>1</td>
<td>3390</td>
<td>2310</td>
</tr>
<tr>
<td>P(NIPAAm/IA)-2</td>
<td>2</td>
<td>5890</td>
<td>5160</td>
</tr>
<tr>
<td>P(NIPAAm/IA)-3</td>
<td>3</td>
<td>11,200</td>
<td>7580</td>
</tr>
</tbody>
</table>

*Swelling % performed by gravimetrically (Taşdelen et al., 2004b).*
exponents $n$ and $k$ values were calculated from the slope and intercept of the lines, respectively. The data were collected in Table 2. It is clear from the analysis that as the IA content in the gel structure increases, the diffusional release kinetic exponent $n$ increases from 0.53 to 0.70 for P(NIPAAm/IA) hydrogels. Hence, the diffusion of 5-FU solutions into PNIPAAm and P(NIPAAm/IA) hydrogels was assumed to be non-Fickian character.

Diffusion coefficients are important penetration parameters of some chemical species to polymeric systems. Using “$n$” and “$k$”, the diffusion coefficient ($D$) of solvent in the matrix could be calculated using the following equation (Peppas et al., 1980; Korsmeyer and Peppas, 1983):

$$k = 4D / \pi r^2,$$

$$4D^n = k(\pi r^2)^n,$$

$$D^n = (k / 4)(\pi r^2) \ldots,$$  \hspace{1cm} (4)

where “$D$” is the diffusion coefficient and “$r$” is the radius of gel disc.

Diffusion coefficients of hydrogels in aqueous solutions of 5-FU are also listed in Table 2. As expected, the diffusion coefficients increase with an increase in equilibrium mass swelling of the present hydrogel in the solutions.

3.3. Equilibrium water and equilibrium water/5-FU content

The water absorbed by PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels is quantitatively represented by the equilibrium water content (EWC) (Karadağ et al., 2004):

$$\text{EWC} = (W_{eq} - W_0) / W_{eq},$$  \hspace{1cm} (5)

where $W_{eq}$ is the weight of the swollen gel at time $t$ (equilibrium) and $W_0$ is the weight of the dry gel at time 0. The EWC values of the hydrogels and equilibrium 5-FU content were calculated and tabulated in Table 3. All EWC values of the hydrogels (0.88–0.98) were greater than the percent values of body about 0.6. Thus, the PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels exhibited fluid contents similar to those of living tissues.

3.4. Drug loading

For the investigation of drug uptake behavior of P(NIPAAm/IA) copolymeric hydrogels, the uptake capacities were determined by measuring the mass of adsorbate per unit mass of adsorbent ($q_c$). $q_c$ values (mg/g) are calculated from the following equation (Şen et al., 2000):

$$q_c = [(C_i - C) \times V_t] / m,$$  \hspace{1cm} (6)

where $C_i$ and $C$ are the initial and equilibrium concentration of solution of adsorbate, $V_t$ the volume of solution treated, and $m$ is the mass of dry adsorbent in gram.

Table 4 shows the effect of comonomer concentration on the drug uptake capacities of PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels. As can be seen from this table, 5-FU uptake into the hydrogels increases with increase in IA content. Since PNIPAAm is non-ionic hydrogel, ionizable groups on the polymer were increased by addition of IA groups to the hydrogel. It is obvious that, the gel with ionic constituent swells in aqueous drug solution much more than the corresponding neutral gel due to the electrostatic repulsion between charged groups on the network chain as well as due to the osmotic pressure of the mobile ions within the gel phase. Due to the higher swelling capacity of ionic gels, a large amount of drug can penetrate into the gel phase.
3.5 Release behavior of hydrogels

The release profiles of 5-FU in pure PNIPAAm and P(NIPAAm/IA) copolymeric gels in water at 37 °C are shown in Fig. 2. For all gels, the 5-FU release increases rapidly at first and then gradually reaches the equilibrium value in approximately 4 h. The release percent increases with the increase of IA content in the gel structure. Fig. 3 show the fractional 5-FU release, expressed as $M_t/M_\infty$, where $M_t$ and $M_\infty$ are the amounts of drug released at the times $t$ and infinite, respectively, as a function of time for the hydrogels. In this figure, the drug release during the first stage could be influenced for the relaxation of polymer chains. Thus, the hydrogels show an initial non-Fickian behavior indicating similar rates of Fickian diffusion and polymer relaxation (Martellini et al., 2003).

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 the body temperature (37 °C). Fig. 4 shows the 5-FU release behavior from pure PNIPAAm and P(NIPAAm/IA)-3 hydrogels in water at two different temperatures (20 and 37 °C). Release of the adsorbed 5-FU 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 (Zhang et al., 2004; Maolin et al., 1998).

4. Conclusion

This study has shown that the equilibrium percentage swelling of the P(NIPAAm/IA) hydrogels in 5-FU solutions increased from 470 to 7580 as the mol% of IA content increased from 0 to 3. Furthermore, it has been found that the drug uptake capacity of the hydrogels both increases 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. In the diffusion transport mechanism study, the number to determine the type of diffusion ($n$) is found to
be over 0.5 for the hydrogels. This implies that the swelling transport mechanism is a non-Fickian transport. The fractional cumulative release of the drug from the hydrogels have showed an initial non-Fiction behavior, probably indicating a comparable rates of Fiction diffusion and polymer relaxation.

References