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5-Fluorouracil trapping in poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels: in vitro drug delivery studies

Olga García, M. Dolores Blanco, Juan Antonio Martín, José M. Teijón*

Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain

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Abstract

The influence of gel formulation on the diffusion of 5-fluorouracil (5-FU) included in poly(2-hydroxyethyl methacrylate-co-acrylamide) (poly(HEMA-co-A)) hydrogels is reported. The synthesis of these copolymers, in different monomeric proportions, each one of them crosslinked with different proportions of ethylenglycol dimethacrylate (90 HEMA/10 A, 75 HEMA/25 A and 50 HEMA/50 A), allows the inclusion of 5-FU in the feed mixture of polymerization, up to 16 mg/disc, without any chemical drug alteration; and it also makes possible the control of its release over a wide range of release times that vary between 7 h and 9 days, just by modulating the crosslinking degree of the copolymer as well as their comonomeric composition, maintaining sufficiently high hydrate degrees (66–24 wt%).

Keywords: Hydrogel; Poly(2-hydroxyethyl methacrylate-co-acrylamide); Controlled release; Swelling; 5-Fluorouracil

1. Introduction

The evolution of the pharmaceutical technology has been based, in recent years, on the development of new drug administration methods as well as in the design and application of controlled dosage systems, and on vectorization or activity targeting systems of a certain drug. In particular, the application of polymeric systems provides, in a great number of selected cases, a clear optimization in the dosage methods to get the desired therapeutic result in the required target, as well as the optimization of the control drug release in order to obtain the maximum result and the minimum adverse effects [1].

The release of a drug incorporated in a polymeric system takes place by migration of the solute to the medium that surrounds the system by molecular diffusion through the support or by diffusion through micropores of the polymeric matrix. This makes the solute solubility in the polymer an important factor in the control of its migration. Drug diffusion from monolithic systems can be analyzed using Fick's Second Law of Diffusion [2,3]. The systems of controlled release by diffusion are based on the principle

* Corresponding author. Tel.: +91-394-14-47; fax: +91-394-16-97.

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of the permeability of the polymeric matrix after the swelling process in a hydration medium. The swelling kinetic and, therefore, the release rate depends on the matrix swelling degree. Matrix is constituted by polymeric systems based on hydrophilic polymers crosslinked with acrylic monomers [4] or, alternatively, for biocompatible copolymers of hydrophilic and hydrophobic monomers with the appropriate composition to control the release rate of the drug [5].

In the case of the hydrogels, the use of copolymeric systems prepared starting from two monomers of different hydrophilic character is another possibility for controlling the swelling degree of the gel and the solute diffusion rate from the matrix. In this case, the composition of the copolymeric system determines the swelling behavior [6].

The copolymerization of 2-hydroxyethyl methacrylate (HEMA), a monomer of wide applicability in the biomedical field because of the high biocompatibility matrices that forms, with more hydrophilic monomers produces hydrogels whose content in water can exceed 40 wt% when they are not crosslinked, as occurs when HEMA is copolymerized with *N*-vinyl-2-pyrrolidone (VP) [5,7]. On the other hand, the poly(acrylamide) hydrogels have a very limited applicability because of their poor mechanical properties due to their high hydration degree; a characteristic which for the copolymers based in acrylamide plays an important role in the field of the hydrogels for biomedical applications [8,9].

The copolymerization of acrylamide (A) with HEMA has been assayed in order to obtain polymeric matrices that maintain high hydrate degrees in water, making it possible to modulate the 5-fluorouracil (5-FU) release to the medium, by variation of the monomeric composition and the crosslinking density of the hydrogels. 5-FU is an antineoplastic agent of extensive use in clinical chemotherapy for the treatment of solid tumors which is an appropriate candidate for this type of system of drugs administration due to the large number of secondary effects that accompany its conventional administration [10].

2. Materials and methods

2-Hydroxyethyl methacrylate (HEMA) (Merck) was purified by vacuum distillation at 315–318 K and 3.7 mmHg (vacuum pump: Eduar 8). Acrylamide (A) (Merck), ethylene glycol dimethacrylate (EGDMA) (Merck), ammonium peroxodisulfate ($(NH_4)_2S_2O)_8$ (Merck), dimethyldichlorosilane solution (BDH), sodium chloride (Merck), and sodium hydroxide (Probus), were used as received.

5-Fluorouracil (5-FU) ($C_4H_3N_2O_2F$, of molecular weight 130.1 Da) was kindly supplied by Roche Laboratories as a crystalline powder.

2.1. Synthesis of poly(2-hydroxyethyl methacrylate-co-acrylamide)

The synthesis of copolymeric hydrogels of poly(2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A) have been carried out in different monomeric proportions (wt/wt): 90 HEMA/10 A, 75 HEMA/25 A and 50 HEMA/50 A, and in turn each one of them was crosslinked with different proportions of EGDMA between 1 and 7 wt% for 90 HEMA/10 A gels, 1–13 wt% for 75 HEMA/25 A gels and 1–10 wt% in the case of 50 HEMA/50 A copolymers, expressed as a percentage of the total weight of the two comonomers present in the gel. The highest proportions of crosslinker correspond with the maximum crosslinking degree that allows the synthesis of the hydrogels including 5-FU in the polymerization mixture.

The synthesis was controlled by bulk polymerization in small glass vials previously siliconized using ammonium peroxodisulfate (0.05 g/ml) as initiator of the reaction. A volume of 0.65 ml of the feed mixture, formed by the monomers and crosslinker (60 wt%) and aqueous initiator solution (40 wt%), was poured into small glass vials. After de-aeration by gaseous nitrogen for 5 min, the vials were sealed. Polymerizations were then performed in an oven at 323 K for 3 h. The vials were then broken and the polymer discs were removed. These were dried to a constant weight for 1 week. The conversions were: 87 wt% for 75 HEMA/25 A–1% EGDMA; 89 wt% for 50 HEMA/50 A–1% EGDMA, 75 HEMA/25 A–13% EGDMA and 90 HEMA/10 A–1% EGDMA; 94 wt% for 50 HEMA/50 A–10% EGDMA; and 98 wt% for 90 HEMA/10 A–7% EGDMA.

Disc dimensions were determined with a micrometer and they were stored in an anhydrous atmosphere and in the dark until their use, in order to prevent a possible deterioration of the drug.

The synthesis of these gels were carried out taking as a base the method described by Davis and Huglin [11,12] for the synthesis of PHEMA hydrogels, beginning the reaction with an inorganic persulfate, as it is the ammonium peroxodisulfate, to obtain high conversion degrees. This initiator is employed in polymerizations that require the presence of water in the feed mixture.

Xerogel discs of 4.03 ± 0.23 mm thickness and 11.76 ± 0.16 mm diameter for 90 HEMA/10 A gels, of 3.99 ± 0.16 mm thickness and 11.66 ± 0.19 mm diameter for the gels of 75 HEMA/25 A and of 4.24 ± 0.31 mm thickness and 11.46 ± 0.26 mm diameter for 50 HEMA/50 A copolymeric gels were obtained.

2.2. 5-Fluorouracil inclusion in the copolymers

The 5-FU was trapped in the gels by its inclusion in the polymerization mixture. To incorporate the 5-fluorouracil (5-FU) to the feed mixture of polymerization, water solutions of 5-FU were used and neutralized equivalent to equivalent with sodium hydroxide, with the purpose of increasing the solubility of the drug, since the sodium salt of the 5-FU is a pharmacologically active compound [13]. In this way discs were obtained whose quantities of 5-FU, as sodium salt, were between 1 and 16 mg by disc corresponding to 0.2–3.5 wt% of the total formulation.

2.3. Swelling of the copolymers in saline solution

The swelling of the discs in saline solution was carried out, for all of hydrogels synthesized, at 310 K. The xerogel discs (without drug) were placed into a bath of saline solution (0.9 wt% NaCl) at a constant temperature and the swelling degree was calculated in water at different times (W_t) by means of the following expression [11,12]:

$$W_t = \frac{\text{weight of swollen disc} - \text{weight of dry disc}}{\text{weight of swollen disc}} \times 100 \quad (1)$$

The time taken by the different gels in reaching the equilibrium swelling degree, W_∞, depended on the temperature and the composition of the matrices.

The sol fraction (wt%) was determined for each hydrogel. After hydrogels reached the equilibrium swelling they were dried to obtain a constant weight in an unhydrated environment. The values obtained were: 2.8 wt% for 50 HEMA/50 A–1% EGDMA; 7.5 wt% for 50 HEMA/50 A–10% EGDMA; 10.0 wt% for 75 HEMA/25 A–1% EGDMA; 7.2 wt% for 75 HEMA/25 A–13% EGDMA; 4.9 wt% for 90 HEMA/10 A–1% EGDMA; and 5.0 wt% for 90 HEMA/10 A–7% EGDMA.

The medium used for swelling of the hydrogels up to equilibrium was scanned to obtain its UV–vis spectrum (Unicam 8700 Spectrophotometer). Also the UV–vis spectra of aqueous solutions of known concentration of HEMA, EGDMA and acrylamide were determined. The wavelength of maximum absorbance was 208.0 nm for HEMA, 207.2 nm for EGDMA and 203.2 nm for acrylamide. The spectra of samples were almost the same as those of acrylamide with a maximum at 202.4 nm. If the compound released was acrylamide, its amount would be between 0.03 μg, from 90 HEMA/10 A gels crosslinked with 1 wt% and 7 wt% EGDMA, and 0.49 μg from 75 HEMA/25 A gels crosslinked with 13 wt% EGDMA, which means a very small amount of total acrylamide in the feed mixture.

2.4. 5-Fluorouracil release from the copolymers

The system used to carry out the experiments of 5-FU release consists of the same thermostatized recipient used in the swelling experiments. Copolymeric discs were placed on a widely perforated glass support so the position of the disc in the vessel was at the same height for all experiments. The total volume of solution in the vessel was 100 ml and the stirring rate was always constant (VELP Scientifica Multistirrer).

At intervals, 50 μl samples were drawn from the solution in order to follow the change in 5-FU concentration; a maximum of 20 aliquots were taken so the vessel volume was considered constant. Drug release was maintained under sink conditions [14], which means the amount of 5-FU released should not exceed 10% of its solubility in the medium.

The concentrations of 5-FU were determined by UV–vis spectroscopy (Unicam 8700 series spectrophotometer) using a 1 cm path length microcuvette (50 μl volume) at 270 nm [15,16]. 5-FU standards of 0.1–50 μg/ml in saline solution were used to obtain a calibration curve.

Neither during the synthesis of the gels with the included drug, nor during the drug release was 5-FU degradation observed. All the discs with 5-FU were optically transparent and the released drug showed an absorption spectrum which belonged to 5-FU; this fact was confirmed by HPLC, samples showing a single peak in the chromatograph which belonged to 5-FU [17,18].

3. Results and discussion

The chemical structure of a polymer can be modified to obtain a product with certain properties. In this sense, copolymerization is used to get physical properties and intermediate mechanics among those of the homopolymers, allowing the synthesis of an almost limitless number of different products to vary the nature and the relative quantities of the monomers that form the copolymer [19,20]. In this case, it is the composition of the copolymeric system that determines the swelling behavior [6].

3.1. Swelling studies of the xerogels in saline solution

The swelling of poly(2-hydroxyethyl methacrylate-co-acrylamide) copolymers crosslinked with ethylenglycol dimethacrylate (EGDMA) ((HEMA/A)–%(EGDMA)) was conducted in saline solution (0.9 wt% NaCl) at a constant temperature of 310 K in

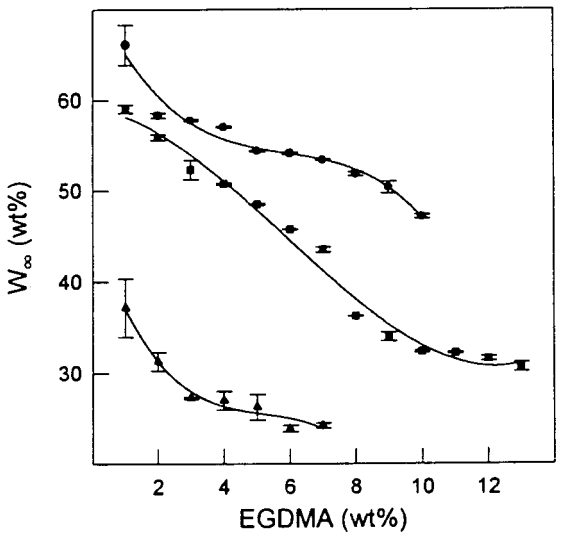

Fig. 1. Equilibrium swelling degree, W_∞, in saline solution reached by poly(2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A–% EGDMA) gels, as a function of the crosslinker percentage, EGDMA, present in the copolymers: 50 HEMA/50 A (●), 75 HEMA/25 A (■) and 90 HEMA/10 A (▲) at 310 K.

order to study the hydrogel behavior in conditions similar to in vivo systems.

The swelling degree, W_t, was determined at different times by means of Eq. (1). In Fig. 1 the equilibrium swelling degree, W_∞, is represented for the three copolymer types as a function of EGDMA percentage present in each gel. Starting from the data shown in Fig. 1, it can observed that the increase of the acrylamide proportion in the copolymer gives way to an important increment of the W_∞, for the crosslinking degree. This way, for the smallest concentration of studied crosslinker (1 wt% EGDMA), the swelling degree varies from 66.1 ± 2.2 wt% for the copolymers 50 HEMA/50 A up to 37.2 ± 3.2 wt% for the gels 90 HEMA/10 A. When a copolymer type is considered, in all cases, the increase of EGDMA percentage gives place to a decrease of the equilibrium swelling degree, being the obtained minimum value 24.2 ± 0.3 wt% for 90 HEMA/10 A–7% EGDMA gels. This behavior is the result of densely crosslinked matrices that do not expand in water as much as hydrogels with lower crosslinking degree [21].

When the swelling values obtained are compared with those of poly(2-hydroxyethyl methacrylate-co-*N*-vinyl-2-pyrrolidone) hydrogels crosslinked with EGDMA, these last present smaller equilibrium swelling values for the percentage of HEMA in the comonomeric mixture and for same crosslinking degree in the gel [5,7]; this way, for example, when the crosslinking percentage is 5 wt% of EGDMA the 75

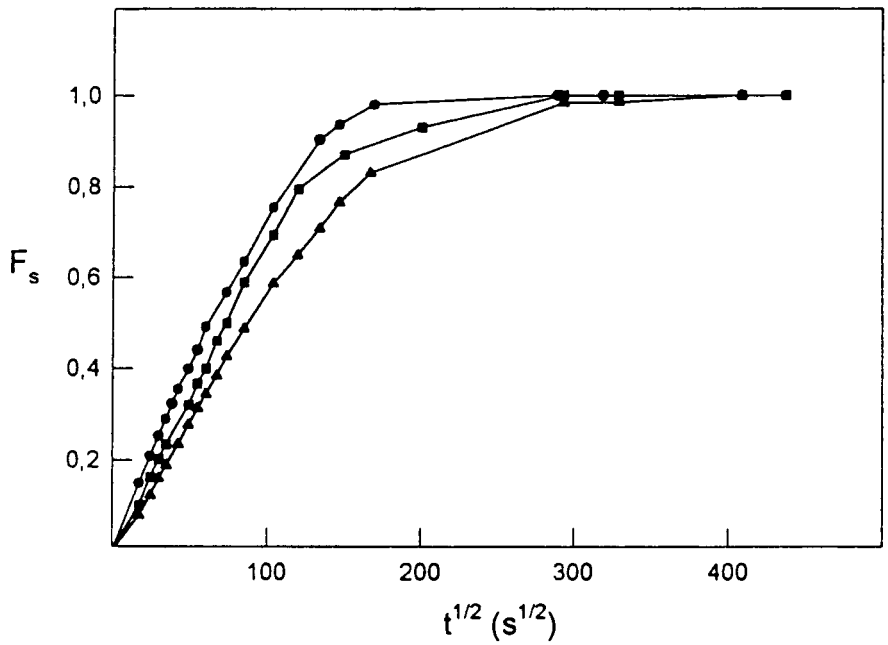

Fig. 2. Representation of the fractional swelling in saline solution (F_s) of poly(2-hydroxyethyl methacrylate-co-acrylamide) gels crosslinked with ethylenglycol dimethacrylate (HEMA/A–% EGDMA) as a function of the square root of the time, $t^{1/2}$, at 310 K: (●) 50 HEMA/50 A–7% EGDMA; (■) 75 HEMA/25 A–7% EGDMA; and (▲) 90 HEMA/10 A–7% EGDMA.

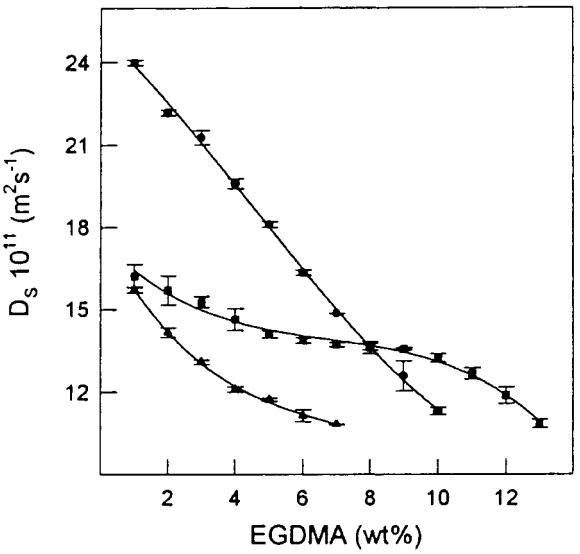

Fig. 3. Variation of the apparent diffusion coefficient for saline solution uptake (D_s) in poly(2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A–% EGDMA) hydrogels as a function of their percentage of EGDMA at 310 K: (●) 50 HEMA/50 A; (■) 75 HEMA/25 A; and (▲) 90 HEMA/10 A.

HEMA/25 VP and 50 HEMA/50 VP gels show swelling degrees of 39 and 49 wt%, respectively, while the gels of 75 HEMA/25 A and 50 HEMA/50 A show a hydration degree of 48.5 ± 0.1 wt% and 54.4 ± 0.1 wt%, respectively. On the other hand, the equilibrium swelling degrees obtained for copolymeric gels with HEMA are, in all cases, inferior to those obtained for copolymeric hydrogels of poly(acrylamide-co-monoalkyl itaconates) independently of the monoitaconate type and its proportion in the gel [17,18].

The time necessary for poly(HEMA-co-A) copolymers to reach equilibrium of swelling in saline solution depends on the hydrogel monomeric composition and its crosslinking degree as well as disc thickness, which was kept fixed. Thus, as the EGDMA percentage increases, for monomeric composition and the acrylamide proportion diminishes in the copolymer, for crosslinking degree, the swelling process is slower. These times varied from 22.7 ± 0.1 h up to 31.2 ± 0.1 h for the copolymers 50 HEMA/50 A, from 28.0 ± 0.2 h up to 127 ± 1 h for 75 HEMA/25 A hydrogels and from 30.1 ± 0.1 h up to 53.0 ± 0.1 h for 90 HEMA/10 A copolymers.

For a controlled diffusion process, the fraction of swelling due to the uptake of the solvent medium (saline solution), $F_s = W_t/W_\infty$, can be expressed by the following equation [22,23]:

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

where D_s, is the apparent diffusion coefficient for the transport of saline solution toward the interior of the hydrogel, h the xerogel thickness 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 for simple geometric forms (discs, cylinders and spheres) [22,23]. When the swelling fraction F_s, is represented in front of the square root of the time, $t^{1/2}$, linearity is obtained in the first stages of the process, corresponding to values of $F_s < 0.5$; just as is shown in the example represented in Fig. 2. This fact allows calculation of an apparent diffusion coefficient for the uptake of saline solution in the gel, D_s, of the slope of the corresponding straight line, by the application of Eq. (2).

The values of D_s for these copolymers are shown in Fig. 3. They clearly show the influence of the crosslinking degree and the monomer composition in the swelling process. The increase of EGDMA concentration originates a decrease of the D_s value, in all gels, as it corresponds to a process of slower swelling, which is the result of more densely crosslinked matrices. On the other hand, the increase of the acrylamide proportion in the polymerization mixture does not always originate an increase in the D_s values, for a crosslinker percentage. Thus, 75 HEMA/25 A gels at 9 and 10 wt% of crosslinker show slightly higher D_s values with regard to those of 50 HEMA/50 A. Thus, the swelling rate of the gels is less affected for the increase of crosslinking in 75 HEMA/25 A than in 50 HEMA/50 A. With the exception of the mentioned gels, when the hydrophilic character of the gel increases, its swelling in aqueous medium turns out to be quicker.

In the case of copolymers of poly(HEMA-co-VP) [7] the values of D_s are of the same order of magnitude as those of poly(HEMA-co-A), although the D_s calculated in this study are always numerically superior to those presented for the hydrogels of poly(HEMA-co-VP); for example 50 HEMA/50 VP and 75 HEMA/25 VP gels at 5 wt% of EGDMA show D_s values of 6.9×10^{-11} and 5.33×10^{11} $m^2\ s^{-1}$, respectively; however, for the same monomeric composition and the same crosslinking degree poly(HEMA-co-A) gels exhibit values of $18.1 \pm 0.1 \times 10^{-11}$ and $14.1 \pm 0.2 \times 10^{-11}$ $m^2\ s^{-1}$.

Three different diffusion mechanisms have been described, for crosslinked PHEMA hydrogels, starting from studies of water absorption [24,25]: for gels with a small crosslinker content (between 0 and 2.5 mol% approximately), the diffusion takes place through pores; for gels with a superior crosslinker concentration, up to 4 mol%, the transport is controlled fundamentally by the water interaction with the matrix of the gel. An intermediate mechanism of transport between both previous mechanisms is obtained for

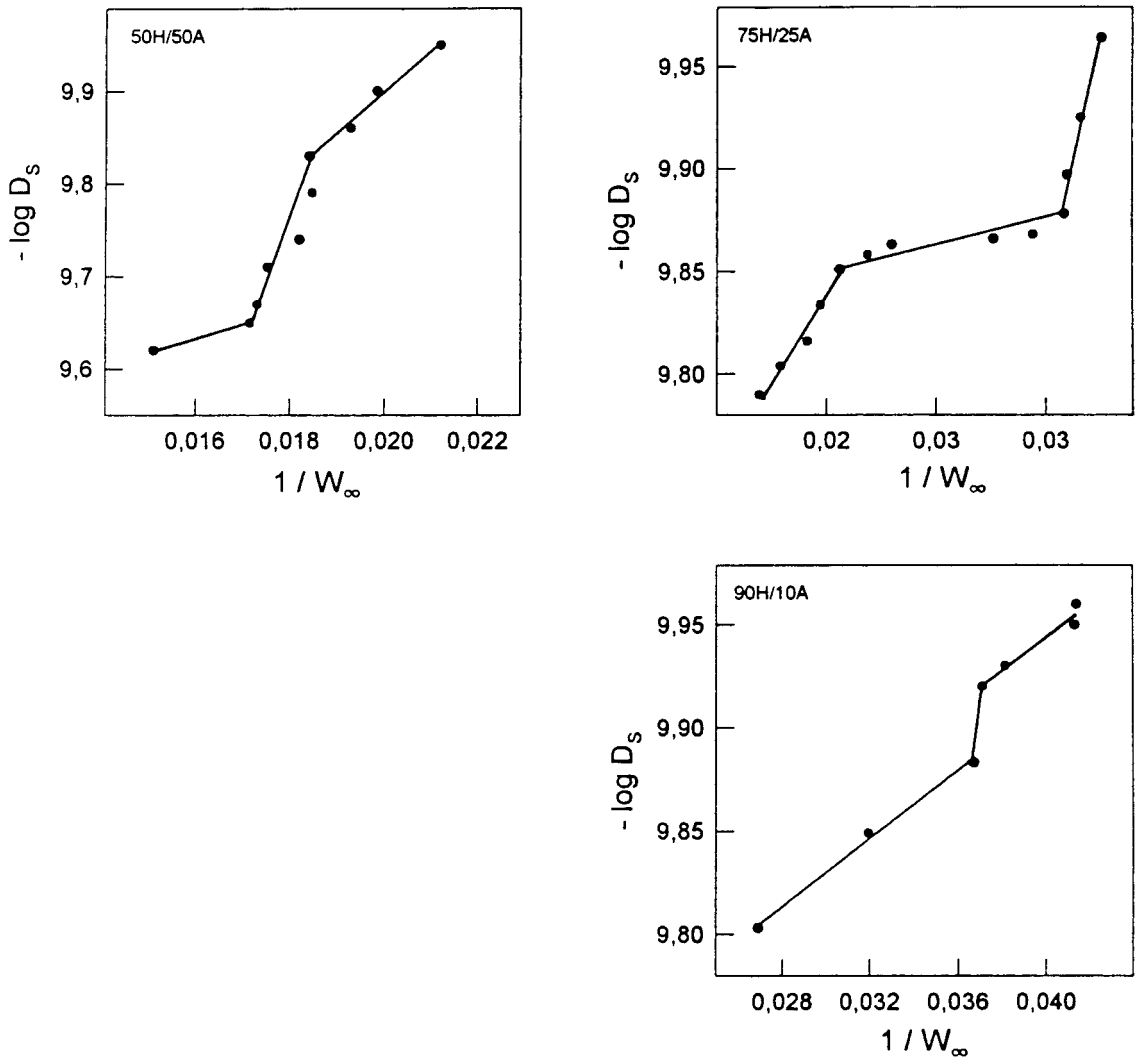

Fig. 4. Representation of the apparent diffusion coefficient logarithm for saline solution uptake, -log D_s, as a function of the inverse of equilibrium swelling degree, $1/W_\infty$, for the copolymeric hydrogels: (A) 50 HEMA/50 A; (B) 75 HEMA/25 A; and (C) 90 HEMA/10 A.

crosslinker concentrations from 2.5 to 4 mol%. Yasuda and coworkers [26] related the different diffusion mechanisms through the matrix of a gel to the hydration degree, since the crosslinking of the gels controls this parameter, finding that the diffusion coefficient, D, is a function of the inverse of the equilibrium swelling degree, $1/W_\infty$, according to the equation [26,27]:

$$\log D_s = \log D_0 - K[(1/W_\infty) - 1] \quad (3)$$

where D_s is the diffusion coefficient for the uptake of saline solution, D_0 the diffusion coefficient of water in pure water and K a proportionality constant.

When the dependence of the equilibrium swelling degree, W_∞, with the apparent diffusion coefficient, D_s, for the saline solution uptake in the gel according to Eq. (3) was studied for these copolymers, different diffusion mechanisms could be observed for these hydrogels as a function of W_∞ value, which means as a function of EGDMA proportion present in each copolymer (Fig. 4).

For 50 HEMA/50 A copolymers (Fig. 4A), the two slope changes correspond to crosslinker concentrations of 2 wt% ($1/W_\infty = 1.71 \times 10^{-2}$) and 7 wt% ($1/W_\infty = 1.84 \times 10^{-2}$). Therefore, at this monomeric composition, for crosslinker concentrations lower than 2 wt%, the diffusion would happen through pores in

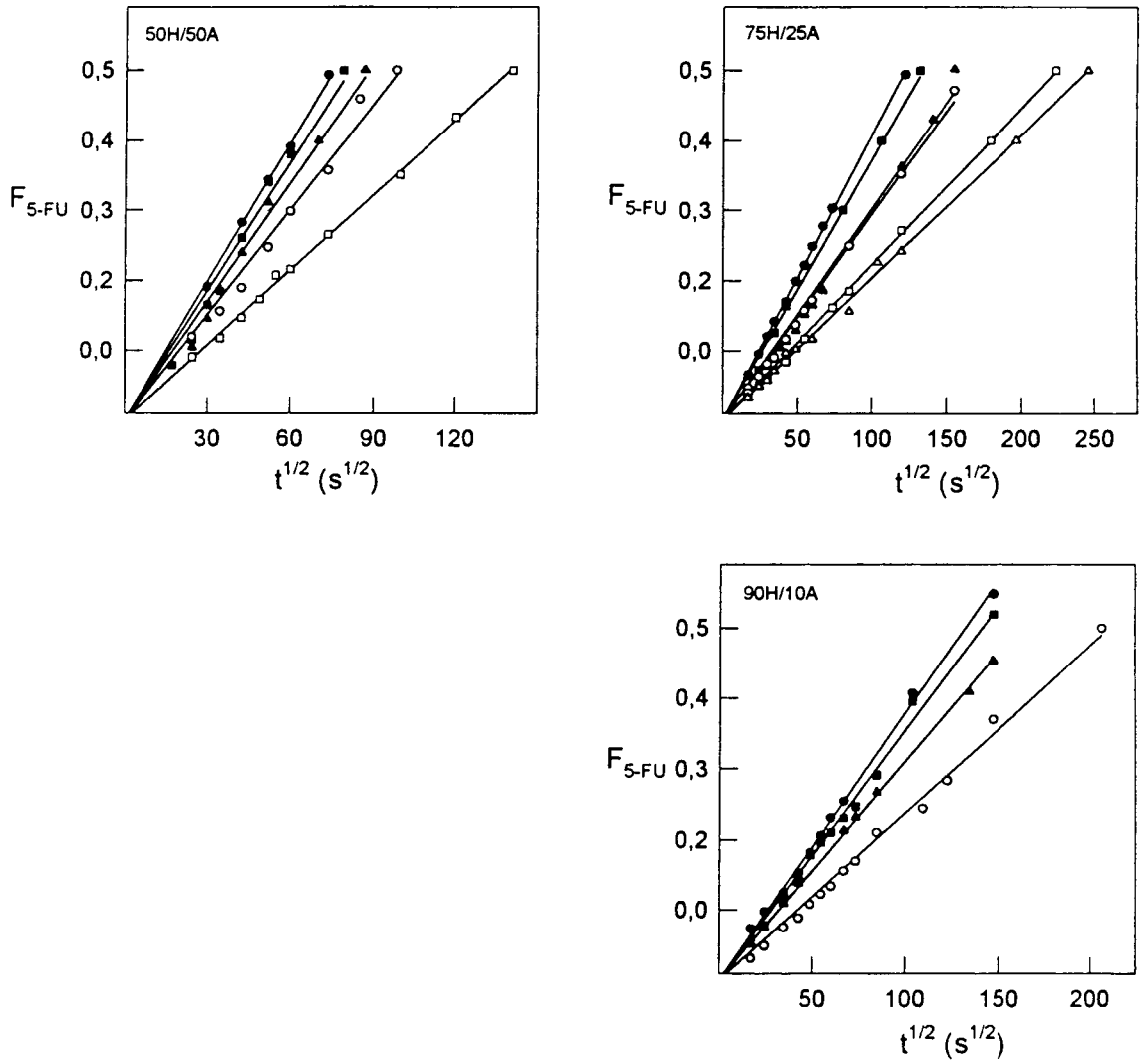

Fig. 5. First stages of the 5-FU release kinetics from poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels. The linear stretch of the variation of the fractional 5-FU release, $F_{5\text{-FU}}$, versus the square root of the time at 310 K is plotted for: (A) 50 HEMA/50 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt%, (○) 7 wt% and (□) 10 wt% of EGDMA; (B) 75 HEMA/25 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt%, (○) 7 wt%, (□) 10 wt% and (△) 13 wt% of EGDMA; and (C) 90 HEMA/10 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt% and (○) 7 wt% of EGDMA.

the matrix, for crosslinker concentrations higher than 7 wt% the diffusion mechanism would be governed by the interaction of the saline solution with the matrix and at EGDMA concentrations between 2 and 7 wt%, the diffusion mechanism would be intermediate between both these mechanisms. This type of diffusion behavior as a function of the crosslinking degree is the same as has been described by Winsniewski et al. [25] and by Yasuda et al. [26,27] for PHEMA hydrogels crosslinked with EGDMA.

In 75 HEMA/25 A gels these limits are different (Fig. 4B), thus the first slope change is obtained for an EGDMA percentage higher than that of the previous case, 6 wt% ($1/W_\infty = 2.18 \times 10^{-2}$), and the second slope change is observed when the concentration of this component is 10 wt% ($1/W_\infty = 3.08 \times 10^{-2}$). In this case, for EGDMA concentrations higher than 10 wt% in the gel the diffusion mechanism would be directed by solution saline–polymeric matrix interactions.

Lastly, for 90 HEMA/10 A gels (Fig. 4C), two slope changes are also observed, the first one for a EGDMA concentration of 3 wt% ($1/W_\infty = 3.67 \times 10^{-2}$) and the second one for 4 wt% of EGDMA ($1/W_\infty = 3.71 \times 10^{-2}$).

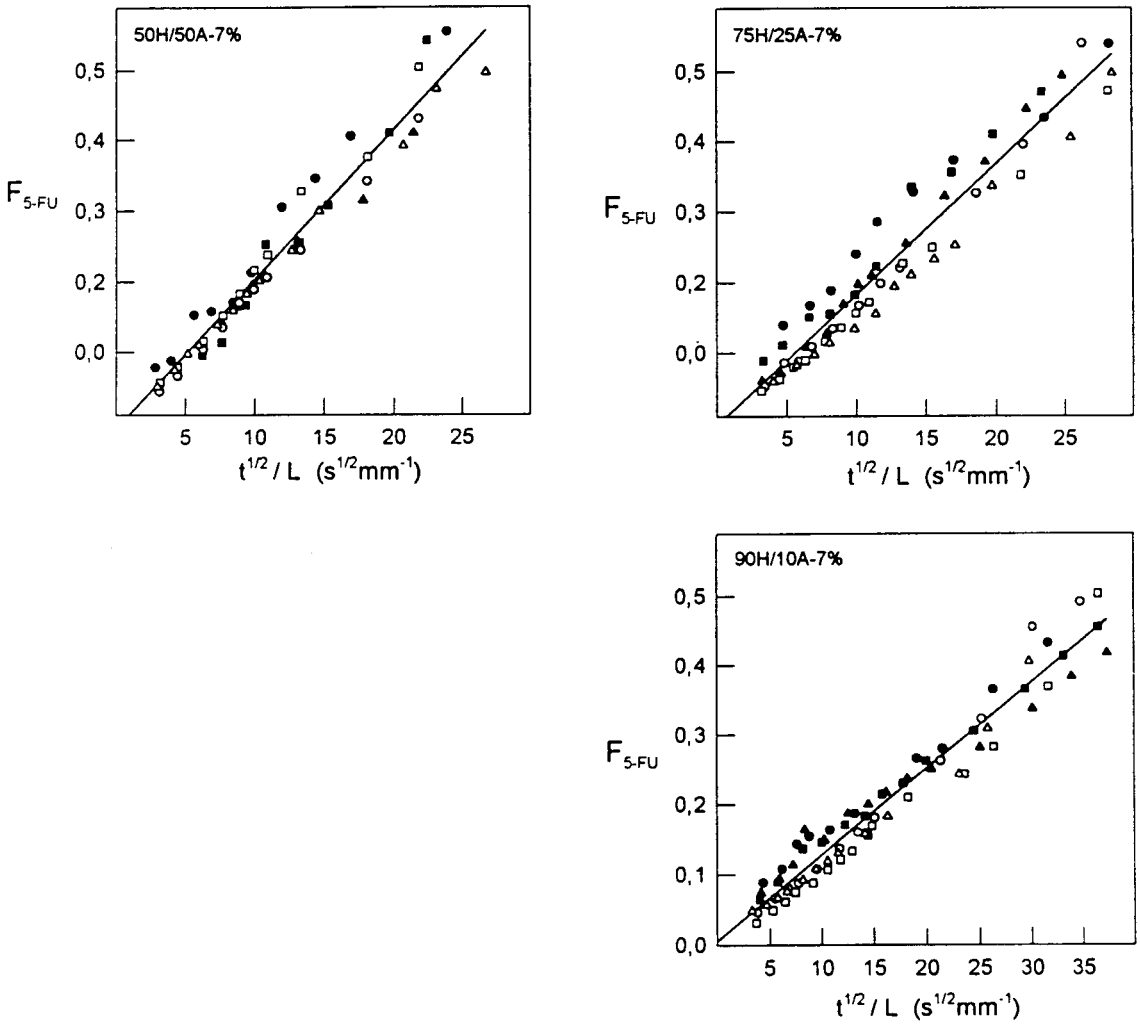

Fig. 6. Fractional release of 5-FU, $F_{5\text{-FU}}$, from poly(2-hydroxyethyl methacrylate-co-acrylamide) gels as a function of (time)$^{1/2}$/thickness of hydrated hydrogel disc, $t^{1/2}/L$. (A) 50 HEMA/50 A–7% EGDMA; (B) 75 HEMA/25 A–7% EGDMA; and (C) 90 HEMA/10 A–7% EGDMA at 310 K. The amount of 5-FU included in the discs were (●) 1 mg, (■) 3 mg, (▲) 6 mg, (○) 9 mg, (□) 12 mg and (△) 16 mg per disc.

This way, as the HEMA proportion increases in the matrix, the diffusion behavior of the copolymeric hydrogels changes, the slope changes taking place at smaller intervals of hydration values (W_∞).

3.2. 5-FU release studies in saline solution from the copolymeric hydrogels

The 5-fluorouracil (5-FU) release experiments from poly(2-hydroxyethyl methacrylate-co-acrylamide) copolymers crosslinked with EGDMA were carried out in saline solution at a constant temperature of 310 K. Each gel, in the form of discs, was prepared with six different 5-FU quantities between 1 and 16 mg, which means 0.2–3.5 wt% of the formulation.

An equation for drug diffusion derived from Fick's Second Law can be applied to these copolymers:

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

where $D_{5\text{-FU}}$ is the apparent diffusion coefficient for 5-FU transport from the hydrogel to the medium and h is the xerogel thickness with the antineoplastic included in the matrix.

This way, for each copolymer, the $F_{5\text{-FU}}$ variation with $t^{1/2}$ is linear for the first stages of the release pro-

Table 1
Apparent diffusion coefficients for 5-FU release, $D_{5\text{-FU}} \times 10^{11}$ $m^2\ s^{-1}$, from poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels with different crosslinker percentages, EGDMA, for each drug amount included in each xerogel disc

Hydrogel	EGDMA	5-FU (mg) 1	3	6	9	12	16
50 HEMA/50 A	1%	6.86	7.23	7.96	8.72	9.95	10.89
	3%	6.71	6.99	7.88	8.51	9.13	10.07
	5%	6.32	6.36	6.50	7.56	8.08	9.31
	7%	6.15	6.26	6.37	6.76	7.42	7.74
	10%	3.86	5.01	5.49	6.21	6.36	6.49
75 HEMA/25 A	1%	4.32	5.43	6.24	6.85	7.14	7.80
	3%	4.28	4.55	4.96	5.15	5.88	6.25
	5%	3.80	4.28	4.71	5.05	5.41	5.77
	7%	3.56	3.71	3.72	3.78	3.83	4.38
	10%	1.91	2.20	2.94	3.06	3.16	4.02
	13%	1.63	1.96	2.41	2.88	2.98	3.50
90 HEMA/10 A	1%	3.46	4.19	4.49	4.61	4.78	5.17
	3%	3.40	3.51	3.52	3.58	3.94	4.39
	5%	3.02	2.99	3.15	3.16	3.45	3.77
	7%	1.26	1.74	2.09	2.24	2.32	2.50

cess, which correspond to $F_{5\text{-FU}}$ values inferior to 0.5, independently of 5-FU load and the crosslinking density of the gels. This behavior can be appreciated in the example illustrated in Fig. 5. The apparent diffusion coefficient for 5-FU release, $D_{5\text{-FU}}$, can be determined.

Fickian behavior implies that for similar equilibrium swelling, W_∞, the diffusion kinetics of the drug must be independent of the load and the thickness of the hydrogel discs [28]. Fig. 6 shows an example of this behavior for each copolymer. This plot of the fractional release of 5-FU, $F_{5\text{-FU}}$, versus $t^{1/2}/L$, where L is the thickness of the hydrated disc, yields a linear relationship and so the independence of drug load within the studied range can be considered. Therefore, the Fickian approach is correct in the case of our systems.

In Table 1 the values of the apparent diffusion coefficient for 5-FU release, $D_{5\text{-FU}}$, from the different copolymers, obtained using Eq. (4) are shown. The slower the release process is smaller the slope from the linear region is, which corresponds to smaller 5-FU diffusion coefficients; this behavior is observed when the crosslinking degree of the gels, for a monomer composition, increases as well as when the acrylamide percentage in the copolymer, for a crosslinking degree and a certain drug quantity, diminishes.

Likewise, for gels with the same monomeric composition and crosslinking density, when the amount of 5-FU in the discs increases the release process is accelerated, thus the load of the discs turns out to be an additional driving force that influences in the release from the drug to the medium. Thus, a diffusion coefficient independent of the drug load must be determined. Starting from Eq. (4), since $M_\infty = \mathscr{A}V = \mathscr{A}Sh$, where V is disc volume and S its surface, we can obtain [29,30]:

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

$M_t t^{-1/2} S^{-1}$ being the release rate per unit area and $\mathscr{A}$ the 5-FU xerogel disc load.

When the 5-FU release rate per unit disc area, $M_t t^{-1/2} S^{-1}$, is plotted versus drug load, $\mathscr{A}$, (Eq. (5)) a linear relationship is obtained between both variables for all the copolymers studied independently of the crosslinking degree (Fig. 7). From the slope of these straight lines the 5-FU diffusion coefficient is independent of the drug load included in the discs, whose values appear reflected in Fig. 8, can be determined. The more crosslinker the hydrogel possesses the more slowly it allows the drug included in the matrix to diffuse. Also the greater the acrylamide proportion present in the gel the more quickly 5-FU is released to the medium.

The crosslinker effect in the drug diffusion can be interpreted in terms of: (a) mobility of the polymeric chain; (b) average pore size; and (c) mobility of the aqueous medium in the gel. The reduction in the mobility of the polymeric chain due to the crosslinking will diminish the range of pore sizes and, also, the size distribution will be smaller [31]. Therefore, since the 5-FU is released from 50 HEMA/50 A gels with 1–10 wt% of EGDMA, from 75 HEMA/25 A gels crosslinked with 1–13 wt% and from 90 HEMA/10 A gels with 1–7 wt% of EGDMA, probably the decrease of the $D_{5\text{-FU}}$ values with the crosslinking increase is due, fundamentally, to a reduction of average pore size in the gel. Another phenomenon that explains the $D_{5\text{-FU}}$ decrease with the increment in crosslinking density is that the mobility of the solvent inside the gel decreases. When a hydrogel is polymerized with water in the feed mixture of polymerization, this can be in the form of freezing water or nonfreezing water. The crosslinking diminishes the percentage of freezing water in the gel, increasing the proportion of nonfreezing water [32,33]. It is believed that the hydrophilic solutes mainly diffuse through the freezing water [34], thus the effect of increasing the crosslinking density is translated in reducing the effective free volume of the polymer, and the release process of the drug is slower.

The values of $D_{5\text{-FU}}$ independent of the disc load are, in all cases, of the same magnitude as those obtained for other copolymers. However, these values are numerically smaller than that determined for 5-FU release from copolymeric hydrogels of poly(acrylamide-co-monoalkyl itaconates) [17,18], and higher than that from PHEMA hydrogels [21,35].

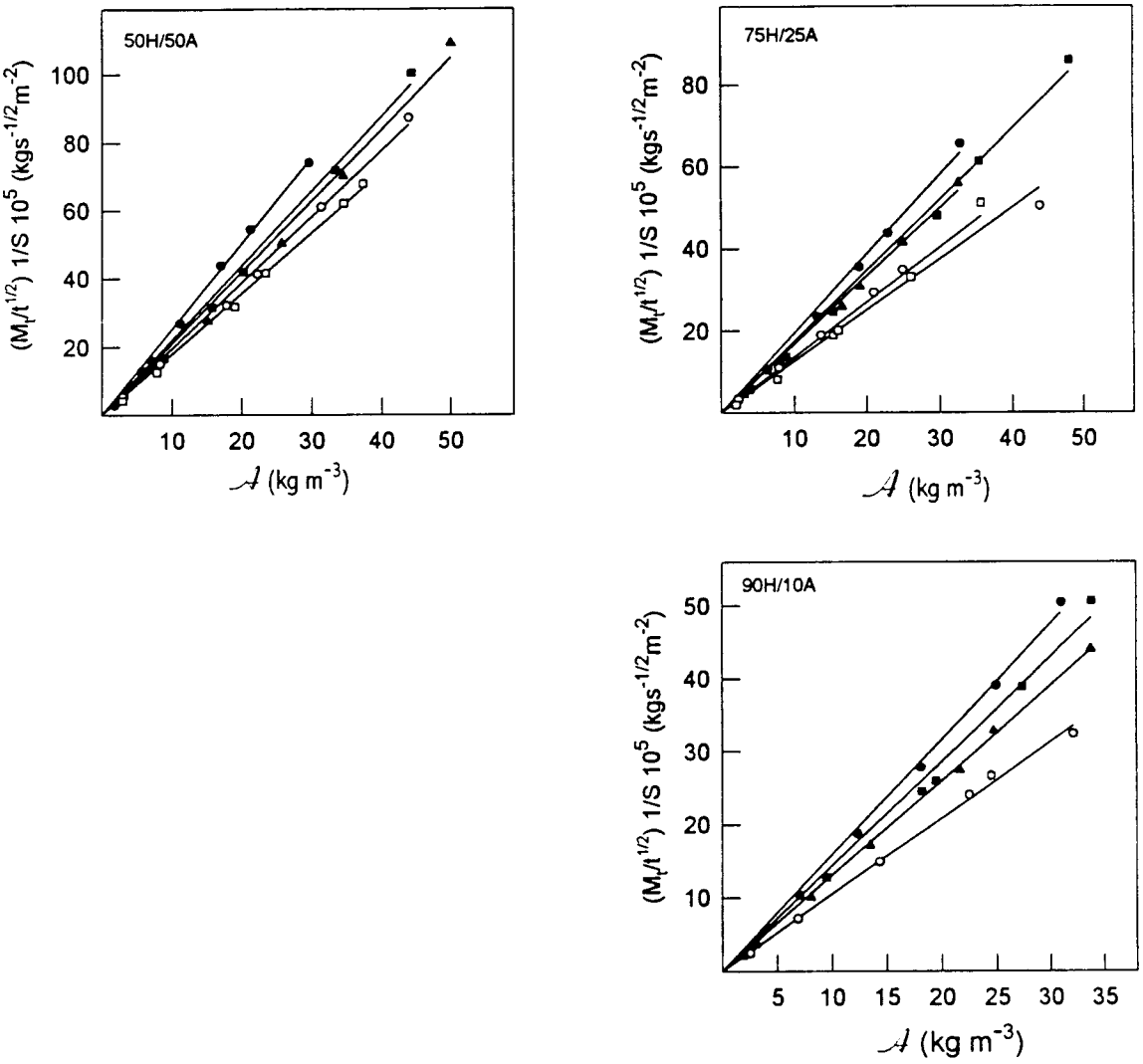

Fig. 7. Representation of the 5-FU release rate in saline solution at 310 K per unit disc area ($M_t t^{-1/2} S^{-1}$) as a function of the 5-FU load included. (A) 50 HEMA/50 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt%, (○) 7 wt% and (□) 10 wt% of EGDMA; (B) 75 HEMA/25 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt%, (○) 7 wt%, (□) 10 wt% and (△) 13 wt% of EGDMA; and (C) 90 HEMA/10 A gels crosslinked with (●) 1 wt%, (■) 3 wt%, (▲) 5 wt% and (○) 7 wt% of EGDMA.

Independently of the 5-FU load included in the discs, the release of drug from copolymers varies, in the extreme cases, between 7 h for the discs of 50 HEMA/50 A–1% EGDMA and 9 days for 90 HEMA/10 A–7% EGDMA gels, approximately. This way, as a function of the monomers composition and of the crosslinking degree, a very wide range of times of drug release is obtained. In Table 2 the interval of loads studied for each gel and the time necessary for complete 5-FU release from each copolymer are shown.

These release times diminish with the acrylamide percentage in the copolymer increases, except in the case of 50 HEMA/50 A–10% EGDMA gels with regard to that of 75 HEMA/25 A. The crosslinker influence at higher EGDMA concentrations is less decisive in the diffusion behavior for 75 HEMA/25 A gels than the monomeric composition of the hydrogel, which agrees with the values of D_s obtained for the swelling of these gels in saline solution (Fig. 3). In the rest of the studied cases, the presence of acrylamide gives place to gels with higher W_∞ and they swell more quickly. On the contrary, when increasing the EGDMA percentage, more densely crosslinked

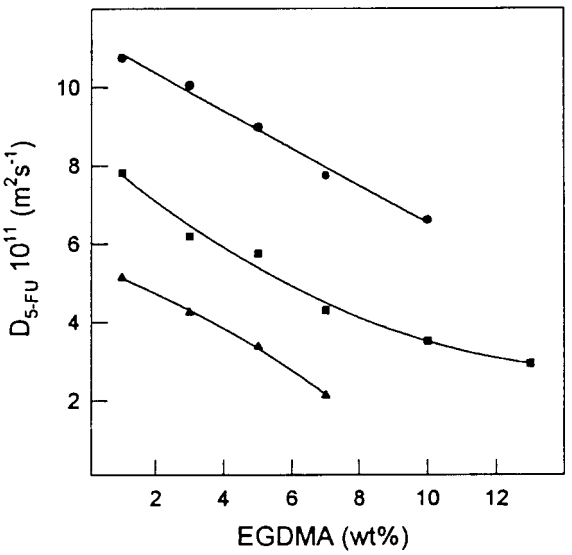

Fig. 8. Variation of the diffusion coefficient independent of the disc load for the 5-FU release in saline solution ($D_{5\text{-FU}}$) from poly(2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A–% EGDMA) hydrogels as a function of the crosslinking degree at 310 K: (●) 50 HEMA/50 A; (■) 75 HEMA/25 A; and (▲) 90 HEMA/10 A.

matrices are obtained, with smaller pores and therefore the swelling in saline solution is restricted, decreasing the drug diffusion rate and giving place to an increase of the 5-FU release total time, in all cases.

5-FU has already been object of release studies from different polymeric supports, among which are different copolymeric hydrogels also with HEMA in their composition, for example poly(HEMA-co-bisglycol acrylate) gels polymerized as spherical beads of 3 mm diameter. This gel can include a maximum of 0.53 mg of 5-FU/bead; from these hydrogels the total drug release takes place in about 20 h [15]. Another type of copolymeric hydrogels based on PHEMA, from which 5-FU release has been assayed, is variable collagen concentration in its structure with the purpose of improving biocompatibility. From these gels complete 5-FU release is reached in a maximum of 10 days using 5-FU loads of 45 mg/bead [16,36].

When 5-FU release has been carried out from new synthesis gels of poly(acrylamide-co-monoalkyl itaconate), the disc dimensions of which are 4.11 ± 0.15 mm of thickness and 10.42 ± 0.38 mm of diameter, the total time of drug release is between 25 ± 1 h from poly(acrylamide-co-monobutyl itaconate) (85/15 w/w%) hydrogels and 100 ± 3 h for poly(acrylamide-co-monopropyl itaconate) (75/25 w/w%) hydrogels. The poly(2-hydroxyethyl methacrylate-co-acrylamide) copolymers have allowed to enlarge the interval of total time of 5-FU release between 6.9 ± 1.4 h and 214.5 ± 10.5 h (9 days). This variety of release times becomes feasible in the case of poly(2-hydroxyethyl methacrylate-co-acrylamide) copolymers due to both the monomeric composition of the gels and their range of crosslinking. In these gels, the crosslinking degree is the more important modulator factor that allows the 5-FU release to be controlled, while in the case of the hydrogels derived from monoalkyl itaconates the crosslinking proportion (2 wt% *N*,*N*′-methylenbisacrylamide) was very limited due to the crosslinker solubility in the polymerization mixture [17,18].

Starting from the exposed results, we can conclude that the synthesis of poly(2-hydroxyethyl methacrylate-co-acrylamide) copolymers not only allows the inclusion of the 5-fluorouracil in the feed mixture of polymerization, up to 16 mg/disc without any chemical drug alteration detected neither during the polymerization process, nor during the drug release, but rather they also make possible the control of its release in a wide range of release times that vary between 7 h and 9 days, just by modulating the crosslinking degree of the copolymer as well as their comonomeric composition, maintaining sufficiently high hydration degrees, between 66 and 24 wt% to assure, at least a priori, a good biocompatibility of these matrices to their possible applicability in in vivo systems.

Table 2
Time, t, in hours necessary to obtain the complete 5-FU release from poly(2-hydroxyethyl methacrylate-co-acrylamide) discs with different crosslinker percentages, EGDMA ($\mathscr{A}$: range of 5-FU loads used)

Hydrogel	EGDMA (wt%)	$\mathscr{A}$ (kg m^{-3})	Time (h)
50 HEMA/50 A	1	2.9–42.2	6.9 ± 1.4
	3	3.2–44.3	7.5 ± 0.8
	5	2.8–50.0	28.5 ± 3.5
	7	3.0–43.9	74.9 ± 0.1
	10	2.9–37.2	185.5 ± 15.5
75 HEMA/25 A	1	2.3–32.8	23.0 ± 1.7
	3	3.9–48.1	23.8–1.7
	5	3.1–32.6	28.8 ± 2.7
	7	2.4–33.1	75.3 ± 3.1
	10	2.0–35.7	170.7 ± 2.8
	13	2.1–34.3	198.5 ± 16.5
90 HEMA/10 A	1	2.9–31.0	29.2 ± 1.8
	3	1.9–33.8	37.0 ± 9.5
	5	2.9–33.8	59.9 ± 14.9
	7	2.6–32.2	214.5 ± 10.5

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