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Release of 5-fluorouracil from poly(acrylamide–co-monopropyl itaconate) hydrogels

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Abstract

The aim of this work was to test the application of copolymeric poly(acrylamide–co-monopropyl itaconate) (A–MPI) hydrogels on the release of 5-fluorouracil (5-FU). The equilibrium degree of swelling in saline solution was 83 ± 2%. 5-FU, as the sodium salt, was trapped in gels by placing it in the polymerization feed mixture. The diffusion coefficients for both swelling of the gels and the release of 5-FU were determined, in addition to the activation energies for both processes. To determine the applicability of these copolymers, A–MPI (75:25) gel was subcutaneously implanted in rats and the drug plasma concentration was determined by HPLC.

Keywords: Hydrogels; 5-Fluorouracil; Poly(acrylamide-co-monopropyl itaconate)

1. Introduction

Classically, anticancer drugs are administered intravenously and their distribution throughout the body is a function of physico-chemical properties of the molecule. A pharmacologically active concentration is reached in the tumour tissue at the expense of massive side-effects in the rest of the body. For cytostatic compounds, this poor specificity raises a toxicological problem that presents a serious obstacle to effective therapy. Efforts made to develop more rational approaches to specific cancer therapy have resulted in the concept of controlled-release systems from different matrices. The main polymers used for both biomedical applications and controlled-release systems of many drugs are hydrogels [1,2].

Hydrogels are characterized by their capacity to absorb water and other solvents. The absorption of a solute into the network and the controlled release of the solute into an aqueous environment are both closely related to the swelling properties of the network involved [3]. The utility of the hydrogels as biomaterials lies in the similarity of their physical properties to those of natural tissue in its biological environment [4]. This resemblance is based on their water content, soft and rubbery consistency and low interfacial tension with water or biological fluids [5]. More-

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over, the water molecules included in the polymer seem to be associated within the three-dimensional network to form a quasi-organized structure similar to the extracellular matrix [6].

One of the most commonly used anticancer drugs in the treatment of solid tumours of breast, stomach, colon and pancreas is 5-fluorouracil (5-FU) [7–9]. However, this drug exhibits very high toxicity and can cause severe side-effects [10]. In an attempt to decrease these side-effects, different systems have been designed that enable the pharmacokinetics of the drug and its tissue distribution to be controlled [11–13].

Among the systems used to this end are the hydrogels. Thus, poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels cross-linked with different percentages of ethylene glycol dimethacrylate (EGDMA) have been employed to obtain a controlled release of 5-FU [14]. Likewise, experiments on 5-FU release from collagen–PHEMA hydrogels [5], copolymers of 2-hydroxyethyl methacrylate (HEMA) and bisglycol acrylate (BGA) cross-linked by γ-radiation have also been carried out [15].

Although hydrogels of PHEMA or copolymers with HEMA are usually used to release 5-FU owing to their good HEMA biocompatibility, we began our study on copolymeric hydrogels of acrylamide and itaconic acid monoesters as matrices for 5-FU release. Acrylamide hydrogels exhibit a very high capacity for water absorption, are permeable to oxygen and also have good biocompatibility [15–17]. The monoesters derived form itaconic acid are structurally similar to the acrylic derivates and have hydroxylic groups in their molecule, which makes them highly hydrophilic [16]. Poly(acrylamide–co-monomethyl itaconate) hydrogels show very high degrees of equilibrium swelling, permitting 5-FU to be trapped inside the polymer matrices and drug release takes place in about 70 h [18].

Continuing the study on the application of this kind of hydrogel on 5-FU release, the aim of this work was to synthesize a new hydrogel based on acrylamide (A) and monopropyl itaconate (MPI) copolymers, and to study their swelling kinetics in saline solution and their 5-FU release in both in vitro and in vivo systems.

2. Experimental

2.1. Materials

Itaconic acid (methylene succinic acid) (Merck, Darmstadt, Germany), acrylamide (Merck), N,N′-methylenebisacrylamide (NBA) (Merck), ammonium peroxodisulfate [$(NH_4)_2S_2O_8$] (Merck), sodium disulfite ($Na_2S_2O_5$) (Merck), dimethyldichlorosilane solution (BDH, Poole, UK), sodium chloride (Panreac, Barcelona, Spain), sodium hydroxide (Probus, Barcelona, Spain), propanol (Panreac), acetyl chloride (Merck), dichloromethane (Panreac), hydrochloric acid (Panreac), anhydrous sodium sulphate (Panreac), toluene (Panreac), monobasic sodium phosphate (NaH_2PO_4) (Probus) and monobasic potassium phosphate (KH_2PO_4) (Scharlau, Barcelona, Spain) were used as received.

The antineoplastic drug 5-fluorouracil (5-FU) ($C_4H_3N_2O_2F$) was supplied by Roche Laboratories (Madrid, Spain) as a crystalline powder.

Doubly distilled and deionized water (Milli-Q system, Millipore, Bedford, MA, USA) was used.

2.2. Monopropyl itaconate synthesis

Monopropyl itaconate (MPI) was obtained by esterification of itaconic acid with propanol, according to the following procedure: 5 ml of freshly distilled acetyl chloride, used as a catalyst, was added dropwise to a mixture of propanol (3 mol) and itaconic acid (1 mol). The mixture reaction was refluxed until complete consumption of the itaconic acid, which was monitored by thin-layer chromatography. The reaction mixture was cooled to room temperature and, after having removed the unreacted propanol under reduced pressure, it was washed with 0.5 *M* NaOH in order to obtain the aqueous phase. This was separated, treated with 0.5 *M* HCl to neutralize the solution and then extracted with dichloromethane. After removing the solvent under reduced pressure, the monopropyl itaconate was crystallized from toluene as a white powder [12]. The monomer was characterized by

Fourier transform IR, ^{1}H NMR and ^{13}C NMR spectrometry [12,13].

2.3. Synthesis of poly(acrylamide–co-monopropyl itaconate) hydrogels

The polymerization of acrylamide–monopropyl itaconate was carried out in cylindrical, siliconized [19,20] glass ampoules. In the feed mixture the monomer-to-water ratio was 60:40 (w/w). Three different acrylamide (A)–monopropyl itaconate (MPI) compositions were studied: 90:10, 75:25 and 60:40 (w/w) . The cross-linking agent was N,N′-methylenebisacrylamide (NBA) (2 wt.% owing to its water solubility) and the initiator was the redox pair $(NH_4)_2S_2O_8$ (1 wt.%)–$Na_2S_2O_5$ (0.4 wt.%); their contents were based on the total monomer mass. The mixture was made up gravimetrically and degassed with nitrogen. The glass ampoules were sealed and placed in an oven at 333K for 2 h.

In order to obtain thin xerogel discs, the amount of the feed mixture components was adjusted to be diluted with 0.5 ml of water in each glass ampoule. After polymerization, the discs were dried at room temperature for 1 week in a reduced environment. The xerogel discs were 4.4 ± 0.3 mm thick and 10.4 ± 0.3 mm in diameter.

2.4. Trapping of 5-fluorouracil in the copolymers

The 5-FU was trapped in the hydrogels by including it in the feed mixture. The solubility of 5-FU in water is low (13 mg/ml) [14]. However, the solubility of the sodium salt increases to 65 mg/ml. In order to trap the maximum amount of 5-FU in the xerogel discs, aqueous solutions of 5-FU neutralized with NaOH were used in the feed mixture instead of water, obtaining disc loads of between 1 and 16 mg of 5-FU, higher than loads obtained when the hydrogel discs are immersed in drug solutions [14]. After polymerization, the samples were optically transparent, showing complete solubility of 5-FU in the copolymeric matrix. The sodium salt of 5-FU is pharmacologically active [21].

2.5. In vitro diffusion studies

2.5.1. Swelling experiments with copolymers in saline solution

The swelling experiments with xerogels (without 5-FU) were conducted at four temperatures between 288 and 310 K in 100 ml of saline solution (0.9 wt.% NaCl) for the three compositions (90:10, 75:25 and 60:40) of poly(acrylamide–co-monopropyl itaconate) hydrogels, in a bath at constant temperature.

The degree of swelling (W_t) was obtained by withdrawing the discs, lightly drying with filter-paper and weighing quickly in a tared sample bottle at different times [22,23]:

$$W_t = \frac{\text{mass of swollen discs} - \text{mass of dry discs}}{\text{mass of swollen discs}} \cdot 100 \quad (1)$$

The equilibrium degree of swelling (W_∞) was reached between 150 and 500 h after immersion of the discs in saline solution, and depended on the temperature and the amount of monopropyl itaconate in the copolymer.

The swelling fraction due to the saline solution uptake, $F_s = W_t / W_\infty$, can be expressed as [24]

$$F_s = 4(D_s t / \pi h^2)^{1/2} \quad (2)$$

where D_s is the apparent diffusion coefficient for the transport of saline solution into the gel, h the thickness of the xerogel disc and t the time. This equation is a solution of Fick's second law under simple boundary conditions, such as swelling in water or biological fluids and simple geometrical forms (discs, cylinders and spheres) [24,25].

The influence of the temperature on the swelling process can be evaluated using the Arrhenius equation:

$$D_s = A \exp(-E_a / RT) \quad (3)$$

where E_a is the activation energy of the process, R the gas constant and T the absolute temperature.

2.5.2. 5-Fluorouracil release experiments

5-FU release from copolymers was determined by placing each xerogel disc with the drug on a holder into a saline solution bath at constant temperature (288, 298, 303 or 310 K) and stirring rate. The volume of saline solution in the vessel was 100 ml. At different times, 50-μl aliquots were withdrawn from the medium in order to monitor 5-FU release; a maximum of 20 aliquots were taken so the vessel volume was considered constant. Drug release was maintained under "sink" conditions [26].

The concentration of 5-FU was measured by high-performance liquid chromatography (HPLC) (Spectra-Physics SP 8800 HPLC pump, SP 100 UV absorbance detector and SP 4400 computing integrator). The stationary phase was Spherisorb ODS, 5-μm (ColoChrom, Cagny, France) (220 × 4.6 mm I.D.). The eluent was 1/75 *M* KH_2PO_4–Na_2HPO_4 buffer solution of pH 7.0 [23]. The flow-rate was 1.0 ml/min and the detection wavelength was 270 nm. 5-FU standards of 1–100 μg/ml were measured to construct a calibration graph. This calibration was computed in the integrator. The 5-FU retention time was 4.8 ± 0.1 min.

Degradation of 5-FU was not observed either during gel synthesis with trapped 5-FU or throughout the entire process of drug release. All the xerogel discs with 5-FU were transparent and all the samples showed a single peak on the chromatograph which corresponded to 5-FU [9,14,27].

Release of 5-FU from the three copolymeric hydrogels was studied at four temperatures (288–310 K) and at six different 5-FU loads at 310 K. Experiments on 5-FU release as a function of temperature (range 288–310 K) were carried out using discs with the same amount of 5-FU in the gel (12 mg of drug per disc) and a similar thickness (4.10 ± 0.15 mm) for each of the three gel compositions. To determine the influence of 5-FU load on drug release from the gels, discs of similar thickness (4.03 ± 0.15 mm) with different drug loads (range 2.65–55.23 kg m^{-3}) were used; the experiments were carried out at 310 K.

The fractional release of 5-FU, $F_{5\text{-FU}}$, can be expressed as [21,27,28]

$$F_{5\text{-FU}} = M_t/M_\infty = 4(D_{5\text{-FU}}t/\pi h^2)^{1/2} \quad (4)$$

where M_t and M_∞ are the amount of drug released at time t and the maximum amount of 5-FU released, respectively, $D_{5\text{-FU}}$ is the apparent diffusion coefficient for 5-FU release from the hydrogel and h is the thickness of the drug-loaded xerogel.

Taking into account that $M_\infty = AV = ASh$, where V is the xerogel disc-loaded volume, S its surface and A the drug load, another expression can be obtained from Eq. 4 [20,29]:

$$\frac{F_{5\text{-FU}}}{t^{1/2}} \cdot Ah = \frac{M_t}{t^{1/2}} \cdot \frac{1}{S} = 4(D_{5\text{-FU}}/\pi)^{1/2}A \quad (5)$$

and $M_t t^{-1/2} S^{-1}$ is the release rate per unit area.

2.6. In vivo diffusion studies

2.6.1. Animals

Male Wistar rats weighing 250 ± 10 g, obtained from the animal department of the Universidad Complutense of Madrid, were used. The animals were kept on a 12-h light, 12-h dark schedule and were fed standard rat food and water ad libitum.

2.6.2. Drug administration

The 75:25 A–MPI composition was chosen, owing to its in vitro diffusion characteristics, for the in vivo studies. The animals were divided into three groups: one group consisted of rats implanted with two xerogel discs (without 5-FU). The animals were anaesthetized with diethyl ether (Panreac) and a single incision 1–2-cm long was made on their backs; blunt-scissor dissection was then used to create lateral implant site by tunnelling immediately beneath the skin in a lateral direction. The implants were then inserted a distance from the incision and it was sutured. This was the control group.

In the second group, two xerogel discs with 5-FU were implanted subcutaneously in the backs of the rats according to the above-described method. Each xerogel disc contained 12.5 mg of 5-FU so the total amount of drug was 25 mg, signifying a dose of 100 mg/kg.

The third group of animals was injected daily intraperitoneally with a 5-FU saline solution

freshly prepared each day. The total amount of drug administered was also 25 mg (total dose 100 mg/kg), and the doses were evenly distributed over the total number of days that 5-FU was released from the implanted gels.

2.6.3. Plasma 5-fluorouracil determination

At predetermined times after drug administration, the animals were anaesthetized with diethyl ether. Blood (1 ml) was collected by puncture of the jugular vein in heparinized (75 units = 15 μl) polypropylene tubes (Leo Laboratories, Madrid, Spain).

The heparinized blood was centrifuged at 8000 *g* for 10 min in a Sigma 202M centrifuge, immediately after collection so as to obtain plasma, which was then stored at −20°C.

Plasma was chromatographed without further treatment. In order to determine 5-FU concentration, saline solutions of 5-FU with concentrations between 0.1 and 100 μl/ml were used as standards for calibration, the same as in the in vitro experiments.

Blood samples were taken from rats implanted with gels 2, 8 and 24 h after the implant and then at 24-h intervals thereafter. When 5-FU was administered intraperitoneally, two samples were taken daily, the first 15 min and the second 2 h after drug injection.

The animals were killed with diethyl ether and then an incision on their backs was made to remove the implanted gel discs. The gel discs were weighed to determine the degree of swelling equilibrium, W_∞. The gel discs used for 5-FU release were placed in a vessel with 100 ml of saline solution, and 1 week later an aliquot was chromatographed to determine the total in vivo 5-FU release from the hydrogel.

3. Results

3.1. In vitro diffusion studies

Swelling of three compositions (90:10, 75:25 and 60:40) of the poly(acrylamide–co-monopropyl itaconate) hydrogel in saline solution (0.9% NaCl) was carried out at four temperatures (range 288–310 K).

The equilibrium degree of swelling (W_∞) for the three gels was 83 ± 2%, independent of their monomer composition. However, the time required to obtain the equilibrium swelling depended on their composition; thus, at 310 K the 90:10 gel required 150 h, whereas 75:25 and 60:40 compositions required 360 and 340 h, respectively. These times increase as the environmental temperature decreases with all gels requiring 500 h to reach an equilibrium degree of swelling at 288 K, indicating that at the lower temperatures studied gel expansion is more dependent on the medium temperature than on the gel composition. In spite of the considerably high equilibrium degree of swelling of these gels, no degradation or structural changes of the discs were observed.

A linear relationship between F_s and $t^{1/2}$ for F_s values less than 0.5, at all temperatures studied, was observed for all gels; an example is shown in Fig. 1a. D_s can be determined from the corresponding slopes. The D_s values (Fig. 2) decrease with increase in temperature for discs of similar thickness and the same monomer composition in the copolymer, indicating that the swelling process is hindered by lower temperatures. There is no linear relationship between D_s values for the swelling of the three copolymers and the amount of MPI of each and the 75:25 gel is the matrix with the slowest saline solution uptake and therefore the smallest D_s; this result is in accordance with the time that this gel requires to reach the swelling equilibrium. These facts could be explained by a differing reactivity of the monomers that make up the gel; thus, when the percentages of these monomers in the feed mixture are changed, this causes a different distribution of monomers in the resulting gel. Each gel can thus be considered as different from the others and to have different diffusion properties. The effect of the monomer composition of the copolymeric hydrogels on their diffusion behaviour has also been observed in HEMA–co-VP gels [30]. The difference in monomer distribution in the poly(acrylamide–co-monopropyl

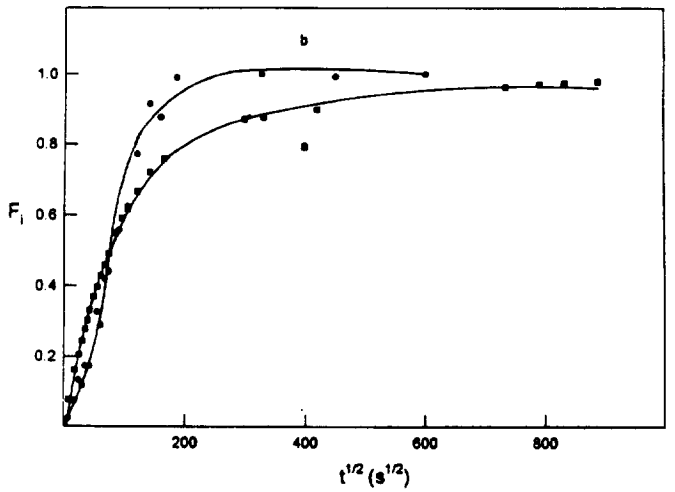

Fig. 1. Representation of the fractional (F_i) (a) swelling in saline solution of an A–MPI (75:25) disc of 4.41 mm thickness and 10.74 mm diameter (for $F_s < 0.5$, $F_s = 0.04 + 6.31 \cdot 10^{-3}\, t^{1/2}$, $r = 0.993$, $D_s = 15.22 \cdot 10^{-11}$ m^2 s^{-1}); and (b) 5-FU release from a 75:25 disc of 4.11 mm thickness and 10.23 mm diameter with 9 mg of 5-FU (for $F_{5\text{-FU}} < 0.5$, $F_{5\text{-FU}} = -0.01 + 5.24 \cdot 10^{-3}\, t^{1/2}$, $r = 0.992$, $D_{5\text{-FU}} = 9.10 \cdot 10^{-11}$ m^2 s^{-1}) versus (time)$^{1/2}$ at 310 K.

itaconate) hydrogels as a function of the monomer ratio is probably due to the size of the itaconate side-chain, since the poly(acrylamide–co-monomethyl itaconate) hydrogels exhibit a linear diffusion behaviour as a function of the amount of monomethyl itaconate in the copolymer, which could be due to the smaller size of the methyl group [18].

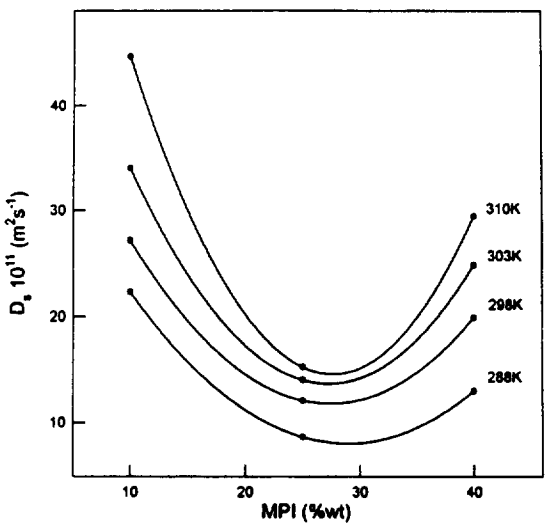

Fig. 2. Variation of the apparent diffusion coefficient (D_s) for saline solution uptake into poly(acrylamide–co-monopropyl itaconate) (A–MPI) gels as a function of the percentage of monopropyl itaconate (MPI) in the gels at four temperatures.

According to the Arrhenius equation (Eq. 3), the relationship between $-\ln D_s$ and $1/T$ exhibits a linear dependence for all gels: 90:10, $-\ln D_s = 10.87 + 3311\ T^{-1}$, $r = 0.991$; 75:25, $-\ln D_s = 13.99 + 2641\ T^{-1}$, $r = 0.995$; and 60:40, $-\ln D_s = 11.03 + 3374\ T^{-1}$, $r = 0.993$. Thus, from each slope, the activation energy (E_a) for saline solution uptake into the gel is obtained (Fig. 3a). The E_a values indicate that the influence of temperature on the swelling kinetics of the gels is much smaller for the 75:25 composition. Again, no linear relationship between the monomer composition of the gels and their behaviour is obtained, which is in accordance with the different monomer distributions in the gels; in poly(acrylamide–co-monomethyl itaconate) hydrogels, the E_a values decrease as the percentage of monomethyl itaconate monomer increases in the copolymer, with similar values to those obtained in this study [18].

In the delivery experiments, the release of 5-FU from each of the three poly(acrylamide–co-monopropyl itaconate) copolymers takes place by penetration of the drug-loaded polymer ma-

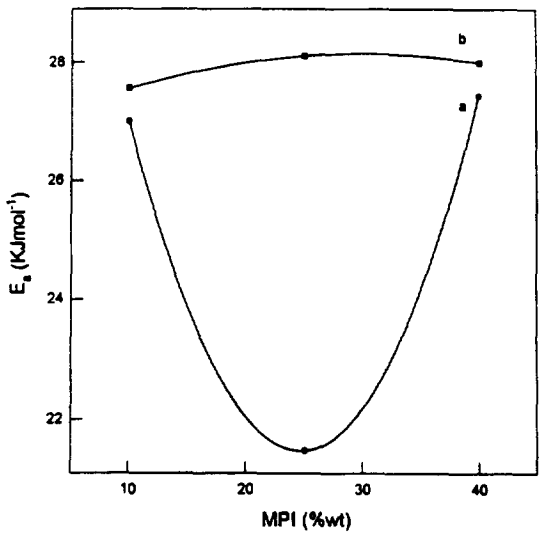

Fig. 3. Dependence of activation energy (E_a) on (a) swelling in saline solution and (b) 5-FU release, with percentage of monopropyl itaconate (MPI) of the gels.

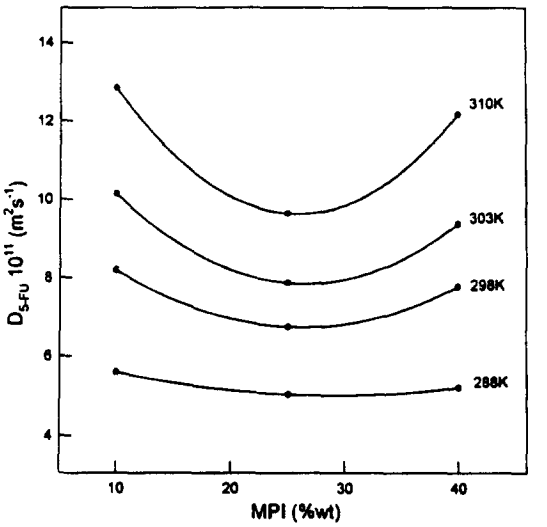

Fig. 4. Variation of the apparent diffusion coefficient ($D_{5\text{-FU}}$) in saline solution for 5-FU release from poly(acrylamide–co-monopropyl itaconate (A–MPI) gels as a function of the percentage of monopropyl itaconate (MPI) in the gels at four temperatures.

trix by the saline solution, which causes it to swell, thus permitting drug delivery.

The fractional release of 5-FU ($F_{5\text{-FU}}$) exhibits a linear behaviour with the square root of time ($t^{1/2}$) for values of $F_{5\text{-FU}}$ less than 0.5 for the three gels studied; an example is given in Fig. 1b. Thus, the 5-FU delivery shows a Fickian diffusion mechanism and the $D_{5\text{-FU}}$ values can be obtained from Eq. 4 [14,27,28]. In all cases, total release of 5-FU was achieved, so no strong interaction of the drug with the gel components takes place during polymerization.

The disc thickness influences the diffusion behaviour and these gels exhibit a large degree of swelling. This means that their initial dimensions change. The Fickian diffusion behaviour would be corroborated if the plot of $F_{5\text{-FU}}$ versus $t^{1/2}/L$, where L is the thickness of the hydrated discs, was linear for each gel and for each 5-FU load of the discs for one gel [31]. In our study, linear relationships were obtained for all three gels: 90:10, $F_{5\text{-FU}} = 0.14 + 0.018t^{1/2}/L$, $r = 0.966$; 75:25, $F_{5\text{-FU}} = -0.02 + 0.047t^{1/2}/L$, $r = 0.975$; and 60:40, $F_{5\text{-FU}} = -0.001 + 0.047t^{1/2}/L$, $r = 0.962$.

The dependence of the diffusion coefficient values for 5-FU release ($D_{5\text{-FU}}$) on the percentage of MPI in the gel at each temperature (Fig. 4) shows that temperature increase improves drug delivery. The slowest 5-FU release takes place from the 75:25 gel. Moreover, at 288 K, 5-FU delivery from the gels is not affected by their monomer composition. Drug solubility in saline solution, which decreases with increase in temperature, is probably a more important factor in release at this temperature (Fig. 4), since the swelling process of the gels in saline solution depends on the gel composition at all temperatures (Fig. 2). In all cases, the diffusion coefficient values for 5-FU release are lower than those obtained for simple swelling in saline solution, which becomes a more complex process.

Application of the Arrhenius equation (Eq. 3) to the 5-FU delivery from the gels shows a linear dependence between $-\ln D_{5\text{-FU}}$ and T^{-1} for each gel: 90:10, $-\ln D_{5\text{-FU}} = 11.84 + 3381\ T^{-1}$, $r = 0.999$; 75:25, $-\ln D_{5\text{-FU}} = 11.72 + 3456\ T^{-1}$, $r = 0.999$; and 60:40, $-\ln D_{5\text{-FU}} = 11.71 + 3446\ T^{-1}$, $r = 0.999$. Thus, from each slope, the activation energy (E_a) for 5-FU release from the gel is obtained (Fig. 3b). These E_a values are independent of the MPI percentage of the gel; the same

behaviour has been observed for the release of 5-FU from poly(acrylamide–co-monomethyl itaconate) hydrogels [18]. When swelling and release processes are compared, from an energetic point of view, the similarity of the activation energies for swelling and their 5-FU delivery values for the 90:10 and 60:40 gels show these two processes to be similar; however, drug release is energetically less favourable than swelling for the 75:25 gel, reflected in the increase in E_a brought about by the drug delivery with respect to the simple uptake of saline solution into the gel (Fig. 3b).

On the other hand, in all cases the drug release process is slower than simple swelling in the early stages (Fig. 1). However, later, when $60 \pm 5\%$ swelling has been reached, the drug delivery behaviour is inverted in all cases, probably owing to the pore size at this degree of swelling being large enough to allow free diffusion of the drug into the saline medium. This is supported by the close similarity of the E_a values for drug release from the three copolymers (Fig. 3b).

In order to determine the influence of the 5-FU load on drug release from the gels, the release rate per unit area ($M_t t^{-1/2} S^{-1}$) versus disc load (A) was plotted (Eq. 5). A linear relationship was obtained for each gel: 90:10, $M_t t^{-1/2} S^{-1} = -2.26 \cdot 10^{-6} + 2.60 \cdot 10^{-5}\ A$, $r = 0.999$; 75:25, $M_t t^{-1/2} S^{-1} = -3.57 \cdot 10^{-6} + 2.45 \cdot 10^{-5}\ A$, $r = 0.997$; and 60:40, $M_t t^{-1/2} S^{-1} = -1.44 \cdot 10^{-6} + 2.59 \cdot 10^{-5}\ A$, $r = 0.998$. Also, a diffusion coefficient independent of drug load can be obtained from the slope, the values being $13.29 \cdot 10^{-11}$ $m^2\ s^{-1}$ for the 90:10 gel, $11.79 \cdot 10^{-11}$ $m^2\ s^{-1}$ for the 75:25 gel and $13.20 \cdot 10^{-11}$ $m^2\ s^{-1}$ for the 60:40 gel. These results show that the gels with both high and low MPI content exhibit very similar drug delivery behaviour, whereas the 75:25 gel releases 5-FU more slowly, as reflected by the diffusion coefficient independent of the load.

3.2. In vivo diffusion studies

In order to determine the applicability of these copolymers, the 75:25 gel was subcutaneously implanted in rats. This monomer gel composition was chosen because it exhibits the best control of 5-FU release in in vitro experiments.

The influence of the method of 5-FU administration on the plasma level of this drug was studied. The pharmacokinetics of 5-FU administered via subcutaneously implanted hydrogels were compared with those obtained when it was administered intraperitoneally. In both cases, the 5-FU dose was 100 mg/kg. This dose is in accordance with those used in 5-FU perfusion experiments in rats of between 0.5 and 80 mg/kg · h every 6 h [32]. Likewise, the dose used in this study is similar to that recommended for average risk patients, that is between 12 and 15 mg/kg per day for 9 days administered by rapid injection [33].

The 5-FU could no longer be detected in the plasma of the implanted rats approximately 72 h after the gel implant and the animals were killed on the sixth day after the implant. This was carried out in order to observe the effect of this copolymeric hydrogel on the surrounding tissues. From a macroscopic point of view, the hydrogel was well tolerated (Fig. 5).

The degree of swelling of the implanted hydrogels was $87.5 \pm 0.5\%$, slightly higher than that determined in in vitro experiments for this copolymer composition, hence it is probably due to other physiological substances rather than to the

Fig. 5. Photograph of A–MPI (75:25) hydrogel discs after 6 days of subcutaneous implantation in a male Wistar rat.

contribution of the saline solution to the disc swelling. The hydrogels reach the equilibrium degree of swelling when they are subcutaneously implanted, hence this physiological environment hydrates the gel sufficiently for 5-FU to be completely released from the copolymer discs.

The release of 5-FU from the implants was followed by HPLC via the measurement of plasma concentrations at each given time. In the implant (75:25 composition) rat group (Fig. 6), the 5-FU plasma concentration decreases from 0.79 ± 0.30 to 0.43 ± 0.20 μg/ml, with the latter concentration remaining constant on the second day; 5-FU was not detected on the third day.

Because release of 5-FU from the implants takes place in about 48 h, intraperitoneal administration of 5-FU was carried out over two days, the total dose being 100 mg/kg (Fig. 6).

As described for GC [34] and HPLC [35], after intravenous injection, the average time of plasma clearance of this drug is 10–20 min. About 1.5–2 h later it is not detected in plasma. The plasma level of 5-FU after intraperitoneal injection was thus determined by taking two blood samples at 15 min and at 2 h. The 5-FU concentration was 28.3 ± 4 μg/ml 15 min after drug administration and no drug was detected after 2 h.

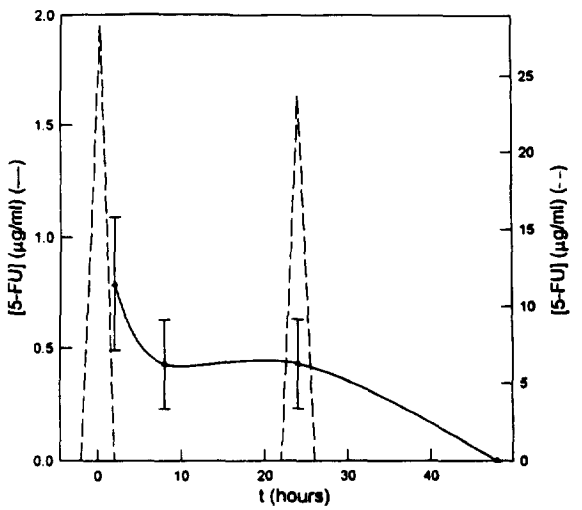

Fig. 6. Representation of plasma concentration of 5-FU versus time of treatment (t) for the rat group implanted with A–MPI (75:25) gel discs (solid line) and those injected intraperitoneally (dashed line).

4. Discussion

One of the most important characteristics of a hydrogel is its equilibrium water content because the water it contains is proportional a priori to its biocompatibility. Moreover, the mechanical properties of a hydrogel have to permit it to maintain its structure without degradation or cracking.

The poly(acrylamide–co-monopropyl itaconate) hydrogels exhibit the latter characteristic because they have a high equilibrium degree of swelling and in this swelling state they maintain their structure. Swelling degrees similar to those obtained for these gels have also been determined for PVP hydrogels cross-linked with 5% (w/w) EGDMA [36] and for HEMA–co-VP copolymers with 70% VP without cross-linker [30], which contrasts with the lower degree of swelling in the PHEMA hydrogels [14], which are those most frequently used for medical and pharmaceutical applications [37]. Although poly(acrylamide–co-monopropyl itaconate) hydrogels have a large equilibrium degree of swelling, their apparent diffusion coefficients for uptake of saline solution are similar to those recorded for PHEMA hydrogels cross-linked with EGDMA, whose equilibrium degree of swelling in water is about 30 wt.% [14,29]. Thus, these hydrogels have a similar swelling rate to that of PHEMA gels but have a greater amount of aqueous solution in their polymeric matrix, which makes them more similar to biological tissues.

One of the most common antineoplastic drugs used for the treatment of several malignancies is 5-FU. Owing to its high toxicity, it is a good candidate for controlled-release technology in order to obtain a therapeutic effect in situ and minimize collateral effects of the drug.

Different hydrogels have been used to release 5-FU, some of which are based on HEMA. Thus,

5-FU has been included in PHEMA hydrogels cross-linked with EGDMA using the absorption method, and a maximum of 9 mg of 5-FU were included in discs of 1.4 ± 0.3 mm thick with a diameter ranging from 12.2 to 14.8 mm [14]. The total release of 5-FU from these hydrogels takes place over 44 h when the degree of cross-linking is 5 wt.%.

Likewise, poly(2-hydroxyethyl methacrylate–bisglycolacrylate) copolymeric hydrogels, polymerized as spherical beads (3 mm diameter) with a maximum of 0.53 mg of 5-FU per bead, were used, and the release took place in about 20 h when the equilibrium degree of swelling of the copolymer was 85 wt · % [15].

The poly(acrylamide–co-monopropyl itaconate) hydrogels allow 5-FU to be trapped in the polymerization feed mixture and a maximum of 16 mg of drug can be included in each disc. Total release of the drug takes place in 100 h from 75:25 hydrogels at 310 K. Hence these copolymeric hydrogels not only allow more 5-FU to be trapped in the gel but also the total release time increases compared with hydrogels with HEMA.

Although some hydrogels have been designed as a polymeric matrix for 5-FU release and 5-FU delivery from these polymeric devices has been studied in vitro, there are few studies on its release from hydrogels in in vivo systems. Thus, 5-FU was included in collagen–poly(2-hydroxyethyl methacrylate) hydrogels with a small amount of collagen and 5-FU-loaded hydrogel pellets were subcutaneously implanted in female Wistar rats in the proximity of a solid fibrosarcoma. Total 5-FU release took place in about 10 days in in vitro experiments but no 5-FU plasma level determinations were carried out when 5-FU-loaded hydrogels were implanted subcutaneously. Histopathological studies of these implanted hydrogels (without 5-FU) indicated that the tissue response progressed from initial acute inflammation to a chronic inflammatory response after 7 days. After 2 weeks, the onset of capsule formation was observed around the implant. Also, a tissue inflammatory response and a very thin fibrous capsule around the implant have been described when PHEMA hydrogels or ones using HEMA as a base were implanted in the backs of Wistar rats [5,28].

Six days after subcutaneous implantation, the poly(acrylamide–co-monopropyl itaconate) hydrogels, from a macroscopic point of view, exhibited good biocompatibility because no thin fibrous capsule was formed around the discs and no inflammatory response of the surrounding tissues was observed (Fig. 5). The hydrogel discs were totally transparent and no degradation was seen. The discs maintained their structure and fissures and mass leakage were not observed in any case (Fig. 5). The large amount of aqueous solution that these poly(acrylamide–co-monopropyl itaconate) hydrogels can take up into their structure make them very similar to living tissues and this could explain the fact that the 75:25 polymeric matrix is well tolerated.

The application of the 75:25 hydrogel to 5-FU release in vivo results in plasma 5-FU concentrations between 0.43 ± 0.2 and 0.79 ± 0.30 μg/ml. When cancer patients are treated with intravenous infusion of 5-FU, the normal levels of this drug in the plasma are between 10 and 500 ng/ml [38]; however, 5-FU concentrations of 30 μg/ml are predictive of toxicity from a clinical point of view, and are often associated with myelosuppression and gastrointestinal toxicity [39]. Thus, the constant plasma level of 5-FU that results from drug release from 75:25 hydrogels is not toxic, whereas if 5-FU is administered intraperitoneally the plasma concentration of the drug obtained can be considered predictive of toxicity.

The results of this work show that the administration of 5-FU in implants of 75:25 copolymeric hydrogel from which the drug is released results in, from a therapeutic viewpoint, suitable levels of 5-FU in plasma for a definite period of time, despite the short average half-life of this drug. Thus, owing to the high equilibrium swelling degree of these copolymeric hydrogels, which makes them a priori highly biocompatible, implantation of these slow-release devices containing 5-FU would permit the drug dosage to be close to its target, for example a solid tumour, and would decrease, at least in part, the secondary effects of this drug.

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