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New biodegradable hydrogels based on a photo-cross-linkable polyaspartamide and poly(ethylene glycol) derivatives. Release studies of an anticancer drug

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Abstract The functionalization of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) with glycidyl methacrylate (GMA) gives rise to a water-soluble photosensitive copolymer PHEA-GMA (PHG). Aqueous solutions of PHG alone or in combination with various concentrations of poly(ethylene glycol) dimethacrylate or poly(ethylene glycol) diacrylate (PEGDA) have been exposed to a source of UV rays at 313 nm in order to obtain polymeric networks. All samples have been prepared both as water-swellable microparticles and as gel systems. Microparticles have been characterised by Fourier transform IR spectrophotometry, dimensional analysis and swelling measurements in aqueous media mimicking biological fluids. In vitro chemical and enzymatic hydrolysis studies showed that all the prepared samples undergo a partial degradation at pH 1, 7.4 and 10 as well as after incubation with enzymes such as esterase. The effect of the cross-linking density on the rheological behavior of gel systems has also been investigated. PHG/PEGDA hydrogel is able to incorporate, during UV-irradiation, 5-fluorouracil (5-FU), chosen as a model drug, and to release it in simulated biological fluids, as confirmed by in vitro drug release studies at pH 1 and 7.4. PHG/PEGDA gel containing 5-FU is able to release this drug in a prolonged way, more slowly than a commercial ointment, as confirmed by in vitro studies at pH 5.5 and 7.4 using a Franz diffusion cell system and a synthetic membrane.

Key words Biodegradable hydrogels · 5-Flourouracil · Anticancer drug · Kinetics of release

Introduction

Special attention has been paid, in recent years, to polymeric hydrogels owing to their excellent molding and shaping properties as well as to a variety of physical and chemical characteristics which justify their applications in biomedical and pharmaceutical fields [1–3]. These applications include controlled-release drug delivery systems, sensor catheter membranes, cell-incorporating networks and hybrid artificial organs [4–7]. Covalently cross-linked polymers often form hydrogel networks, but ionic bonds and van der Waals forces can also lead to water-swellable materials [8, 9]. Conventional chemical cross-linking has been extensively used as a hydrogel preparation method; in contrast, there are few reports on the preparation of hydrogels via photo-*cross*-linking of water-soluble polymers [10–14]. In effect, UV- induced cross-linking of hydrophilic polymers represents a practical method for producing well-defined networks and provides significant advantages over conventional chemical cross-linking, such as the easiness (the synthesis is often carried out in a single step and without the presence of initiators), safety and low cost [15].

The principle used is based on cross-linking via

1. Photodimerization of photosensitive groups such as cinnamate, stilbazolium or coumarin, which are

added as terminal functional groups to hydrophilic polymers [15, 16].
2. ''Free-radical type'' polymerization of a polymer containing photo-cross-linkable groups (e.g. derivatives of acrylic or methacrylic acid) [11, 17]. Obviously these polymers would be stable during storage and processing and could be cross-linked by simple exposure to a suitable source of radiation.

In order to follow the latter approach, in a previous work α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA), a biocompatible polymer [18, 19], was partially modified by reaction with glycidyl methacrylate (GMA) to obtain PHEA-GMA (PHG), a water-soluble photosensitive copolymer, carrying methacryloyl groups in the side chain [20]. Successively, we have verified that aqueous solutions of PHG in the presence or not of *N*,*N*′-methylenebisacrylamide (BIS) are converted into hydrogels by free-radical polymerization using UV radiation [21]. These hydrogels showed a rapid swelling behavior and a partial degradation when incubated in the presence of hydrolytic enzymes or aqueous media at various pH values. We have also demonstrated that PHG aqueous solutions can be cross-linked by exposure to a γ-ray source [22]. As a continuation of these studies, in the present article we report the synthesis and characterization of different types of hydrogels based on PHG alone or in mixture with poly(ethylene glycol) diacrylate (PEGDA) or with poly(ethylene glycol) dimethacrylate (PEGDMA) which, in turn, are photosensitive polymers [23]. Moreover, PEGDMA and PEGDA have been chosen in this study because of the known biocompatibility of polymers having poly(ethylene glycol) (PEG) moieties. The combination of PHG with PEGDA and PEGDMA could give rise to new hydrogels with a degree of swelling potentially controlled by the amount of PEGDA or PEGDMA in the network. Therefore, aqueous solutions of PHG were irradiated for 3.5 h under argon at 313 nm in the presence or not of PEGDA or PEGDMA at different concentrations. All samples were prepared both as water-swellable microparticles and as gel systems; their physicochemical characterization is reported here.

In vitro studies of chemical and enzymatic hydrolysis on the networks obtained were carried out to confirm their potential biodegradability.

In addition, the potential of PHG/PEGDA hydrogel as a novel drug-release system was investigated using 5-fluorouracil (5-FU) as a model drug. This drug is widely used for the treatment of colon carcinoma [24] and can be considered an ideal candidate for a drug-delivery system [25, 26]. In effect, 5-FU has a short plasma half life (10 min) following intravenous administration [27] and its administration causes several side effects owing to the lack of selective action, such as leukopenia, gastrointestinal ulceration, alopecia, cardiotoxicity, vomiting and nausea [28, 29]. 5-FU is also used topically for the treatment of superficial basal cell carcinoma and multiple actinic keratoses when conventional methods are impracticable [30]. Hydrogels designed to release 5-FU in a prolonged and/or controlled way theoretically provide many advantages, such as an optimization of pharmacokinetics and, in addition, for topical treatment, the drug release in the disease site.

Therefore, 5-FU was incorporated into PHG/PEGDA hydrogel and the drug-release rate was evaluated by performing in vitro studies in simulated gastric and extracellular fluids. A gel of PHG/PEGDA containing 5-FU was also prepared and characterized by rheological measurements and in vitro release studies using a Franz cell system and a synthetic membrane. The results were compared with those obtained from a commercial ointment containing 5-FU (EFUDIX).

Materials and methods

Chemicals

All reagents were of analytical grade, unless otherwise stated. D,L-aspartic acid, ethanolamine, *N*,*N*-dimethylformamide (DMF), anhydrous *N*,*N*-dimethylacetamide (DMA), BIS and 5-FU were from Fluka (Italy). GMA, 4-dimethylaminopyridine (4-DMAP), PEGDA (M_n 258), PEGDMA (M_n 330) and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (Italy). Pepsin from porcine stomach mucosa 3500 U (mg/protein), α-chymotrypsin from bovine pancreas 49 U (mg/protein) and esterase from porcine liver 250 U (mg/protein) were purchased from Sigma (Italy). EFUDIX was from INC Pharmaceuticals (Germany).

PHEA was prepared by reaction of a polysuccinimide, obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution, purified and characterized according to a procedure elsewhere reported [31]. The batch of PHEA used in the present study had a weight-average molecular weight of 56,900 ($M_w/M_n = 1.79$).

Derivatization of PHEA with GMA (PHG copolymer) was carried out in the organic phase (anhydrous DMA), using 4-DMAP as a catalyst, purified and characterized according to a procedure elsewhere reported [20]. The degree of derivatization (DD) of the PHG prepared, determined by ^{1}H NMR and calculated according to the method elsewhere reported [20] was 28 ± 1 mol%. The weight-average molecular weight of PHG copolymer determined by light scattering measurements, was 71,000 ($M_w/M_n = 1.86$).

Cellulose acetate membrane (Schleicher & Schuell) type OE 67 was obtained from Bracco (Italy).

Apparatus

The molecular weights of the starting PHEA and PHG copolymer were determined by light scattering measurements, using a Spectra Physics Dawn DSP-F laser spectrometer.

^{1}H NMR spectra were obtained with a Bruker AC-250 instrument operating at 250.13 MHz. Samples were solubilized in D_2O.

Fourier transform (FT) IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Perkin-Elmer 1720 FT spectrophotometer. The resolution was 1 cm^{-1}. The number of scans was 100.

UV irradiation was performed using a Rayonet reactor equipped with a Rayonet carousel motor assembly and 16 mercury lamps of 8 W at medium pressure with an emission at 313 nm.

The particle size distribution was studied using a Leica Quantimet Q 500 image processing and analysis system equipped with a Leica Wild 3D stereomicroscope.

Viscosity measurements were carried out using a Brookfield DV-III rotatory viscometer model with a SC4-16 cylindrical spindle. The temperature was controlled during the test with a Neslab RTE-110 thermostat.

Centrifugation was performed with an International Equipment Company Centra MP4R equipped with an 854 rotor and temperature control.

High-pressure liquid chromatography (HPLC) analyses were carried out using a Varian 9012 liquid chromatograph equipped with a Rheodyne 7125 injector (fitted with a 10-μl loop), a Kontron HPLC 432 detector and a Hewlett-Packard 3394 integrator. For the analyses a reversed-phase C_{18} column (μBondapak; 10 μm of 250 × 4.6 mm internal diameter, obtained from Waters) was used.

X-ray diffraction analysis was performed using a Philips PW 1729 X-ray generator diffractometer. The experimental parameters were set as follows: Cu Kα radiation, tube setting 40 kV, 20 mA; angular speed 2° (2θ/min); range recorded 10–40° (2θ/min); time constant 1 s, chart speed 2 cm/min.

The battery system with six Franz diffusion cells was obtained from Laboratory Glass Apparatus (Berkeley, Calif., USA). The temperature was controlled during the test with a Neslab RTE-110 thermostat.

Preparation of polymeric networks

Solutions of PHG copolymer (60 g/l, sample a) in double-distilled water were placed in Pyrex tubes each equipped with an internal Pyrex piston in order to have a sample of about 2-mm in thickness of sample then irradiated for 3.5 h under argon at 313 nm. PHG was also irradiated in combination with PEGDMA (10 and 20 mol% relating to the moles of GMA residues linked to PHEA, samples b and c) and PEGDA (10 and 20 mol% relating to the moles of GMA residues linked to PHEA, samples d and e).

Each gel was purified by several washes with distilled water, centrifuging, from time to time, at 12000 rpm at 4 °C for 20 min. After further washing with acetone, the powder obtained was dried at 10^{-1} mmHg in the presence of P_2O_5 until its weight remained constant.

Evaluation of irradiation effects on 5-FU

An aqueous solution of 5-FU (10 g/l) with or without PEGDA (8.88 g/l) was irradiated for 3.5 h under argon at 313 nm.

After irradiation, the solution was analyzed by HPLC, then lyophilized and the solid residue characterized by FT-IR analysis.

The absence of alteration of 5-FU was confirmed by comparing the HPLC chromatogram and IR spectrum with those of non-irradiated 5-FU. In particular, the HPLC analysis was performed on a reversed-phase C_{18} column with H_3PO_4 0.05 vol%/methanol 98:2, v/v as eluant at 1.0 ml/min and the eluate was monitored at 266 nm.

Preparation of PHG/PEGDA 20% microparticles containing 5-FU (sample e-5-FU)

The polymeric microparticles containing 5-FU were prepared by irradiating with a UV source a solution of PHG copolymer (60 g/l), PEGDA (20 mol% relating to the moles of GMA residues linked to PHEA) and 5-FU (10 g/l) in double-distilled water. The irradiation was carried out for 3.5 h under argon at 313 nm.

After irradiation, the sample (e-5-FU) was washed once with distilled water, centrifuged (12000 rpm; 4 °C; 10 min), then after a fast washing with acetone, the powder obtained was dried to constant weight.

Preparation of gels

Solutions of PHG copolymer (60 g/l) in double-distilled water were irradiated with a UV source, under argon, for 3.5 h at 313 nm (sample A).

PHG aqueous solutions were also irradiated in the presence of PEGDMA (10 and 20 mol% relating to the moles of GMA residues linked to PHEA, samples B and C) and PEGDA (10 and 20 mol% relating to the moles of GMA residues linked to PHEA, samples D and E). After 3.5 h of irradiation, the gels obtained (A, B, C, D and E) were washed once with distilled water then characterized by rheological measurements.

Preparation of gel E containing 5-FU

A polymeric gel containing 5-FU was prepared by irradiating, at 313 nm, a solution of PHG copolymer (60 g/l), PEGDA (20 mol% relating to the moles of GMA residues linked to PHEA) and 5-FU (10 g/l) in double-distilled water (sample E-5-FU). The irradiation was carried out under argon at 313 nm for 3.5 h. After this time, the gel E-5-FU was washed once with distilled water, then characterized.

Determination of the amount of drug trapped in samples e-5-FU and E-5-FU

Aliquots of 20 mg of the dried sample e-5-FU or 500 mg of gel sample E-5-FU were extensively extracted at room temperature with distilled water. The amount of 5-FU released was determined by HPLC using the conditions reported earlier. The amounts of 5-FU found were 8% w/w for the dried sample e-5-FU and 0.54% w/w for the gel sample E-5-FU.

Swelling studies

The swelling ability of polymeric microparticles (samples a, b, c, d and e) was determined at 37 °C in double-distilled water, HCl 0.1 N (pH 1) and phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) at pH 7.4 and 6.8. In particular, aliquots of each dried and exactly weighed sample were kept in contact with the penetrant medium until the equilibrium swelling was reached; then each swollen sample was filtered, plugged with blotting paper and weighed. The weight swelling ratio was calculated as follows:

$$q = W_s/W_d \ ,$$

where W_s and W_d are the weights of swollen and dry sample respectively.

Each experiment was performed in triplicate and the results were in agreement within ±2% error.

Chemical hydrolysis

Chemical hydrolysis of samples a, b, c, d and e was investigated in HCl 0.1 N (pH 1), phosphate buffer solution pH 7.4 (NaCl, Na_2HPO_4, KH_2PO_4) and NaOH 10^{-4} N (pH 10). Samples (25 mg) were dispersed in 10 ml of liquid medium, then kept in a water bath at 37 ± 0.1 °C with continuous stirring (100 rpm) for 10 days. When pH 7.4 was used, the hydrolysis was investigated as a function of time (5, 10, 15, 20, 25 days). After the hydrolysis, the samples were neutralized (when necessary), centrifuged at

12000 rpm at 10 °C for 15 min and the supernatant was separated. For each sample, the remaining hydrogel was washed five times with distilled water under continuous stirring at 37 °C for 1 h to extract some soluble polymer chains and electrolytes remaining inside the network. Finally, the remaining hydrogel was washed with acetone and centrifuged at 12000 rpm at 10 °C for 15 min. The solid residue recovered was dried, weighed and characterized by FT-IR analysis and swelling studies in double-distilled water.

Each experiment was performed in triplicate and the results were in agreement within ±2% error.

Enzymatic hydrolysis

Accurately weighed aliquots of samples a, b, c, d and e (25 mg) were incubated under continuous stirring (100 rpm) at 37 ± 0.1 °C for 5, 10, 15, 20 and 25 days with

1. HCl solution (2 ml, 0.01 N) at pH 2 containing pepsin (final enzyme concentration 0.8 g/l) for 24 h.
2. Tris(hydroxymethyl)aminomethane buffer solution (2 ml) at pH 8 containing $CaCl_2$ 0.1 M and α-chymotrypsin (final enzyme concentration 0.8 g/l) for 24 h.
3. Phosphate buffer solution (2 ml, NaCl, Na_2PO_4, KH_2PO_4) containing esterase (final enzyme concentration 0.5 g/l).

For the experiments carried out in the presence of esterase, a new dose of enzyme was added after each 24 h. Enzyme solutions were prepared immediately before the experiment. After each experiment, the samples were neutralized (when necessary), purified and characterized by the same methods used for the samples obtained after chemical hydrolysis. Control experiments were carried out under the same reaction conditions without any enzyme.

Each experiment was performed in triplicate and the results were in agreement within ± 3% error.

Rheological study

The viscosity measurements of the gels prepared (samples A, B, C, D, E and E-5-FU) were performed at 37 ± 0.01 °C. The rheograms (viscosity as a function of the angular speed) were obtained using an angular speed ranging from 0.5 to 20 rpm (0.5 rpm/10 s) for all the samples. The rheogram of an ointment containing 5-FU (EFUDIX) was also obtained under the same conditions as reported previously. All measurements were carried out in triplicate and the results agreed with each other within ±2% error.

In vitro release studies

Aliquots (10 mg) of sample e-5-FU were dispersed in flasks containing 25 ml phosphate buffer (pH 7.4, NaCl, Na_2HPO_4, KH_2PO_4) or 0.1 N HCl solution (pH 1). The solutions in the flasks were stirred continuously (100 rpm) and maintained at 37 ± 0.1 °C. Sink conditions were maintained during the experiments. At suitable times, the samples were filtered through a 0.45-μm. Millipore filter and assayed by HPLC analysis using the conditions reported earlier. All experiments were carried out in triplicate and the results agreed with each other within ±2% error.

In vitro diffusion studies

The 5-FU diffusion rate in the E-5-FU gel was evaluated using a battery system of six Franz diffusion cells, each with a receiving compartment volume of 4.2 ml and an effective diffusion area of 0.95 cm^2. The receptor phases used were phosphate buffers at pH 7.4 (NaCl, Na_2HPO_4, KH_2PO_4) or at pH 5.5 (Na_2HPO_4, KH_2PO_4), stirred continuously (600 rpm) by a rotating Teflon-coated magnet placed inside the cell and thermostatted at 37 ± 0.1 °C.

Aliquots (1.018 g) of gel (sample E-5-FU) or 110 mg of the ointment EFUDIX were placed in the donor compartment on a cellulose acetate membrane with pore size of 0.45 μm and a thickness of 115 μm which had previously been moistened with the receptor phase. Samples (50 μl) of the receptor phase were withdrawn at different times and replaced with the same volume of fresh buffer solution. Sink conditions were maintained during the experiments. The amount of 5-FU released as a function of time was determined by HPLC using the conditions reported earlier.

All experiments (six for each sample) agreed with each other within ±4% error.

Results and discussion

The derivatization of PHEA with GMA is a useful method to introduce pendent double bonds and ester groups in side chains of this macromolecule. The presence of GMA residues makes the starting polymer properly reactive towards a reaction activated by UV rays in order to obtain cross-linked structures [21]. The exposure of aqueous solutions of functionalized polymer (PHG) at a wavelength of 313 nm gives rise to hydrogel formation. Polymeric networks were also prepared by irradiation of PHG aqueous solutions in the presence of PEGDMA and PEGDA, i.e. photosensitive macromolecules which can participate in a cross-linking process giving rise to the formation of hydrogels with a mixed PHG/PEGDMA or PHG/PEGDA structure. In order to evaluate the effect of different amounts of PEG derivatives on the properties of these hydrogels, various concentrations of PEGDMA or PEGDA were used (10 and 20 mol% relating to the moles of GMA residues linked to PHEA) in the reaction mixture. The chemical structures of PHG, PEGDA and PEGDMA and a schematic representation of the networks obtained by UV irradiation are shown in Fig. 1. These networks were prepared both as water-swellable microparticles and as gels (Table 1).

Microparticles

The values of the percentage yield of the microparticles obtained are reported in Table 2. As can be observed, the presence of 10 mol% PEGDMA or PEGDA (samples b and d) causes an increase in yield in comparison with PHG alone, i.e. PEG derivatives improve the cross-linking efficiency. In particular, when PEGDA is used the highest yield is obtained. This behavior could be due to the formation of acrylic radicals belonging to PEGDA during UV irradiation, which are less stable, therefore more reactive, in comparison with methacrylic radicals belonging to PHG and PEGDMA, which, on the other hand, are sterically more hindered. A concentration of PEGDMA or PEGDA of 20 mol% (samples c and e) causes a further increase in the network yield owing to the higher

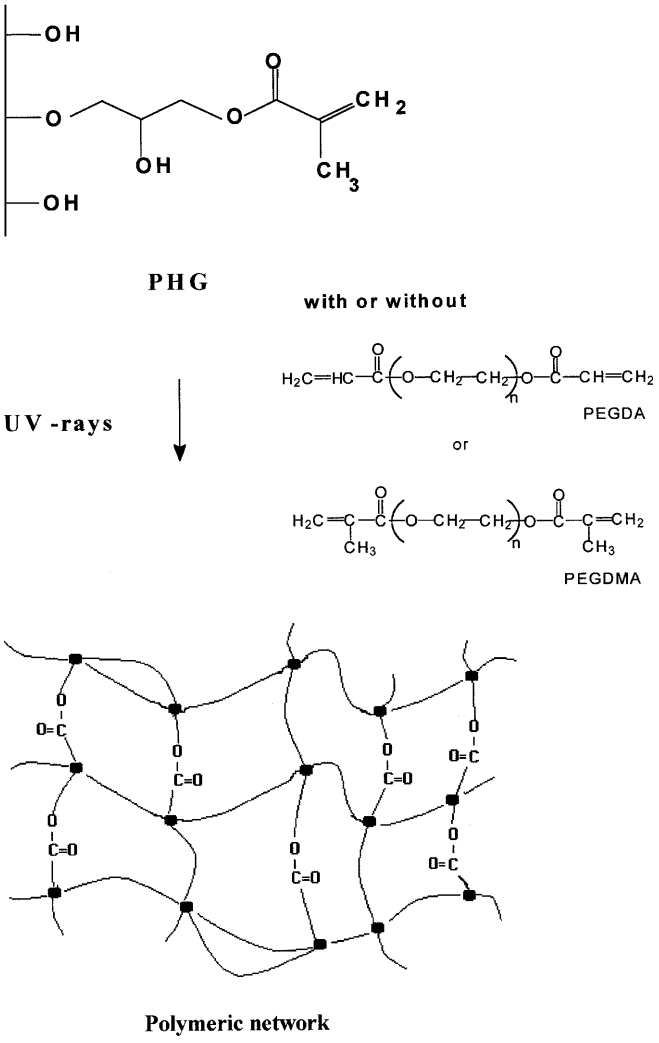

Fig. 1 Chemical structure of the copolymer of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide and glycidyl methacrylate (*PHG*), poly(ethylene glycol) dimethacrylate (*PEGDMA*) and poly(ethylene glycol) diacrylate (*PEGDA*) and schematic representation of the polymeric network obtained by UV irradiation

amounts of reactive functionalities (methacrylic or acrylic groups) in the reaction mixture.

All the networks obtained were insoluble in water and in common organic solvents, such as dichloromethane, acetone, ethanol, dimethyl sulfoxide, dimethyl acetamide, DMF.

FT-IR analysis was used to confirm that the photo-cross-linking reaction happens. In effect, FT-IR spectra of PHG networks obtained alone or in combination with PEGDMA or PEGDA show different peaks in comparison with starting PHG, PEGDMA and PEGDA before irradiation (Fig. 2a, b).

In particular, the more interesting feature is the complete disappearance of peaks related to double bonds, i.e. 1408 cm^{-1} (scissoring –C=C–) and 956 cm^{-1} (wagging –C=C–) of starting PHG and 1638 cm^{-1} (stretching –C=C–conjugated to carbonyl), 1406 cm^{-1} (scissoring –C=C–) and 816 cm^{-1} (wagging of –C=C–) of starting PEGDMA or PEGDA. This suggests that the cross-linking reaction induced by UV rays involves the opening of double bonds, probably through the formation of free radicals which give rise to inter- and intrapolymeric chain cross-linked bonds. In addition, after irradiation, the shift of the ester group asymmetric stretching from 1720 cm^{-1} to about 1730 cm^{-1} confirms the lack of conjugation with the double bonds of methacrylate residues of PHG and PEGDMA or acrylate residues of PEGDA. Finally, the intensity of the band at 1387 cm^{-1} (symmetrical bending of C–H bond in the methyl group) is higher when PHG is irradiated in combination with PEGDMA and it increases on increasing the PEGDMA concentration.

A typical particle size distribution of PHG, PHG/PEGDMA and PHG/PEGDA microparticles is shown in Fig. 3. It can be observed that there is an asymmetric particle distribution with a maximum value of equivalent diameter in the range 3–6 μm for all samples.

In addition, the image processor used for the determination of the particle size distribution also calculates the roundness index, which was always below 1.3.

Table 1 Polymeric networks prepared as microparticles or gels by UV irradiation at 313 nm for 3.5 h. For all samples the concentration of the starting copolymer of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide and glycidyl methacrylate (*PHG*) was 60 g/l. Poly(ethylene glycol) dimethacrylate: (*PEGDMA*); poly (ethylene glycol) diacrylate (*PEGDA*); 5-fluorouracil (*5-FU*)

Aqueous solutions irradiated	Sample	
PHG	Microparticles	a
PHG + PEGDMA 10 mol%	Microparticles	b
PHG + PEGDMA 20 mol%	Microparticles	c
PHG + PEGDA 10 mol%	Microparticles	d
PHG + PEGDA 20 mol%	Microparticles	e
PHG + PEGDA 20 mol% + 5-FU	Microparticles	e-5-FU
PHG	Gel	A
PHG + PEGDMA 10 mol%	Gel	B
PHG + PEGDMA 20 mol%	Gel	C
PHG + PEGDA 10 mol%	Gel	D
PHG + PEGDA 20 mol%	Gel	E
PHG + PEGDA 20 mol% + 5-FU	Gel	E-5-FU

Table 2 Yield (%) of microparticle samples obtained after UV irradiation of aqueous solutions of PHG alone or in combination with PEGDMA or PEGDA at various concentration

Sample	Yield (%)
a	84.1
b	86.4
c	89.3
d	96.9
e	98.7

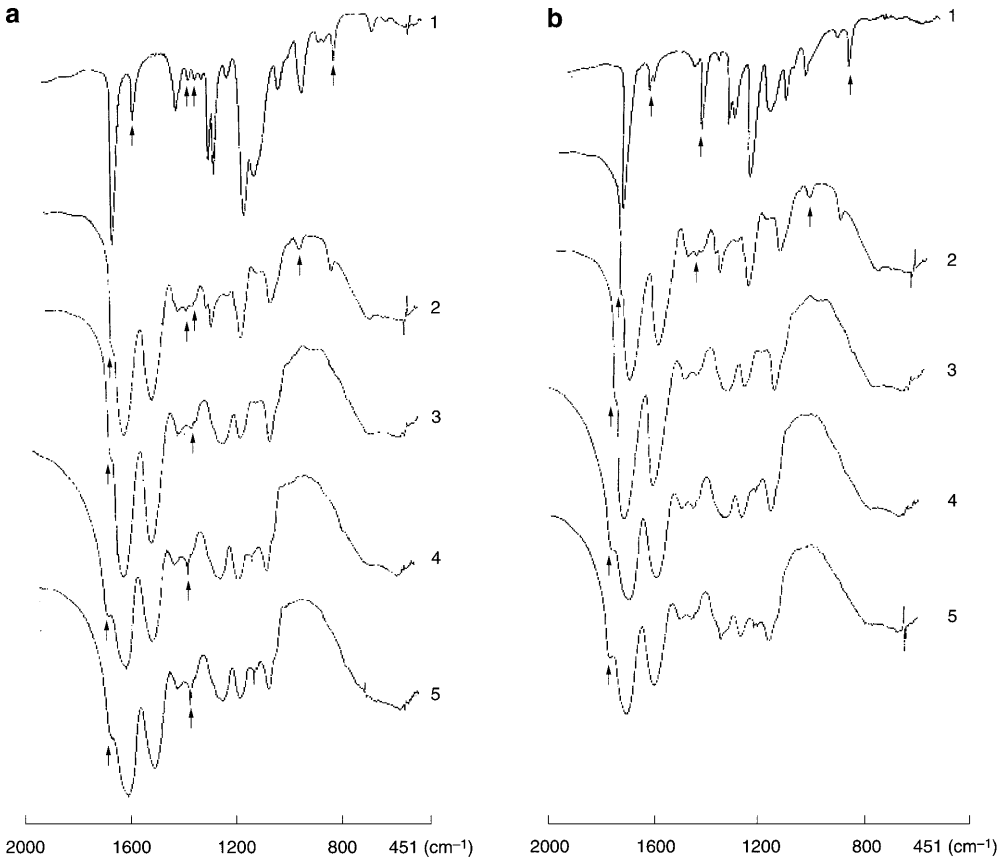

Fig. 2 Fourier transform (*FT*) IR spectra of *1* starting PEGDMA, *2* starting non-cross-linked PHG, *3* cross-linked PHG, *4* cross-linked PHG/PEGDMA 10%, *5* cross-linked PHG/PEGDMA 20%. **b** FT-IR spectra of *1* starting PEGDA, *2* starting non-cross-linked PHG, *3* cross-linked PHG, *4* cross-linked PHG/PEGDA 10%, *5* cross-linked PHG/PEGDA 20%

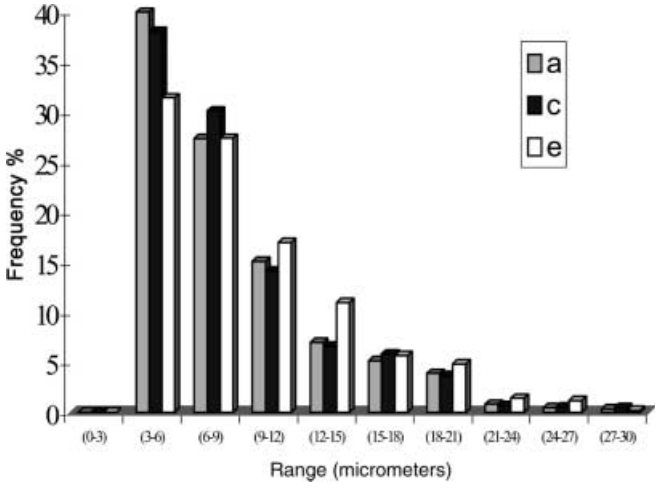

Fig. 3 Size distribution profiles of cross-linked PHG (sample a), PHG/PEGDMA 20% (sample c) and PHG/PEGDA 20% (sample e) microparticles

The roundness index is a parameter which gives information about particle shape. It was calculated using following ratio: $(\text{perimeter})^2/(4 \times \pi \times \text{area} \times 1.064)$, where 1.064 is a correction factor for the angles produced by the image digitalization. Values of the roundness index close to 1 indicate an almost spherical shape.

In order to evaluate the affinity of the networks prepared towards aqueous media, swelling measurements were carried out in double-distilled water at 37 °C on dried PHG, PHG/PEGDMA and PHG/PEGDA microparticles. Swelling measurements were also performed in media at different pH values in order to mimic some biological fluids (simulated gastric, intestinal and extracellular fluids at pH 1, 6.8 and 7.4, respectively). All the results evidenced a remarkable affinity of the microparticles towards the aqueous medium depending on the nature of the network, the pH of the medium and the ionic strength (Table 3).

In particular, the degree of swelling appears to be controlled by the presence of PEGDMA or PEGDA in the hydrogel. As the yield of cross-linked samples increases in the order PHG < PHG/PEGDMA < PHG/PEGDA, we can hypothesize that the rigidity of these gels increases with the same order and, consequently, that the amount of water that they can absorb decreases. The decrease in the weight swelling ratio is probably due to a different degree of cross-linking, which increases in the order PHG < PHG/PEGDMA < PHG/PEGDA in accordance with the different yield of the products obtained (Table 2) and also because the length of the PEGDA bridge is shorter than the PEGDMA one (M_n of PEGDA = 258 whereas M_n of PEGDMA = 330). As

Table 3 Weight swelling ratio (q) values of PHG, PHG/PEGDMA and PHG/PEGDA hydrogels in various media

Sample	Weight swelling ratio (q)			
	H_2O	pH 7.4	pH 6.8	pH 1
a	14.4	7.8	6.4	5.4
b	12.8	7.2	6.2	4.5
c	11.5	6.8	5.7	4.0
d	7.5	5.9	4.5	3.8
e	6.3	5.2	4.2	3.7

far as the effect of the pH of the medium and the ionic strength on the swelling ability is concerned, it is evident that the weight swelling ratio values are higher in double-distilled water, whereas in phosphate buffers at pH 6.8 and 7.4 these values are smaller owing to the osmotic pressure and ionic strength of the penetrant medium. The lowest swelling occurs at acidic pH analogously to that found for PHG hydrogels obtained at 254 nm or through γ irradiation [21, 22].

The presence of chemically or enzymatically degradable bonds, i.e. ester groups in PHG, PHG/PEGDMA and PHG/PEGDA networks (Fig. 1), confers a potential biodegradability to these systems. For this reason, we considered it interesting to evaluate their hydrolysis after chemical or enzymatic treatment. As far as the chemical hydrolysis studies are concerned, small aliquots were placed into aqueous solutions at various pH (pH 1, 7.4 and 10) at 37 °C for 10 days. After this time the percent residual weight was determined (Table 4) and the weight swelling ratio of the remaining residue was measured in double-distilled water (Table 5).

It is evident that in all the cases studied, a degradation of the network structure occurs, as shown by the decrease in the yield after the chemical treatment. At pH 10, as expected, the yield values are lower than for other media, owing to more hydrolysis of ester bonds. Moreover, the remaining residues have a greater swelling ability in comparison with the starting hydrogels (before the chemical treatment) as evidenced by comparing the weight swelling ratio values reported in Table 5 with those reported in Table 3.

Table 4 Yield (%) of hydrogels recovered after chemical treatment (pH 1, 7.4 or 10) for 10 days or incubation with pepsin and α-chymotrypsin for 24 h

Sample	Yield (%)				
	pH 1	pH 7.4	pH 10	Pepsin	α-Chymotrypsin
a	85	84	66	95	96
b	82	76	62	93	90
c	82	74	64	90	92
d	86	78	65	94	93
e	89	77	66	96	95

Table 5 Weight swelling ratio (q) determined in double-distilled water of hydrogels recovered after chemical treatment (pH 1, 7.4 or 10) for 10 days or incubation with pepsin and α-chymotrypsin for 24 h

Sample	Weight swelling ratio (q)				
	pH 1	pH 7.4	pH 10	Pepsin	α-Chymotrypsin
a	30.2	36.6	49.5	15.9	16.6
b	19.1	21.2	37.4	13.5	15.5
c	18.6	19.8	23.6	13.0	14.1
d	17.1	19.2	20.3	7.7	8.7
e	10.8	12.5	13.8	6.4	7.2

On the other hand FT-IR spectra of samples recovered after chemical treatment showed a reduction of ester asymmetric stretching (peak at 1730 cm^{-1}). This reduction appears more marked after treatment at pH 10 because of hydrolysis and formation of carboxylate and alcoholic groups. As an example, in Fig. 4 the FT-IR spectrum of PHG/PEGDMA 20% recovered after treatment at pH 10 is reported and compared with the untreated sample.

A more detailed hydrolytic study was performed under simulated physiological conditions (phosphate buffer pH 7.4, 37 °C) as a function of time. The yield of the remaining hydrogels as a function of hydrolysis time and their swelling ability, as reported in Fig. 5a and b respectively, confirm that all the samples undergo a progressive partial degradation (Fig. 5a, b) since the yield decreases and simultaneously the weight swelling ratio increases with time. In addition it is also evident that the samples containing PEGDMA or PEGDA

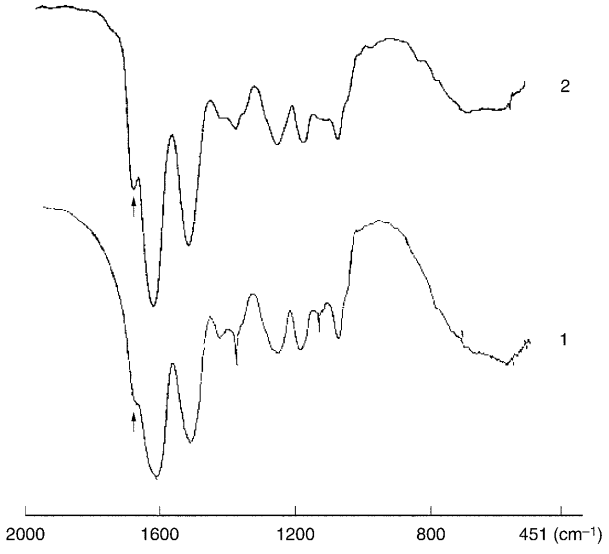

Fig. 4 FT-IR spectra of PHG/PEGDMA 20% sample before (*1*) and after (*2*) treatment at pH 10 for 10 days

moieties undergo a more marked weight decrease with respect to the PHG hydrogel with time.

In order to evaluate the stability of all five samples towards the enzymes of the gastrointestinal tract, hydrolysis assays were performed in the presence of pepsin and α-chymotrypsin for 24 h.

The small decrease in the weight of hydrogels recovered after enzymatic treatment and the small increase in the weight swelling ratio values (Tables 4, 5) suggest the weak action of pepsin and α-chymotrypsin on these networks, i.e. as expected, a scarce degradation process occurs. On the other hand, FT-IR analysis revealed no significant difference in comparison with samples that were not treated.

Owing to the presence of ester bonds, the hydrogels prepared were also incubated in the presence of esterase and in order to obtain information about the rate of hydrolysis caused by this enzyme, the hydrolytic study was performed as a function of time, until 25 days had elapsed.

The yield of the remaining hydrogels as function of incubation time and their swelling ability was taken as a measure of the progressive degradation by esterase (Fig. 6a, b).

In particular, in the presence of esterase it is evident that all the samples undergo a decrease in their weight, whereas their swelling ability increases after incubation with this enzyme. These effects are more marked with respect to those obtained at pH 7.4 but in the absence of esterase (Fig. 5a, b), thus suggesting that this enzyme is able to hydrolyze ester groups present in the network structure. At longer incubation times the hydrolytic effect of esterase is more notable; this suggests a slow action of the enzymes which probably initially hydrolyze the external sites of the network but, as time elapses they have the possibility to penetrate into the network structure, thus hydrolysing also the internal sites.

As far as FT-IR analysis is concerned, the samples treated with esterase showed modification in the spectrum where the intensity of the ester band was reduced. This is evident in Fig. 7, where, as an example, the FT-IR spectrum of PHG/PEGDA 20% recovered after incubation with esterase for 20 days is reported and compared with that of the untreated sample.

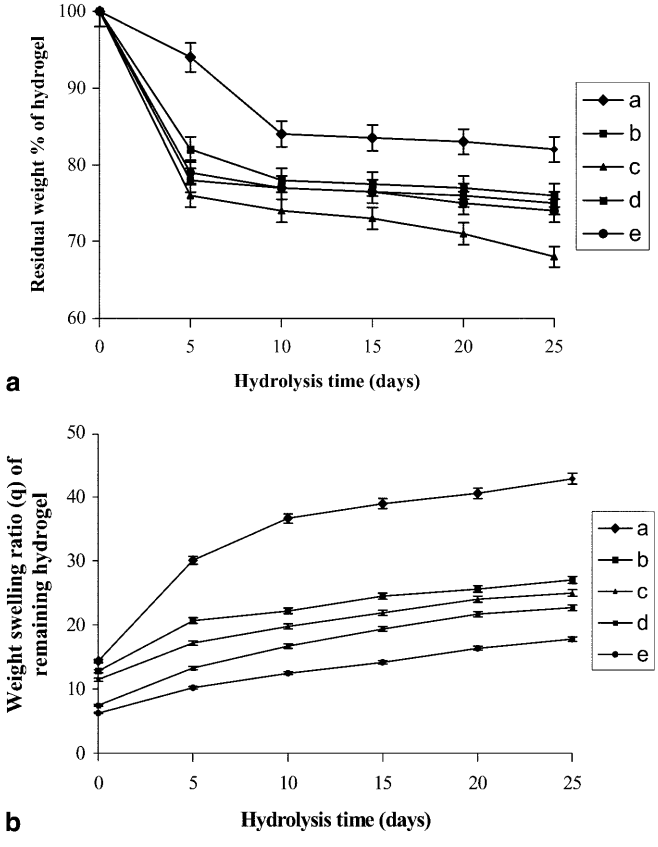

Fig. 5 Residual weight percentage of hydrogels due to hydrolytic cleavage at pH 7.4, 37 °C as a function of hydrolysis time. **b** Weight swelling ratio of hydrogels after hydrolytic cleavage at pH 7.4, 37 °C as a function of hydrolysis time

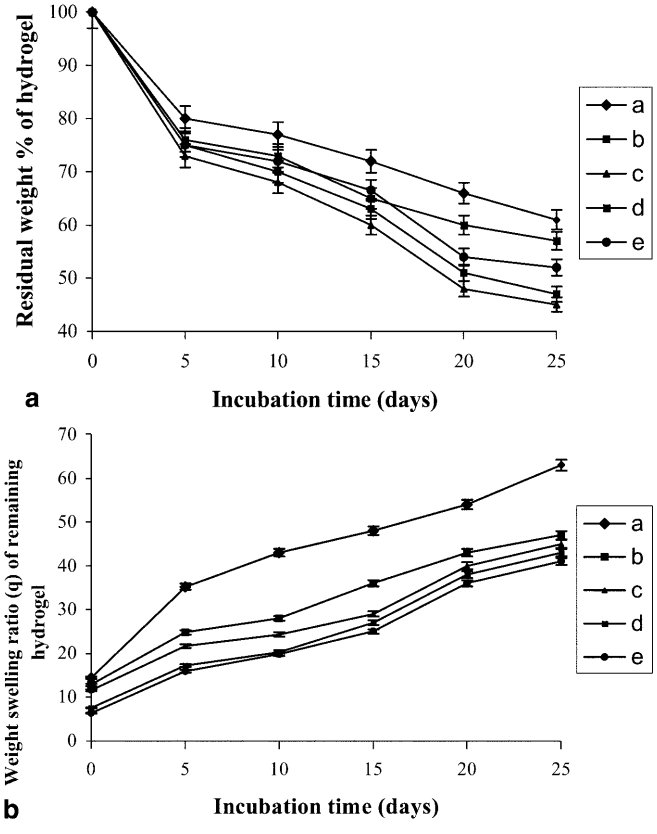

Fig. 6 **a** Residual weight percentage of hydrogels due to hydrolytic cleavage by esterase at 37 °C as a function of incubation time. **b** Weight swelling ratio of hydrogels after hydrolytic cleavage by esterase at 37 °C as a function of incubation time

PHG/PEGDA 20% microparticles as drug-delivery systems

In order to evaluate the ability of the networks prepared to physically trap drug molecules and release them in a physiological medium, PHG/PEGDA 20% microparticles were chosen as a model to estimate their potential application as drug-delivery systems. In addition, in this study, 5-FU, an anticancer agent used in the treatment of colon carcinoma, was selected as an example of a drug of low molecular weight.

For the preparation of 5-FU-loaded PHG/PEGDA 20% microparticles, an aqueous solution of starting polymers (see Materials and methods) was irradiated in the presence of this drug. In order to verify the absence of alteration on 5-FU caused by UV irradiation, a preliminary test was performed by irradiating aqueous solutions of this drug with and without PEGDA and the reaction mixture was analyzed by HPLC, which revealed that 5-FU remains unaltered. The drug loading value was 8% w/w for the microparticle sample (e-5-FU).

The determination of the drug dispersion state in PHG/PEGDA 20% microparticles was performed by X-ray analysis. X-ray diffraction patterns of pure 5-FU and unloaded and drug loaded PHG/PEGDA 20% samples are reported in Fig. 8. It is evident that pure 5-FU is in the crystalline state; in contrast when dispersed in the PHG/PEGDA 20% network it is in the amorphous state like the unloaded sample.

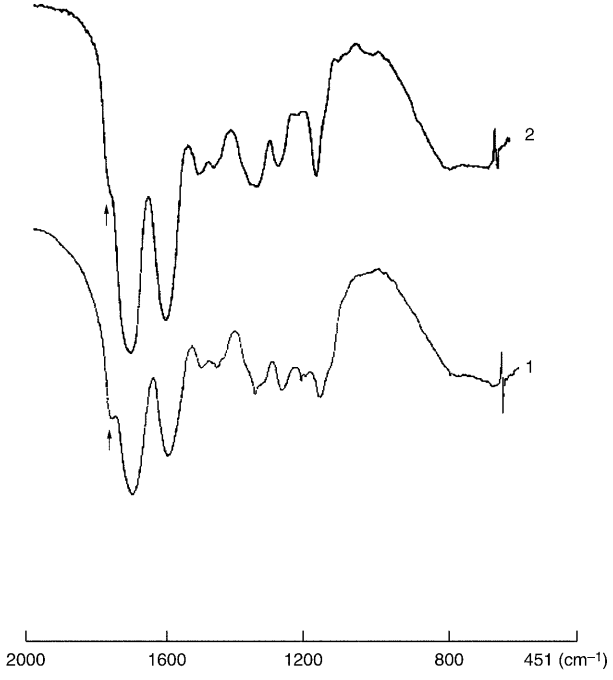

Fig. 7 FT-IR spectra of PHG/PEGDA 20% sample before (*1*) and after (*2*) incubation with esterase for 20 days

The size distribution profile of PHG/PEGDA 20% microparticles containing 5-FU, reported in Fig. 9, is very similar to that of the unloaded sample and analogously, the roundness index was below 1.3.

The evaluation of the drug release was performed in simulated gastric (pH 1) and extracellular (pH 7.4) fluids at 37 °C. The release profiles, reported in Fig. 10, show a complete drug delivery within 70 min at pH 7.4 and 100 min at pH 1.

The higher release rate at pH 7.4 with respect to pH 1 can be related to a higher degree of swelling of the

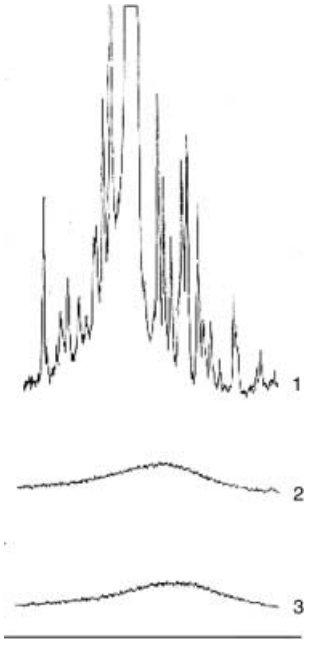

Fig. 8 X-ray diffraction patterns of pure 5-fluorouracil (*5-FU*) (*1*), unloaded PHG/PEGDA 20% sample (*2*) and PHG/PEGDA 20% loaded with 5-FU (*3*)

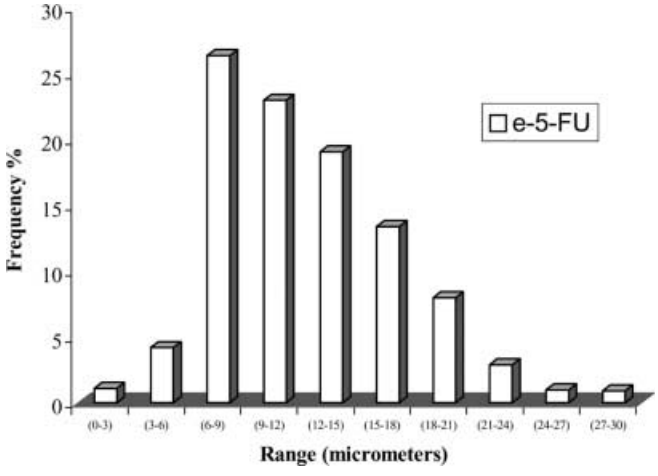

Fig. 9 Size distribution profile of PHG/PEGDA 20% microparticles containing 5-FU (sample e-5-FU)

microparticles in this medium. Obviously, during this short time, polymer degradation cannot influence the release kinetics. Instead, the drug release can be affected by two phenomena, i.e. drug diffusion into swollen polymer and swelling owing to the penetrating medium.

In effect, the kinetics of 5-FU release was analyzed using the empirical exponential equation [32]

$$M_t/M_{inf} = Kt^n \quad , \tag{1}$$

with $M_t/M_{inf} \leq 0.6$. In Eq. (1), M_t/M_{inf} is the drug fraction released at time t, K and n are the constant and the kinetic exponent of drug release, respectively. Although the use of this equation requires detailed statistical analysis [33], the calculated exponent, n, gives an indication of the release kinetics [for spherical matrices it ranges from Fickian ($n = 0.43$) to case II transport ($n = 0.85$), while it is anomalous for intermediate values] [34]. The n values obtained are 0.74 at pH 1 and 0.47 at pH 7.4 (Table 6), indicating that the kinetics of 5-FU release is anomalous, as expected for swellable microparticles. A more informative analysis can be obtained by fitting the data with the model proposed by Peppas and Sahlin [35]. The equation for this model is

$$M_t/M_{inf} = K_1 t^{1/2} + K_2 t \quad , \tag{2}$$

with $M_t/M_{inf} \leq 0.95$. In this equation the first term is the Fickian contribution and the second term is the case II relaxational contribution. K_1 and K_2 values calculated according to Eq. (2) are reported in Table 6. The term $K_1 t^{1/2}$ is greater than the term $K_2 t$ for both pH values, indicating that the predominant mechanism for 5-FU release is the Fickian diffusion through the swollen PHG/PEGDA 20% microparticles.

Finally, in order to calculate the apparent diffusion coefficient, D_i, the final portion of the release profiles ($0.6 \leq M_t/M_{inf} < 1$) was analyzed by means of the approximate diffusion equation for spherical matrices [36]:

$$1 - M_t/M_{inf} = (6/\pi^2)\exp[-(\pi^2 D_i t/r^2)] \quad , \tag{3}$$

r being the mean radius of the swollen particles. We adopted a constant value of $r = 7$ μm at pH 7.4 and $r = 4$ μm at pH 1, determined by observing with a stereomicroscope connected to an image analyzer (see Apparatus) the variation of the microparticle diameter during the release time. The calculated D_i values reported in Table 6 confirm that at pH 7.4 the drug diffusion is faster than at pH 1.

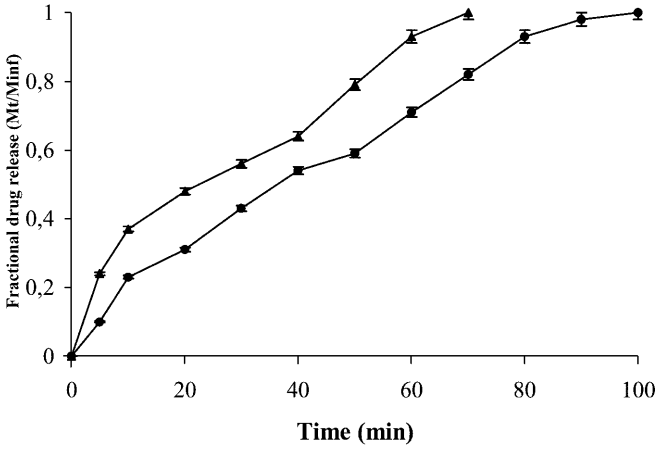

Fig. 10 5-FU release at 37 °C from PHG/PEGDA 20% hydrogel at pH 7.4 (▲) and pH 1 (●) as a function of time

Table 6 Fitting of release data with Eqs. (1), (2) and (3)

$M_t/M_{inf} = Kt^n$ (1)			
pH value	$K\ 10^3$ (min^{-n})	Exponent n	r
1	34.39	0.74	0.993
7.4	118.32	0.47	0.992
$M_t/M_{inf} = K_1 t^{1/2} + K_2 t$ (2)			
	$K_1\ 10^3$ ($min^{-1/2}$)	$K_2\ 10^3$ (min^{-1})	
1	38.77	7.05	0.997
7.4	94.73	2.48	0.987
$1-M_t/M_{inf} = (6/\pi^2)\exp[-(\pi^2 D_i t/r^2)]$ (3)			
	$D_i\ 10^{12}$ ($cm^2 s^{-1}$)		
1	2.79		0.993
7.4	8.50		0.995

Gels

In recent years great attention has turned to gel systems which can be used as dermatological, ophthalmic, rectal or vaginal dosage forms, as well as systems for sustained or controlled drug delivery. For this reason, gels of PHG cross-linked by exposure at 313 nm alone or in the presence of PEGMA and PEGDA at different concentrations (10 and 20 mol% relating to the moles of GMA residues linked to PHEA) were prepared as described in Materials and methods.

All the gels prepared (samples A, B, C, D and E) were characterized by rheological measurements. The rheograms reported in Fig. 11 show that the viscosity decreases as the angular speed increases, thus proving non-Newtonian pseudoplastic behavior. In addition, as expected, the viscosity increases in the order PHG < PHG/PEGDMA 10% < PHG/PEGDMA 20% < PHG/PEGDA 10% < PHG/PEGDA 20% in agreement with the increase in the rigidity of the gel structure discussed earlier.

Since 5-FU is also used topically in the treatment of precancerous dermatoses, especially actinic keratosis, for which it is the drug of choice, we have considered interesting to evaluate the potential ability of PHG/PEGDA 20% as a gel system to release 5-FU. In particular, a gel of PHG/PEGDA 20% containing

0.54% w/w 5-FU was prepared (sample E-5-FU) and characterized by rheological measurements and in vitro release studies. The results were compared with those obtained from a commercial ointment containing 5% w/w 5-FU (EFUDIX).

In particular, the viscosity of the PHG/PEGDA 20% gel containing or not containing 5-FU (samples E and E-5-FU) in the investigated range of angular rate appears to be similar to that of EFUDIX (Fig. 12). The small difference observed can be ascribed to the greater rigidity of the network prepared in comparison with the commercial ointment.

The drug release from the gel or the ointment has been determined through a cellulose acetate membrane using Franz diffusion cells [37] and buffer solutions at pH 7.4 or 5.5 as receptor phases. In order to compare the release data obtained from PHG/PEGDA 20% gel with those of EFUDIX, aliquots of the gel or the ointment placed on the synthetic membrane contained the same amount of 5-FU.

As can be seen in Fig. 13, at pH 7.4 PHG/PEGDA 20% gel is able to release 5-FU in about 15 h, whereas for EFUDIX a complete drug release occurs within 10 h. Moreover, a more marked prolongation of drug release from PHG/PEGDA 20% gel is found at pH 5.5 (value which simulates the skin pH), where 5-FU is released in about 36 h, whereas for EFUDIX the complete drug release occurs within 12 h.

The values of the initial release rate calculated for each sample are the following: 71.40 mg%h^{-1} for EFUDIX at pH 7.4; 63.86 mg%h^{-1} for EFUDIX at pH 5.5; 59.04 mg%h^{-1} for PHG/PEGDA 20% gel at pH 7.4 and 38.02 mg%h^{-1} for PHG/PEGDA 20% gel at pH 5.5. It is evident that from the very beginning of each experiment, 5-FU was released from the gel at a rate lower than EFUDIX.

Finally, as can be seen in Fig. 14, by plotting the amount of 5-FU released as a function of the square root of time, a linear relationship is obtained. The excellent linearity indicates that the kinetics of 5-FU release from PHG/PEGDA 20% gel as well as from EFUDIX agree with the mathematical model postulated by Higuchi [38] for the release of a drug from semisolids, represented by the following equation:

$$q = 2C_0(Dt/\pi)^{1/2} \quad , \tag{4}$$

where q is the amount of drug released into the receptor phase per unit area of exposure, C_0 denotes the drug loading dose initially dispersed in the gel (or ointment),

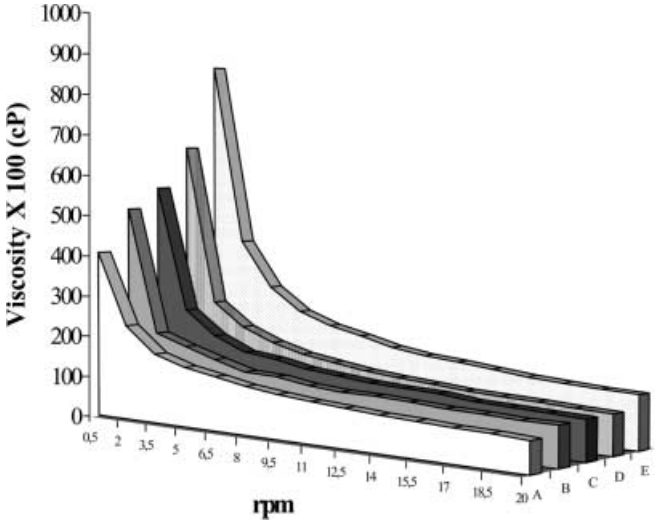

Fig. 11 Plot of viscosity versus angular rate for gels of PHG (*A*), PHG/PEGDMA 10% (*B*), PHG/PEGDMA 20% (*C*), PHG/PEGDA 10% (*D*) and PHG/PEGDA 20% (*E*)

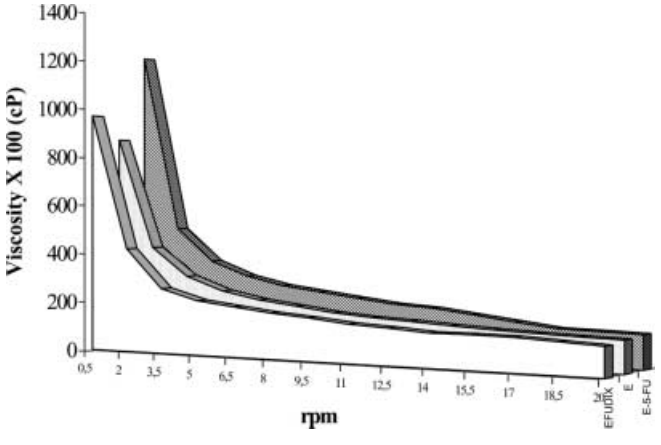

Fig. 12 Plot of viscosity versus angular rate for EFUDIX, PHG/PEGDA 20% gel (sample E) and PHG/PEGDA 20% gel containing 5-FU (sample E-5-FU)

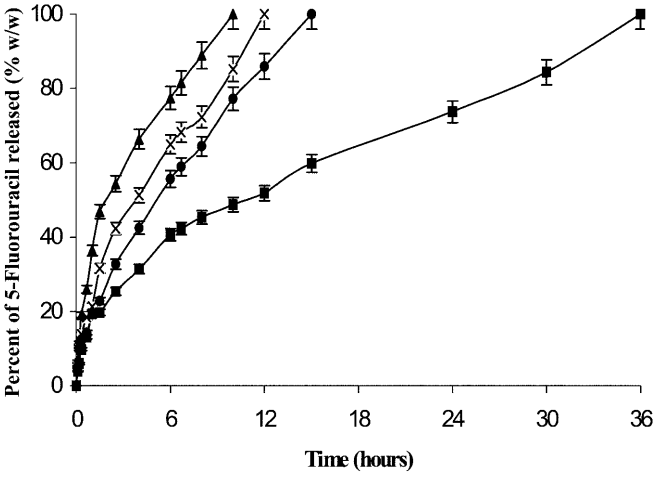

Fig. 13 Release of 5-FU at 37 °C from EFUDIX (×) and PHG/PEGDA 20% gel (■) at pH 5.5 and from EFUDIX (▲) and PHG/PEGDA 20% gel (●) at pH 7.4 as a function of time

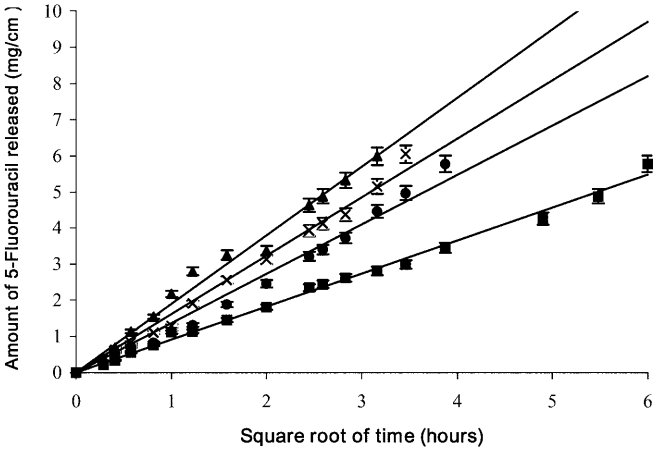

Fig. 14 Amount of 5-FU released from EFUDIX (×) and PHG/PEGDA 20% gel (■) at pH 5.5 and from EFUDIX (▲) and PHG/PEGDA 20% gel (●) at pH 7.4 as a function of the square root of time

D is the apparent diffusion coefficient of the drug and t represents the time elapsed since the start of drug release.

The results obtained show that PHG/PEGDA 20% gel is able to release 5-FU in a prolonged way and suggest the potential use of this gel in the treatment of superficial basal cell epithelioma and multiple actinic keratoses.

Conclusions

Novel biodegradable hydrogels have been prepared by irradiating at 313 nm aqueous solutions of PHG alone or in the presence of PEGDMA and PEGDA. In particular, we have demonstrated that the swelling ability can be controlled by the nature and concentration of unsaturated PEG derivatives used in combination with PHG. The presence of PEGDA 20 mol%, with respect to the moles of GMA groups in PHG, gives rise to a network with the highest yield and the lowest swellability. Owing to the presence of ester bonds in their cross-links, all the hydrogels prepared undergo a partial degradation by chemical and enzymatic treatment. This degradation depends on the pH of the environment, the nature of the enzyme (esterase is effective) and the chemical structure of the network. PHG/PEGDA is able to trap during the irradiation process a bioactive agent, such 5-FU, and to release it in simulated biological fluids (pH 1 and 7.4) by a mechanism controlled essentially by diffusion. Finally, the comparison between PHG/PEGDA gel containing 5-FU and a commercial ointment containing the same drug has been performed. In vitro release studies showed that this gel is able to give a prolonged drug delivery and appears potentially useful for a topical application.

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