J. Pharm. Pharmacol. 1998, 50: 703–712
Received December 11, 1997
Accepted March 4, 1998

Poly(acrylamide-co-monoethyl Itaconate) Hydrogels as Devices for Cytarabine Release in Rats

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Abstract

This study has tested the application of three different copolymeric poly(acrylamide-co-monoethyl itaconate; A/MEI) hydrogels, 90A/10MEI, 75A/25MEI and 60A/40MEI, on the release of cytarabine (ara-C).

The drug was incorporated in gels by placing it in the polymerization feed mixture and discs loaded with 5–50 mg ara-C were obtained. The amount of swelling at equilibrium in saline solution (NaCl, 0·9% w/w) was between 78 and 82% w/w, depending on the composition of the copolymer. The diffusion studies followed Fick's second law. The diffusion coefficients for swelling of the gels were between $9{\cdot}30 \times 10^{-11}$ m^2 s^{-1} and $37{\cdot}42 \times 10^{-11}$ m^2 s^{-1}; those for release of ara-C were between $3{\cdot}42 \times 10^{-11}$ m^2 s^{-1} and $10{\cdot}25 \times 10^{-11}$ m^2 s^{-1}. The activation energies for swelling were in the range $16{\cdot}60 \pm 2{\cdot}59$–$21{\cdot}85 \pm 1{\cdot}78$ kJ mol^{-1}; those for ara-C release were $28{\cdot}13 \pm 3{\cdot}1$–$29{\cdot}7 \pm 4{\cdot}6$ kJ mol^{-1}. To determine the applicability of these copolymers, 75A/25MEI gel was subcutaneously implanted in rats and the plasma concentration of the drug was determined by high-performance liquid chromatography. The concentration of ara-C in plasma (range $17{\cdot}67 \pm 5{\cdot}68$–$10{\cdot}76 \pm 2{\cdot}15$ μg mL^{-1}) was maintained during the first stages (2–8 h) and no drug was detected after 32 h. This route of administration was compared with intraperitoneal injection of the drug.

In conclusion, ara-C can be incorporated in hydrogels and released in a pharmacologically active form. The concentration of ara-C in plasma is maintained for long enough to improve therapeutic results.

Hydrogels are hydrophilic polymer networks capable of absorbing large amounts of water, yet are insoluble because of the presence of physical or chemical cross-links, entanglements or crystalline regions. They can be used in biomedical applications, for example as drug-delivery systems, biosensors, contact lenses, catheters and wound dressings (Peppas & Korsmeyer 1987). Drug-delivery systems are of considerable interest owing to the possibility of releasing bioactive molecules both at a sustained rate and at a specific site, furnishing a high local concentration (Rihova & Kopecek 1986; Roorda et al 1986).

Cytosine arabinoside (1-β-D-arabinofuranosylcytosine; ara-C) is a leading drug for the treatment of haematological malignancies (Vogler 1992; Kufe 1985). It is one of the most thoroughly studied drugs in cancer chemotherapy and, accordingly, its pharmacokinetics, metabolism and mechanisms of action have been extensively reviewed (Cohen 1966; Grant 1990). Nevertheless, it is surprising that there is still no clear consensus on an optimum dose rate or schedule for ara-C administration (Capizzi et al 1991). Although it is rapidly cleared metabolically by deamination to the inactive arabinosyluracil, the relatively high solubility of ara-C enables ara-C dose rates to be varied to achieve the plasma concentrations required for manipulation of the formation of ara-C triphosphate (ara-CTP) (Plunkett & Gandhi 1992).

The aim of this study was to prepare a polymeric hydrogel in which ara-C could be incorporated. The polymers chosen were copolymers of acrylamide (A) and monoethyl itaconate (MEI), a monoester derived from itaconic acid. Monoesters derived from itaconic acid (Katime et al 1993) are structurally similar to acrylic derivatives, and so can be copolymerized with a large number of monomers.

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They have hydroxylic groups in their molecules which make them highly hydrophilic. Because of the hydrophilic character of this copolymer, it is capable of absorbing a large amount of water which makes it resemble living tissue, a characteristic which improves its biocompatibility.

Materials and Methods

Materials

Itaconic acid (methylenesuccinic acid) (Merck), acrylamide (Merck), *N,N'*-methylenebisacrylamide (NBA) (Merck), ammonium peroxodisulphate ($(NH_4)_2S_2O_8$) (Merck), sodium disulphite ($Na_2S_2O_5$) (Merck), sodium chloride (Panreac), ethanol (Panreac), acetyl chloride (Merck), dichloromethane (Panreac), sodium hydroxide (Probus), hydrochloric acid (Panreac), anhydrous sodium sulphate (Merck), toluene (Panreac), heparin (Analema, Vigo, Spain), tetrahydrouridine (THU; Upjohn Farmoquimica, Madrid, Spain), diethyl ether anaesthetic (Panreac), $NaH_2PO_4.H_2O$ (Probus), $Na_2HPO_4.2H_2O$ (Merck) and arabinofuranosiluracilo (ara-U) (Sigma, St Louis, MO) were used as received.

The anticancer drug, cytarabine (ara-C), molecular weight 248, was supplied by Upjohn Farmoquimica (Madrid, Spain) as a crystalline powder, purity 99·7%.

Water was doubly distilled and deionized from a Milli-Q system (Millipore, Bedford, MA).

Monoethyl itaconate synthesis

MEI was obtained by esterification of itaconic acid with ethanol. Freshly distilled acetyl chloride (5 mL), used as catalyst, was added dropwise to a mixture of ethanol (3 mol) and itaconic acid (1 mol) and the reaction mixture was heated under reflux until complete consumption of the itaconic acid (monitored by thin-layer chromatography). The reaction mixture was cooled to room temperature, the unreacted methanol was removed under reduced pressure, and the residue was washed with aqueous 0·5 M NaOH to extract the product into the aqueous phase; this was separated, treated with 0·5 M HCl to neutralize the solution and then extracted with dichloromethane. After removal of the solvent under reduced pressure, the MEI was re-crystallized from toluene as white needles. The monomer was characterized by fourier-transform infrared (FTIR) spectroscopy and by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Wang & Samji 1981; Cesteros et al 1994).

Synthesis of poly(acrylamide-co-monoethyl itaconate) hydrogels

Three different acrylamide–MEI compositions were studied: 90A/10MEI, 75A/25MEI and 60A/40MEI (w/w). In the feed mixture the monomer-to-water ratio was 60/40. The cross-linking agent was *N,N'*-methylenebisacrylamide (NBA) (2% w/w owing to its solubility in water) and the initiator was the redox pair $(NH_4)_2S_2O_8$ (1% w/w)–$Na_2S_2O_5$ (0·4% w/w), the contents being based on the total mass of monomers. The mixture was prepared gravimetrically and degassed with nitrogen. The glass ampoules were sealed and placed in an oven at 313 K for 2 h.

To obtain thin xerogel discs the amounts of the feed mixture components were adjusted so that the final volume of water in each ampoule was 0·5 mL. Thus monomers were dissolved in the solution of cross-linking agent (0·25 mL) and 0·25 mL of aqueous initiator solution was added. To prepare hydrogels containing drug, ara-C was included in the feed mixture by dissolving it in the aqueous solution of initiator. Discs without drug and discs loaded with 5, 10, 25 and 50 mg ara-C were synthesized. After polymerization the samples were optically transparent, showing complete solubility of ara-C in the copolymer matrices. Discs were dried at room temperature for one week in a dehydrating environment. The xerogel discs were $4{\cdot}4 \pm 0{\cdot}4$ mm thick and $10{\cdot}4 \pm 0{\cdot}3$ mm in diameter, thus the coefficients of variation for disc dimensions were 9% for thickness and 3% for diameter.

All experiments were performed in triplicate.

In-vitro diffusion studies

To determine swelling behaviour three poly (acrylamide-co-monoethyl itaconate) hydrogels of different composition (90A/10MEI, 75A/25MEI and 60A/40MEI) (without ara-C) were placed in saline solution (0·9% NaCl; 100 mL) at four different constant temperatures between 288 and 310 K. The amount of swelling (W_t) was determined at different times by withdrawing the discs, lightly drying them with filter paper and quickly weighing them ($\pm 10^{-4}$ g) in a tared sample bottle by means of an electronic balance. W_t was calculated by use of the expression (Kou et al 1988; Allen et al 1992a):

$$W_t = ((\text{weight of swollen disc} - \text{weight of dry disc}) / (\text{weight of swollen disc})) \times 100$$

The extent of equilibrium swelling of the hydrogel (W_∞) was reached when the weight of the swollen disc was constant.

In-vitro release studies

Release experiments were performed by placing the disc in a highly perforated glass holder, in a double-walled vessel (7 cm × 5·5 cm i.d.), connected to a water-recirculating thermostat, so that the position of the disc in the vessel was at the same height in all experiments. The volume of saline solution (NaCl 0·9% w/w) was 100 mL. The vessel was covered with laboratory film (Parafilm). The stirring rate was constant at approximately 500 rev min^{-1} (Velt multi-position electromagnetic stirrer). At different times, samples (50 μL) were withdrawn from the medium to monitor ara-C release; a maximum of 20 samples was taken, so the vessel volume can be considered constant. The concentration of ara-C in the release medium was always less than 10% of the maximum solubility of ara-C (sink conditions) (Song et al 1981; Duerloo et al 1990). The experiments were performed in the dark.

Ara-C concentrations were measured by UV-vis spectroscopy at 272 nm (Unicam 8700 series spectrophotometer) employing a 1-cm path-length microcuvette (50 μL). Ara-C standards of 1–100 μg mL^{-1} were used to obtain a calibration curve.

Ara-C degradation was not observed either during loading of the gels or during drug release. All xerogel discs containing ara-C were transparent and all of the samples gave an absorption spectrum of ara-C. The lack of degradation of ara-C was confirmed by high-performance liquid chromatography (HPLC) (Spectra-Physics SP 8800 HPLC pump, SP 100 ultraviolet detector and SP 4400 computing integrator). The stationary phase was 5-μm Spherisorb ODS2 (25 cm × 0·46 cm); the mobile phase was 0·005 M monobasic sodium phosphate in distilled water containing 5% v/v methanol (Quock & Sakai 1985). The flow rate was set at 1·2 mL min^{-1} and the detector wavelength was 272 nm. The chromatogram of the samples contained a single peak (retention time 2·8 ± 0·4 min) belonging to ara-C (Breithaupt & Schick 1981; Quock & Sakai 1985).

Ara-C release from the three copolymeric hydrogels was studied at four temperatures (288, 298, 303 and 310 K) for discs containing 50 mg ara-C and at one temperature, 310 K, for discs containing four different amounts of ara-C (5, 10, 25 and 50 mg/disc).

In-vivo diffusion studies

Animals. Male Wistar rats, 234 ± 2 g, were obtained from the animal department of the Universidad Complutense de Madrid. The animals were kept on a 12-h light–dark schedule and standard rat food and water were freely available.

Drug administration. The 75A/25MEI copolymer was chosen to study the release of ara-C in-vivo. The animals were divided into three groups of five rats. One xerogel disc without ara-C was implanted in rats in the control group. The animals were anaesthetized with diethyl ether and a single 1–2 cm incision was made on their backs; blunt-scissor dissection was then used to create an implant site by tunnelling immediately beneath the skin in a lateral direction. The implants were then inserted a distance from the incision, which was then sutured.

One xerogel disc containing 50 mg ara-C was implanted subcutaneously in the backs of the rats in the second group, by means of the method described above.

The third group of animals was injected daily, intraperitoneally, with a saline solution of ara-C prepared freshly each day. The total amount of drug administered was also 50 mg (total dose 201 mg kg^{-1}) and the doses were distributed evenly over the total number of days that ara-C was released from the implanted gels.

Plasma ara-C concentration. Ara-C and ara-U plasma levels were determined by HPLC (Breithaupt & Schick 1981). The animals were anaesthetized with diethyl ether, and blood (1 mL), obtained by puncture of the jugular vein, was collected in polypropylene tubes containing heparin (15 μL, 75 units) and tetrahydrouridine (final concentration 10^{-3} M; to prevent deamination of ara-C to ara-U).

Immediately after collection the heparinized blood was centrifuged at 12 000 *g* for 10 min in a Sigma 202 M centrifuge and the plasma obtained was stored at 253 K.

The concentration of ara-C was measured by HPLC after addition of trichloroacetic acid (2 M, 5 μL) to plasma (100 μL) to precipitate plasma proteins. This treatment of the samples did not affect ara-C levels. Saline solutions of ara-C and ara-U with concentrations between 1 and 100 μg mL^{-1} were used as calibration standards. The retention time was 2·8 ± 0·4 min for ara-C and 3·5 ± 0·4 min for ara-U.

Blood samples were taken from rats implanted with gels 2, 5, 8 and 24 h after the implant and then at 24-h intervals. For rats which received ara-C by intraperitoneal administration, two samples were taken daily, the first 30 min and the second 4 h after drug injection.

The pharmacokinetic parameters were calculated as reported by Kreis et al (1985) and Tyle et al (1989).

The animals were killed with diethyl ether and an incision was made on their backs to remove the

implanted gel discs. These were weighed to determine the extent of swelling at equilibrium, W_∞. The gel discs used for ara-C release were placed in a vessel with 100 mL saline solution and one week later a sample was chromatographed to determine the total in-vivo release of ara-C from the hydrogel.

Results and Discussion

In-vitro diffusion studies

Swelling of three compositions (90A/10MEI, 75A/25MEI and 60A/40MEI) of the poly (acrylamide-co-monoethyl itaconate) hydrogel in saline solution (0·9% NaCl) was investigated at four temperatures (range 288–310 K). The extent of swelling at equilibrium (W_∞) for the three gels was 81·5 ± 0·5% w/w (90A/10MEI), 80·2 ± 0·3% w/w (75A/25MEI) and 79·0 ± 1·1% w/w (60A/40MEI). Despite the considerably large amount of swelling of these gels at equilibrium, no degradation or structural changes of the discs were observed. The time required for equilibrium swelling depended on composition; thus at 310 K the 90A/10MEI gel required 75 h, whereas the 75A/25MEI and 60A/40MEI compositions required 160 h and 90 h, respectively. These times increase as the environmental temperature decreases—the gels require 173 h (90A/10MEI), 311 h (75A/25MEI) and 215 h (60A/40MEI) to reach equilibrium swelling at 288 K, indicating that at the lowest temperatures studied gel expansion is more dependent on the temperature of the medium than on the gel composition (Table 1).

Values of W_∞ as high as these have been observed before, e.g. for poly(vinyl pyrrolidone) hydrogels cross-linked with 5% ethylene glycol dimethacrylate (Davis et al 1988) and for hydroxyethyl methacrylate-co-vinyl pyrrolidone copolymers with 70% poly(vinyl pyrrolidone) without cross-linker (Davis & Huglin 1989), which contrasts with the extent of swelling observed for poly(-hydroxyethyl methacrylate) hydrogels (García et al 1994), which have been widely studied. From the point of view of possible in-vivo implantation, the high water content of these poly(acrylamide-co-monoethyl itaconate) copolymeric hydrogels is quite important with regard to their biocompatibility.

For a diffusion-controlled process, the fractional swelling as a result of saline solution, F_S, can be expressed as:

$$F_S = W_t/W = 4(D_S t/\pi h^2)^{1/2} \qquad (1)$$

where D_S is the apparent diffusion coefficient for the transport of the saline solution into the hydrogel, t the time and h the dry thickness of the xerogel discs. Equation 1 is a solution of Fick's second law under simple boundary conditions, such as swelling in water or biological fluids and simple geometrical forms (disc, cylinders and spheres) (Crank & Park 1968; Korsmeyer & Peppas 1983). When F_S values are plotted against $t^{1/2}$, at four temperatures (288, 298, 303 and 310 K) for discs of the same thickness, for the first states of swelling ($F_S < 0·6$), a linear relationship is obtained between F_S and $t^{1/2}$. An example is shown in Figure 1. Thus, D_S can be obtained directly from the corresponding slopes. The results are shown in Table 1.

The diffusion coefficient for the transport of saline solution into the gel decreases when the percentage of MEI in the gel is increased, at each of the temperatures studied. Likewise, the temperature increment produces a larger diffusion coefficient for discs of similar thickness and equal monomer composition, indicating that the temperature increase favours the uptake of saline solution by the gel.

These values of D_S are clearly higher than those obtained for other hydrogels. For example, poly(2-hydroxyethyl methacrylate) hydrogels containing 26% water and 1% ethylene glycol dimethacrylate

Table 1. Time needed by poly(acrylamide-co-monoethyl itaconate) gels to reach equilibrium swelling in saline solution at different temperatures, and values of apparent diffusion coefficient for swelling ($D_S \times 10^{11}$ m^2 s^{-1}) from poly(acrylamide-co-monoethyl itaconate) gels.

Poly(acrylamide-co-monoethylitaconate) gel	Temperature (K)							
	310		303		298		288	
	Time (h)	$D_S \times 10^{11}$	Time (h)	$D_S \times 10^{11}$	Time (h)	$D_S \times 10^{11}$	Time (h)	$D_S \times 10^{11}$
90A/10MEI	75 ± 6	37·42 ± 0·08	144 ± 13	29·95 ± 0·01	166 ± 11	24·69 ± 0·04	173 ± 7	19·54 ± 0·04
75A/25MEI	160 ± 16	19·94 ± 0·01	300 ± 5	18·11 ± 0·02	311 ± 23	14·39 ± 0·03	311 ± 23	12·38 ± 0·01
60A/40MEI	90 ± 13	17·06 ± 0·05	167 ± 18	13·04 ± 0·03	167 ± 18	11·52 ± 0·03	215 ± 5	9·30 ± 0·01

Results are means ± s.d., n = 3.

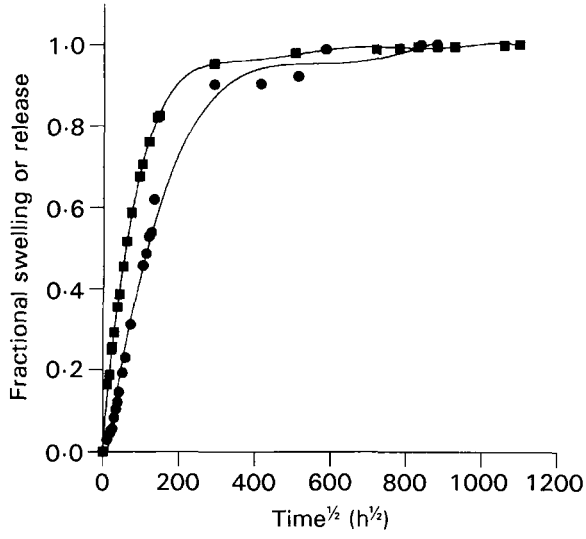

Figure 1. Plot of (■) fractional swelling in saline solution of a 75A/25MEI disc (4·05 mm thick, 10·31 mm diam.) and (●) fractional release of ara-C from a 75A/25MEI disc (4·25 mm thick, 10·15 mm diam.) loaded with 50 mg ara-C at 310 K. The coefficient of variation was 4·5% for swelling and 2·3% for release of ara-C.

in the feed mixture yield a D_S of $2{\cdot}4 \times 10^{-11}$ m^2 s^{-1} (Khaw et al 1976). For poly(2-hydroxyethyl methacrylate) hydrogels synthesized with azo-bis-isobutyronitrile as initiator and 0·5% ethylene glycol dimethacrylate, D_S values are between $1{\cdot}77 \times 10^{-11}$ and $3{\cdot}99 \times 10^{-11}$ m^2 s^{-1} for the temperature range 285–308 K (Trigo et al 1993). However, these values are similar to values obtained with copolymeric hydrogels of itaconic acid (acrylamide-co-monomethyl itaconate and acrylamide-co-monopropyl itaconate) (Blanco et al 1996a, b). This, and their greater swelling, is favourable for high biocompatibility because it increases the similarity of the gels to living tissues.

The Arrhenius equation:

$$D_S = Ae^{(-E_a/RT)} \qquad (2)$$

shows the dependence of the diffusion coefficient on temperature (T), E_a being the activation energy of the diffusion process and R the gas constant. According to equation 2 there is a linear relationship between $\ln D_S$ and 1/T. The correlation equations obtained for these gels were: 90A/10MEI, $-\ln D_S = 13{\cdot}29 + 2619{\cdot}61T^{-1}$, $r = 0{\cdot}99$; 75A/25 MEI, $-\ln D_S = 15{\cdot}92 + 1990{\cdot}58T^{-1}$, $r = 0{\cdot}97$; and 60A/40MEI, $-\ln D_S = 14{\cdot}89 + 2380{\cdot}25T^{-1}$, $r = 0{\cdot}99$. The activation energy (E_a) for uptake of saline solution into the gel is obtained from the slopes (Figure 2); the values indicate that the influence of temperature on the swelling kinetics of the gels is much smaller for the 75A/25MEI composition. This is in accordance with the time needed for this gel to reach the swelling equilibrium. These facts could be explained by the differing reactivity of the monomers that make up the gel; thus changing the percentages of these monomers in the feed mixture results in 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 monomer composition on the diffusion behaviour of the copolymeric hydrogels has also been observed for other hydrogels (Blanco et al 1996b).

The release of ara-C from each poly(acrylamide-co-monoethyl itaconate) copolymer occurs as a result of penetration of the drug-loaded polymer matrix by the saline solution; the polymer starts to swell enabling drug release. For the three gels studied the fractional release of ara-C ($F_{ara\text{-}C}$) is linearly dependent on the square root of time ($t^{1/2}$) for values of $F_{ara\text{-}C} < 0{\cdot}6$ (Figure 1). Thus, ara-C delivery follows a Fickian diffusion mechanism and the $D_{ara\text{-}C}$ values can be obtained from (Korsmeyer & Peppas 1984):

$$F_{ara\text{-}C} = M_t/M_\infty = 4(D_{ara\text{-}C}t/\pi h^2)^{1/2} \qquad (3)$$

where M_t and M_∞ are, respectively, the amount of drug released at time t and the maximum amount of ara-C released (total release of the ara-C trapped in the discs was achieved). $D_{ara\text{-}C}$ is the apparent diffusion coefficient for ara-C release from the hydrogel and h is the thickness of the drug-loaded xerogel. In accordance with equation 3 the slopes of these plots yield $D_{ara\text{-}C}$ for each gel.

Experiments to determine release of ara-C as a function of temperature (between 288 and 310 K) were performed with discs of similar thickness

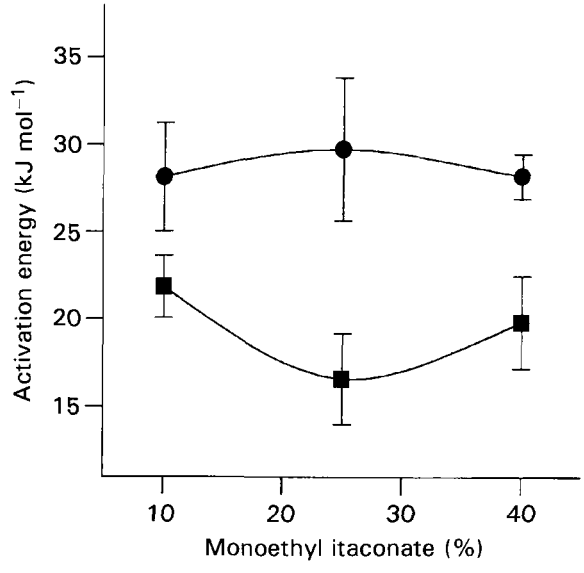

Figure 2. Dependence of activation energy of (■) swelling in saline solution and (●) ara-C release on the amount of monoethyl itaconate in the gels.

(4·37 ± 0·36 mm) containing an equal amount of ara-C (50 mg/disc) for the three gels. The values of $D_{ara\text{-}C}$ are given in Table 2. For each gel, drug release increases with temperature; this is reflected in an increment in the value of the apparent diffusion coefficient. At a constant temperature, as the amount of MEI increases in the gel drug release into the medium is slower.

When diffusion coefficients for ara-C release are compared with those for swelling, it is observed that those for ara-C release are always smaller, because it is a more complex process; this is in accordance with data from similar experiments (García et al 1994; Trigo et al 1994).

With regard to the energetic behaviour of these copolymers in ara-C release, by plotting the Arrhenius equation it can be shown that there is a linear dependence between $-\ln D_{ara\text{-}C}$ and T^{-1} for each gel: 90A/10MEI, $-\ln D_{ara\text{-}C} = 12{\cdot}06 + 3372{\cdot}90T^{-1}$, $r = 0{\cdot}97$; 75A/25MEI, $-\ln D_{ara\text{-}C} = 11{\cdot}57 + 3565{\cdot}16T^{-1}$, $r = 0{\cdot}96$; and 60A/40MEI, $-\ln D_{ara\text{-}C} = 12{\cdot}35 + 3381{\cdot}61T^{-1}$, $r = 0{\cdot}99$. Thus the activation energy (E_a) for ara-C release from the gel is obtained from each slope (Figure 2). The E_a values for release of ara-C from the gels are independent of the amount of MEI in the gel. These E_a values for the release of the drug show that the influence of temperature on the kinetics of release from the gels is almost equal, probably because the pore size of these gels is sufficiently large, at any temperature and any composition studied, from the early stages of the release process, to enable fairly rapid release of ara-C.

For all gels the process of release of ara-C is, energetically, more difficult than swelling, as the activation energy data for the processes show (Figure 2). Alternatively, the release process is slower than swelling for short release times (Figure 1, Tables 1 and 2). On the other hand, total release of ara-C at 310 K occurs between 3 and 7 days; 3 days for 90A/10MEI, 7 days for 75A/25MEI and 4 days for 60A/40MEI, irrespective of the loading of the discs. The time necessary for total release of ara-C from the gels is in accordance with the total swelling. Although the complete release from 25A/75MEI gels occurs in 7 days, release is 80% complete within 24 ± 5 h, so the remaining 20% is released over a period of 6 days (Figure 3).

The influence of loading on ara-C release at 310 K was determined by use of discs of similar thickness loaded with 5–50 mg ara-C. For each gel drug release becomes more favourable as the load increases; this is reflected in an increment of the diffusion coefficient value (Table 2). Because $M_\infty = AV = ASh$, where V is the disc volume and S its surface area, equation 3 can be expressed:

$$(F_{ara-C}/t^{1/2})Ah = (M_t/t^{1/2})(1/S) = 4(D_{ara-C}/\pi h^2)^{1/2}A \quad (4)$$

where $(M_t/t^{1/2})(1/S)$ is the release rate per unit disc area. A plot of this against the drug loading (A) of the disc yields a straight line for each gel (Figure 4) and the slope of these plots gives a diffusion coefficient that is not dependent on disc loading. This enables determination of a unique diffusion coefficient for the release of this drug from each of these hydrogels, irrespective of the amount of ara-C in the discs, so the effect of drug loading on drug diffusion is eliminated. These diffusion coefficients are for each gel: 90A/10MEI, $10{\cdot}65 \times 10^{-11}$ $m^2\ s^{-1}$; 75A/25MEI, $8{\cdot}79 \times 10^{-11}$ $m^2\ s^{-1}$; 60A/40MEI $7{\cdot}78 \times 10^{-11}$ $m^2\ s^{-1}$. Increasing the amount of MEI in the copolymer results in slower release of ara-C from the gel; this is reflected in the value of diffusion coefficients independent of disc loading.

Diffusion coefficients for ara-C release from gels of poly(2-hydroxyethyl methacrylate) (Trigo et al 1994) were smaller than those from the copolymers

Table 2. Apparent diffusion coefficients ($D_{ara\text{-}C} \times 10^{11}$ $m^2\ s^{-1}$) for release of ara-C from poly(acrylamide-co-monoethyl itaconate) gels, as a function of temperature and the amount of ara-C incorporated in the disc.

Temperature (K)	Ara-C (mg/disc)	90A/10MEI	75A/25MEI	60A/40MEI
310	50	10·25 ± 0·05	8·92 ± 0·01	7·79 ± 0·07
303	50	8·31 ± 0·02	7·18 ± 0·01	6·01 ± 0·01
298	50	7·86 ± 0·01	7·02 ± 0·01	5·17 ± 0·01
288	50	4·48 ± 0·01	3·70 ± 0·01	3·42 ± 0·01
310	25	9·08 ± 0·06	8·35 ± 0·01	7·50 ± 0·01
310	10	8·68 ± 0·02	8·01 ± 0·02	7·29 ± 0·05
310	5	7·99 ± 0·02	7·71 ± 0·07	7·18 ± 0·02

Results are means ± s.d., n = 3. The thickness of the disc was 4·06 ± 0·3 mm and the diameter 10·6 ± 0·2 mm.

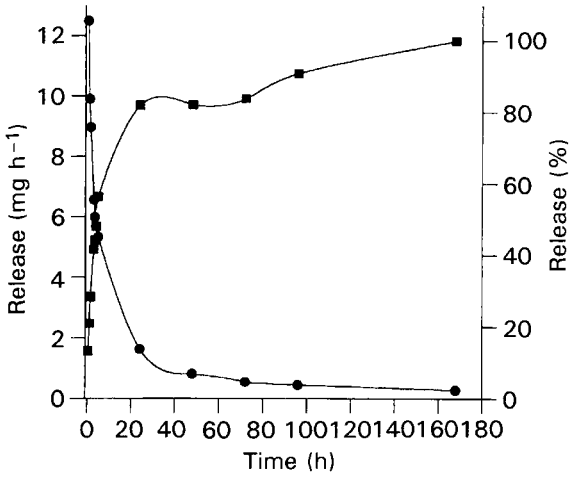

Figure 3. Plots of the rate of release of (●) ara-C (mg h^{-1}) and (■) percentage ara-C released from 75A/25MEI disc loaded with 50 mg ara-C. The experiment was performed at 310 K in saline solution.

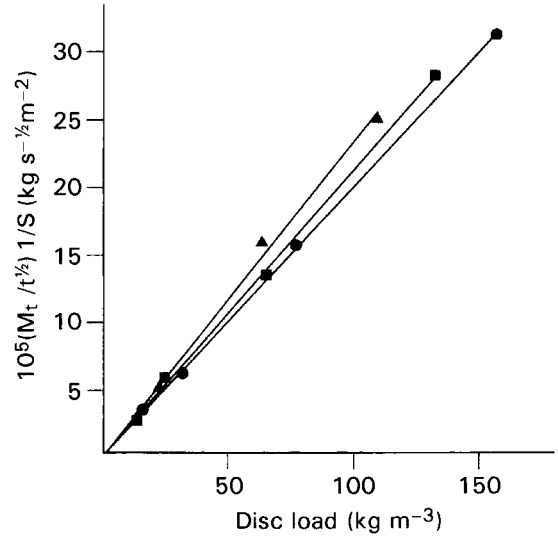

Figure 4. Plot of rate of ara-C release per unit disc area (M_t, $t^{-1/2}$ S^{-1}) as a function of disc loading for poly(acrylamide-co-monoethyl itaconate) gels of three different compositions at 310 K. ▲, 90A/10MEI; ■, 75A/25MEI; ●, 60A/40MEI.

of poly(acrylamide-co-monoethyl itaconate) studied here, because the swelling of this new type of copolymer is greater. In contrast, studies of the release of 5-fluorouracil from copolymers of poly (acrylamide-co-monomethyl itaconate) (Blanco et al 1996a) and poly(acrylamide-co-monopropyl itaconate) (Blanco et al 1996b) furnished diffusion coefficients for ara-C higher than those obtained in this work, probably because of the smaller molecular size of 5-fluorouracil.

In-vivo diffusion studies

Because of the short half-life of ara-C (Zimm et al 1984; Calabresi & Parks 1986), different devices have been developed to maintain suitable levels of ara-C for an optimum period of time in biological fluids. Thus, ara-C has been included in hydrogels (Trigo et al 1994) and liposomes (Kim et al 1970; Allen et al 1992b), and prodrugs (Schleyer et al 1995) have been developed for production of devices with different diffusion characteristics enabling controlled release of ara-C to improve its therapeutic index.

The applicability of these copolymers to controlled release of ara-C was determined by subcutaneous implantation of 75A/25MEI gels in rats. This monomer gel composition was chosen because its equilibrium swelling in-vitro was very high and its release of ara-C was significantly slower than from 90A/10MEI and quite similar to that from 60A/40MEI after short periods. In addition, the period over which ara-C is released from 75A/25MEI is the longest.

The influence of the method of administration on ara-C plasma levels was studied by comparing drug administration in the form of subcutaneously implanted hydrogels with that after intraperitoneal injection. In both cases, the ara-C dose was 201 mg kg^{-1}.

The animals implanted with the xerogel discs without the drug were killed 21 days after the implant to measure the total swelling of the disc and to observe the effect of this copolymeric hydrogel on the surrounding tissues. Histopathological studies showed the tissues were not affected. The biocompatibility of the hydrogels, from a macroscopic point of view, was good—no thin fibrous capsule was formed around the discs and there was no visually apparent inflammatory response of the surrounding tissues. All the gel discs were removed in perfect condition and no degradation was apparent.

The amount of swelling of the implanted hydrogels was 82·5 ± 0·4% w/w, slightly higher than that determined in-vitro for this copolymer (80·2 ± 0·3% w/w). This suggests that some of the swelling is probably a result of other physiological substances rather than to the contribution of the saline solution to the disc swelling. Thus, hydrogels reach equilibrium swelling when they are implanted subcutaneously. The large amount of aqueous solution that these poly(acrylamide-co-monoethyl itaconate) hydrogels absorb into their structure makes them very similar to living tissues and this could explain why the 75A/25MEI polymeric matrix is well tolerated.

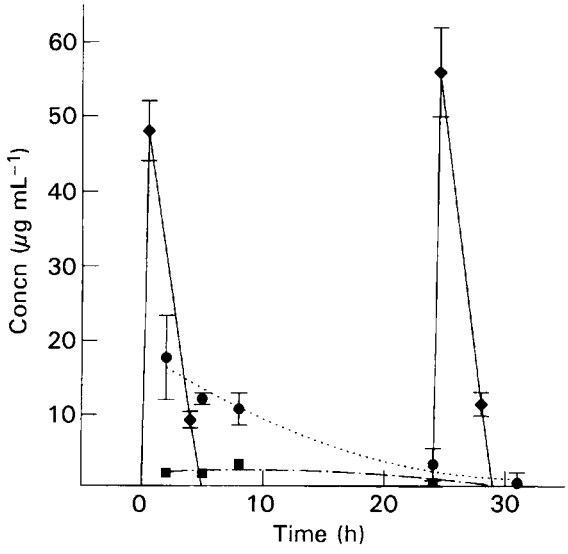

Figure 5. Plot of plasma concentrations of (●) ara-C and (■) ara-U against time of treatment for the group implanted with 75A/25MEI gel disc. Plot of plasma concentration of (◆) ara-C against time for the group injected intraperitoneally.

In the implanted group with xerogels loaded with ara-C, the drug could be detected in plasma approximately 48 h after implant. When the animals were killed the amount of swelling of the discs was 82·4±0·5%. From their macroscopic appearance the biocompatibility of these discs was no different from those without ara-C.

Ara-C was detected in plasma for 32 h (Figure 5). Its concentration was in the range 17·67±5·68–10·76±2·15 μg mL^{-1} between 2 and 8 h after implant; this decreased slowly to 3·39±2·16 μg mL^{-1} after 24 h and 0·9±1·41 μg mL^{-1} after 32 h. The drug was not detected on the third day after implantation of the gel disc. Although in-vitro release of ara-C from this matrix takes place over a period of 7 days, 80% of the drug is released within 24 h (Figure 3). This explains why the drug is not detected in plasma after 32 h in the in-vivo studies—the small amount of ara-C released is very quickly degraded to ara-U.

The ara-C plasma concentration up to 24 h is greater than 10 μmol L^{-1}, thus the amount of intracellular ara-C (60% of extracellular amount; (White et al 1987)) is larger than the K_m of the deoxycitidine kinase for ara-C (1·5 μmol L^{-1}) and formation of ara-CTP is at a maximum (Capizzi et al 1991).

The presence of ara-C in plasma is favoured when its transformation to ara-U is inhibited; this occurs when the concentration of ara-U is greater than 5·6 μmol L^{-1}. When ara-C is released from 75A/25MEI hydrogels the maximum amount of ara-U, obtained after 8 h, is 13·79±9·52 μmol L^{-1} (3·37±2·33 μg mL^{-1}) (Figure 5), thus the deaminase is inhibited and this contributes to maintaining the plasma concentration of ara-C.

Because the release of ara-C from the implants takes place in approximately 48 h, intraperitoneal administration of ara-C was performed over two days, the total dose being 201 mg kg^{-1}. A high concentration of ara-C was measured 30 min after the injection (almost three times higher than the plasma concentration reached when the drug was released from hydrogels). Because of the short average half-life of ara-C in plasma (2·6±0·6 h), the drug concentration is only 18% of that obtained 30 min after the injection at 4 h. Thus, using this method of administration ara-C remains in plasma for a short period of time, its concentration increasing and decreasing quickly, and there is a period of time between consecutive injections during which the plasma contains no drug.

The pharmacokinetic parameters of ara-C after both intraperitoneal injection (25 mg ara-C/injection) and subcutaneous implantation of drug-loaded hydrogel (50 mg ara-C/disc) are shown in Table 3. Administration of ara-C as a hydrogel seems to result in improved therapeutic results. Not only was the area under the plasma concentration–time curve (AUC) larger, but the elimination half-life of the drug was also ten times larger.

In summary, the results of this work show how the administration of ara-C by implant of

Table 3. Pharmacokinetic parameters of ara-C after intraperitoneal injection (25 mg ara-C/injection) and subcutaneous implantation of ara-C-loaded hydrogels (50 mg ara-C/disc).

	Elimination constant (h^{-1})	Elimination half-life (h)	Total area under the blood level–time curve (μg h mL^{-1})	Total body clearance (L h^{-1})	Volume of distribution (L)
Intraperitoneal injection	1·09±0·04	0·64±0·02	123·25±16·68	0·20±0·03	0·19±0·03
Ara-C-loaded hydrogel	0·11±0·02	6·36±1·20	374·01±49·86	0·13±0·02	1·27±0·16

Results are means±s.d., n=3.

75A/25MEI copolymeric hydrogel from which the drug is released results in therapeutically suitable levels of ara-C in plasma for a precise period of time, despite the short average half-life of this drug. Also, these copolymeric hydrogels are, from macroscopic appearances, highly biocompatible, because of the large amount they swelled, a factor that increases the similarity of the gels to living tissues. In addition, ara-C can be incorporated into these hydrogels by addition to the feed mixture used for polymerization; it is then released into a medium in a pharmacologically active form. The implantation of these slow-release devices containing ara-C would enable drug dosage to be close to its target, which should partly reduce the secondary effects of this drug.

Acknowledgements
This work was funded by a grant from the Comision Interministerial de Ciencia y Tecnologia.

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