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Hydrogels containing 5-Fluorouracil obtained by γ-irradiation. Synthesis, characterization and in vitro release studies

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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 copolymer PHEA-GMA (PHG) containing double bonds and ester groups in the side chain. Aqueous solutions of PHG alone or in combination with *N,N′* methylenbisacrylamide (BIS) have been exposed to a γ-ray source at different irradiation doses in order to obtain polymeric networks. All samples have been prepared both as water-swellable microparticles and as gel systems. Microparticles have been characterized by FT-IR spectrophotometry and swelling measurements in aqueous media mimicking biological fluids. The effect of irradiation dose and BIS presence on rheological behavior of the gel systems had also been investigated. All prepared hydrogels are able to incorporate, during γ-irradiation, 5-Fluorouracil, (5-FU) chosen as a model drug and to release it in simulated biological fluids, as confirmed by the in vitro drug release studies at pH 1 and pH 7.4. Gels of PHG containing 5-FU, obtained in the presence or in the absence of BIS, are 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 pH 7.4 using a Franz diffusion cell system and a synthetic membrane.

Key words α,β-Poly(*N*-2-hydroxyethyl)-DL-aspartamide · Glycidyl methacrylate · γ-Irradiation · Swellable microparticles · Gels

Introduction

In recent years the development of novel drug delivery systems (NDDS) using known molecular drugs has received a great impetus [1], not only because of the many therapeutic advantages of these new delivery devices, such as improved efficacy and patient compliance and reduced toxic/side effects, but also because of their relatively low cost with respect to that of a new discovery. In fact, whereas the developmental cost of a new drug may require some hundreds of dollars and take around 12–15 years to reach the market place, an existing drug molecule can "get a second life" with NDDS that can be developed in half the time and at about 20% of the cost of a new drug. As a consequence, some economic estimates suggest that the drug delivery market will grow from 12% of the total pharmaceutical market in 1996 to 20% of the total pharmaceutical market in 2005 [2].

The role of polymeric materials in obtaining new efficient drug delivery systems is becoming more and more important [3]. With this aim, already known polymeric materials are used in different ways; in addition, new polymeric matrices are synthesized with properties that make them "tailor made" for both different and novel applications [3].

Among NDDS, rising interest in both the scientific and industrial world has been noticed for hydrogels, three-dimensional water-swollen structures composed of mainly hydrophilic homopolymer or copolymers. To

date these have been proposed for use as wound dressings, biosensors, contact lenses, and artificial organs [4–6], in addition to use as drug delivery systems.

The goal of using hydrogel-based drug delivery systems that can swell in the presence of a biological fluid is to control properly drug delivery rate as required, through network permeability, swelling behavior, and the size and geometry of the devices [7, 8].

Hydrogels can be prepared by both chemical and physical methods [9] and – among the latter – the use of γ-irradiation has some general advantages [10]; in effect, it offers a simple, compact, and rapid technology to produce hydrogels which are, at the same time, sterilized [10]. γ-Irradiation of a polymer in an aqueous medium offers additional advantages with respect to irradiation of the solid state because of the high mobility of macromolecules and the high yield of reactive species obtained from water radiolysis which can promote the reaction of polymers, thus making the crosslinking process easier [10].

Finally, since the ionizing radiation is absorbed by a system proportionally to the electronic fraction of components, the presence of double bonds makes polymeric materials more reactive towards radical reactions following γ-irradiation to improve the crosslinking process [10]. In other words the presence of unsaturation in the polymeric structures generally decreases the gel dose (D_g), i.e., the dose where the first insoluble fraction of a gel appears during the irradiation process [10]. The possibility of using a low gel dose can make possible the incorporation of drug molecules into a hydrogel structure, also during the crosslinking process, provided that D_g is so low as not to interfere with the drug molecules.

In a previous paper we reported the preparation of a hydrogel by γ-irradiation of an aqueous solution of an acryloylated polyaspartamide [11]. In particular, α,β-poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) [12, 13], a water-soluble and biocompatible polymer with a protein-like structure, was derivatized by glycidyl methacrylate (GMA) to obtain an acryloylated polyaspartamide [14] PHEA-GMA (PHG). γ-Irradiation of aqueous solutions of a PHG sample at 29 mol% of derivatization gave rise to stable hydrogels at D_g values from 0.5 kGy to 2 kGy; this was much lower than that for PHEA [11].

The hydrogels so obtained were characterized by FT-IR spectroscopy, swelling measurements at different pH-values, and chemical and enzymatic hydrolysis studies [11].

In this paper, aqueous solution of a PHG sample with a derivatized degree of 34 mol% have been irradiated at different irradiation doses (2 kGy and 2.5 kGy) in the presence and in the absence of *N,N′*-methylenbisacrylamide (BIS), at a low temperature (0 °C), under a nitrogen atmosphere. Hydrogels so obtained have been kept both in the swollen state (gel) and in the dry state (microparticles). Analogous products containing drug molecules have been prepared incorporating the drug during the irradiation process.

5-Fluorouracil (5-FU) has been selected in this study because it is an anticancer drug with a broad spectrum of activity against solid tumors; it is currently used either in colon carcinoma therapy by intravenous infusion or topically for the treatment of superficial basal cell epithelioma. It is also used in the treatment of old age keratoses when conventional methods are ineffective [15].

However, its intravenous administration is accompanied by disorders of the bone marrow or the epithelium of the gastrointestinal tract [16]. In addition, it has a short plasma circulation half-life as a result of its fast metabolism in the liver [17]; its topical administration by traditional ointment can cause strong burning, itching, pain, and hyperpigmentation, as well as stomatitis, photosensitivity, and leucopenia [18]. Therefore its administration as a hydrogel could potentially minimize the toxic side effect and solve some of these delivery problems. In effect, topical semisolid dosage forms, such as dermatological hydrogels, which are especially useful for application to mucous membrane or ulcerated tissues, can be a suitable alternative therapeutic approach to systemic treatment either because hydrogels can provide a high and effective local concentration of drug or their high water content can reduce the local irritation processes [19]. Moreover, 5-FU can be considered as a small water-soluble drug model.

The ability of PHG microparticles and gels to release drugs has been evaluated by in vitro studies under different experimental conditions mimicking biological compartments.

Experimental

Materials

All reagents were of analytical grade, unless otherwise stated. D,L-Aspartic acid, ethanolamine, *N,N*-dimethylformamide (DMF), anhydrous *N,N*-dimethylacetamide (DMA), *N,N*-methylenebisacrylamide (BIS), and 5-Fluorouracil (5-FU) were from Fluka (Switzerland). Glycidyl methacrylate (GMA), 4-dimethylaminopyridine (4-DMAP), and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA).

EFUDIX was obtained from ICN Pharmaceuticals Germany GmbH.

α,β-Poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution, purified and characterized according to a procedure elsewhere reported [12]. 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 glycidyl methacrylate, to obtain PHG copolymer, was carried out in an organic phase (anhydrous DMA), using 4-DMAP as a catalyst; the product was purified and characterized according to a procedure reported elsewhere [11]. The degree of derivatization (DD) of prepared PHG, determined by ^{1}H-

NMR and calculated according to the method reported elsewhere [11], gave a result of 34 ± 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 membranes (Schleicher & Schuell) type OE 67, were obtained from Bracco (Milano, Italy).

Apparatus

Molecular weights of starting PHEA and PHG copolymer were determined by light scattering measurements, using a Dawn DSP-F Laser Spectra Physics Spectrometer.

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

FT-IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Perkin-Elmer 1720 Fourier Transform Spectrophotometer with a resolution of 1 cm^{-1}; each spectrum was recorded after 100 scans.

UV spectra were obtained using a Perkin-Elmer 330 Instrument, equipped with a 3600 station.

γ-Irradiation was performed using a IGS-3 panoramic 3000 Ci ^{60}Co irradiator, at 0 °C and under nitrogen. The dose rate, measured by a PTW Universal Dosimeter, was 0.5 kGy/h with a variance accepted below 5% in the absorbed dose.

Viscosity measurements were carried out using a Brookfield rotatory viscometer model DV-III 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.

HPLC analyses were carried out using a system consisting of a Varian 9012 Liquid Chromatography unit equipped with a Rheodyne Injector 7125 (fitted with a 10-μl loop) and a Kontron HPLC Detector 432 on line with a computerized HP workstation. For the analyses, a reversed-phase C_{18} column (μBondapak; 10 μm of 250 mm × 4.6 mm i. d., obtained from Waters) was used.

X-rays diffraction analysis was performed using a diffractometer with a Philips PW 1729 X-ray generator. 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 Inc. (Berkeley, Ca USA). The temperature was controlled during the test with a Neslab RTE-110 thermostat.

Preparation of PHG-γ and (PHG + BIS)-γ microparticles free drug by ^{60}Co irradiation

The polymeric microparticles were prepared by irradiating, with a ^{60}Co source, solutions of PHG copolymer (50 mg/ml) in double-distilled water at 0 °C, under nitrogen, using a dose rate of 0.5 kGy/h for different times, corresponding to a total exposing dose of 2 kGy or 2.5 kGy, in the absence of BIS (samples *a* and *b*, respectively) and in presence of BIS (25 mol% relating to the moles of GMA linked to PHEA) (samples *a′* and *b′*, respectively).

Every gel was purified by several washings with distilled water and centrifuging, from time to time, at 12,000 rpm at 4 °C for 20 min. After a further washing with acetone, the powder obtained was dried at 10^{-1} mm Hg 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 (12 mg/ml) was irradiated with a dose rate of 0.5 kGy/h for a total dose of 2.5 kGy. After irradiation the solution was analyzed by HPLC, then lyophilized and the solid residue characterized by FT-IR and UV analyses. The absence of degrading effects on 5-FU was confirmed by comparing HPLC chromatograms, IR-, and UV spectra with those of non-irradiated 5-FU. HPLC analysis was performed by using an H_3PO_4 solution (0.05 vol.%)/MeOH 98/2 mixture, as eluent with a flow of 1.0 ml/min and monitoring the eluate at 266 nm.

Preparation of PHG-γ and (PHG + BIS)-γ microparticles containing 5-FU by ^{60}Co irradiation

Polymeric microparticles containing 5-FU were prepared by irradiating with a ^{60}Co source, solutions of PHG copolymer (50 mg/ml) and 5-FU (10 mg/ml) in double-distilled water, with or without BIS (25 mol% relating to the moles of GMA linked to PHEA). The irradiation was carried out under nitrogen at 0 °C, using a dose rate of 0.5 kGy/h and for different times, corresponding to a total exposing dose of 2 kGy or 2.5 kGy. The following samples were obtained: samples *5-FU-a* and *5-FU-b* in the absence of BIS at 2 kGy and 2.5 kGy respectively, and samples *5-FU-a′* and *5-FU-b′* in the presence of BIS at 2 kGy and 2.5 kGy respectively.

After irradiation, each sample was washed once with distilled water, centrifuged (at 10,000 rpm at 4 °C for 5 min) then treated with acetone. The obtained powder was dried at 10^{-1} mm Hg in the presence of P_2O_5 until its weight remained constant.

Preparation of PHG-γ and (PHG + BIS)-γ gels, drug free, by ^{60}Co irradiation

Solutions of PHG copolymer (50 mg/ml) in double-distilled water were irradiated with a ^{60}Co source, at 0 °C, under nitrogen, using a dose rate of 0.5 kGy/h and for different times, corresponding to a total exposure dose of 2 kGy or 2.5 kGy, in the absence of BIS (samples *d* and *e* respectively) and in presence of BIS (25 mol% relating to the moles of GMA linked to PHEA) (samples *d′* and *e′* respectively). The gels obtained were purified by five washings with distilled water, centrifuged, weighed in order to determine gel fraction, and then characterized by rheological measurements.

Aliquots of these gels were lyophilized for 3 days, then 200 mg of each lyophilized gel was treated with 6 ml of distilled water. Immediately after the water addition, gels were again formed (samples called "swelled") and analyzed by viscosity measurements.

Preparation of PHG-γ and (PHG + BIS)-γ gels containing 5-FU by ^{60}Co irradiation

Solutions of PHG copolymer (50 mg/ml) and 5-FU (10 mg/ml) in double-distilled water were irradiated with a ^{60}Co source, at 0 °C, under nitrogen, using a dose rate of 0.5 kGy/h and for different times, corresponding to a total exposure dose of 2 kGy or 2.5 kGy, in the absence of BIS (samples *5-FU-d* and *5-FU-e* respectively) and in presence of BIS (25 mol% relating to the moles of GMA linked to PHEA) (samples *5-FU-d′* and *5-FU-e′* respectively). After irradiation, gels were weighed and characterized by rheological measurements.

Determination of the amount of drug entrapped in the PHG-γ and (PHG + BIS)-γ samples

Aliquots of 30 mg of the dried samples *5-FU-a*, *5-FU-a′*, *5-FU-b*, *5-FU-b′*, or 100 mg of gel samples *5-FU-d*, *5-FU-e*, 5-FU-d′, *5-FU-e′* were extensively extracted at room temperature with distilled water. The amount of 5-FU delivered was determined by HPLC using the conditions reported above. The amounts of entrapped 5-FU were found equal to about 15 wt% for the dried samples *5-FU-a′* and

5-FU-b′, 10 wt% for the samples *5-FU-a* and *5-FU-b*, and 1.1 wt% for the gel samples *5-FU-d*, *5-FU-e*, *5-FU-d′*, and *5-FU-e′*.

Swelling studies

The swelling ability of polymeric microparticles (samples *a*, *a′*, *b*, and *b′*) 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; ionic strength = 0.187) at pH 6.8 and 7.4.

In particular, aliquots of each dried and exactly weighed sample were kept in contact with the penetrating medium until the equilibrium swelling was reached; then each swollen sample was filtered, plugged by blotting paper and weighed. The weight swelling ratio (q) was calculated as follows:

$$q = w_s/w_d$$

where w_s and w_d are the weights of swollen and dry sample respectively.

All experiments were carried out in triplicate and results agreed with each other within a ±3% error.

Rheological measurements

The viscosity measurements of prepared gels (samples *d*, *d′*, *d swelled*, *d′ swelled*, *e*, *e′*, *e swelled*, *e′ swelled*, *5-FU-d*, *5-FU-d′*, *5-FU-e*, *5-FU-e′*) were performed at 37 ± 0.01 °C using an angular speed ranging from 10 rpm to 260 rpm (10 rpm/min).

The rheogram of an ointment containing 5-FU (EFUDIX) was also obtained in the same conditions as reported above. All measurements were carried out in triplicate and the results agreed with each other within a ±2% error.

In vitro release studies

In vitro release studies of 5-FU from polymeric microparticles (samples *5-FU-a*, *5-FU-a′*, *5-FU-b*, *5-FU-b′*) were carried out by keeping aliquots (10 mg) of each dried sample in 25 ml of phosphate buffer at pH 7.4 (NaCl, Na_2HPO_4, KH_2PO_4), or 0.1 N HCl solution (pH 1). The flasks containing the release media and the hydrogel samples were placed in a 37 ± 0.1 °C thermostatic water bath, under continuous stirring (100 rpm). Sink conditions were maintained during the experiments. At different time intervals, the samples were filtered through a 0.45-μm Millipore filter and assayed by HPLC analysis using the conditions reported above. All experiments were carried out in triplicate and the results agreed with each other within a ±3% error.

In vitro diffusion studies

5-FU diffusion rate in the gels 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; ionic strength = 0.187) or at pH 5.5 (Na_2HPO_4, KH_2PO_4; ionic strength = 0.856), kept at 37 ± 0.1 °C and continuously stirred (600 rpm) by a rotating teflon coated magnet placed inside the cell.

Aliquots of 500 mg of each gel (samples *5-FU-d*, *5-FU-d′*, *5-FU-e*, *5-FU-e′*) 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 thickness of 115 μm, previously moistened with the receptor phase. Samples (50 μl) of the receptor phase were withdrawn at different times and replaced with the same volume of buffer. 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 as reported above.

All experiments (six for each sample) agreed with each other within a ±3% error.

Results and discussion

Hydrogel-based controlled release systems have been studied for over 30 years [6, 9] and proposed for delivery by almost all of the conventional routes [20]. Now, the attention of many researchers is focused on the synthesis of new polymeric materials that greatly simplify the preparation of hydrogels and the entrapment of drugs into their structure [7]. The chemical nature of these polymers can be different and new polymers proposed as parent material to obtain hydrogels include polymethacrylates [21], polysaccharides [22], and polyaminoacids [11]. In this context polyaminoacids offer some advantages with respect to other polymers such as biocompatibility and potential biodegradability. Besides, if synthetic, it is generally quite simple to introduce into their polymeric structure functional groups useful to increase reactivity of the material for some chemical reactions. In this paper we propose a polyaminoacidic derivative as a parent compound to prepare water-swellable micromatrices and gels for the release of 5-Fluorouracil (5-FU), a known antitumor drug. In particular, the reaction between glycidyl methacrylate (GMA) and α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) in DMA solution and in the presence of 4-DMAP as a catalyst gave rise to a PHEA-GMA copolymer (PHG) that, unlike PHEA, presents in the side chain double bonds that make easier its crosslinking by γ-irradiation [11] (Fig. 1).

Then, hydrogels were obtained through γ-irradiation of aqueous solutions of PHG at a polymer concentration of 50 mg/ml with a total irradiation dose of 2 kGy or 2.5 kGy at 0 °C and under nitrogen. Networks obtained were purified as previously described (see Experimental), then simply centrifuged to separate gel phases or dried so as to obtain microparticles.

Microparticles

Table 1 reports the yield % values of samples *a* and *b*.

In Fig. 2 FT-IR spectra of sample *a* (as an example) and non-crosslinked PHG are reported. In particular, the more interesting feature is the complete disappearance of peaks related to double bonds, i.e., 1405 cm^{-1} (scissoring —C=C—) and 951 cm^{-1} (wagging —C=C—) of the starting PHG. This suggests that the crosslinking reaction induced by γ-rays involves the opening of double bonds, probably through the formation of free-radicals which give rise to inter and intra-polymeric chain crosslinked 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.

Analogously, aqueous PHG solutions at 50 mg/ml were irradiated with a total dose of 2 kGy or 2.5 kGy, but in the presence of *N,N′*-methylenbisacrylamide (BIS) in an amount of 25 mol% relating to the moles of GMA residues linked to PHEA to obtain samples *a′* and *b′* respectively. These were purified as previously reported (see Experimental) and weighed in order to determine the values of yield % reported in Table 1. As can be seen, yield values in the presence of BIS are greater than those obtained without BIS due to the participation of this molecule in the crosslinking process. Moreover, with a dose of 2.5 kGy the yield of the crosslinking reaction is about 99%.

FT-IR spectra of samples *a′* and *b′* are no different from those obtained without BIS (*a* and *b* samples) since the bands assigned to BIS are superimposable on those of PHG; furthermore, the double bond bands of BIS disappear after irradiation because they are involved in the crosslinking process.

Samples *a*, *b* and *a′*, *b′* were characterized by swelling measurements in different aqueous media, mimicking some biological environments, i.e., a 0.1 N solution of hydrochloric acid (pH 1, simulated gastric juice) and phosphate buffer solutions pH 6.8 (simulated intestinal fluid) and pH 7.4 (simulated extracellular fluid) as well as in double-distilled water.

Swelling data in terms of *q* are reported in Table 2. As can be seen, all the investigated samples have a very high swelling ability. However, in the presence of BIS,

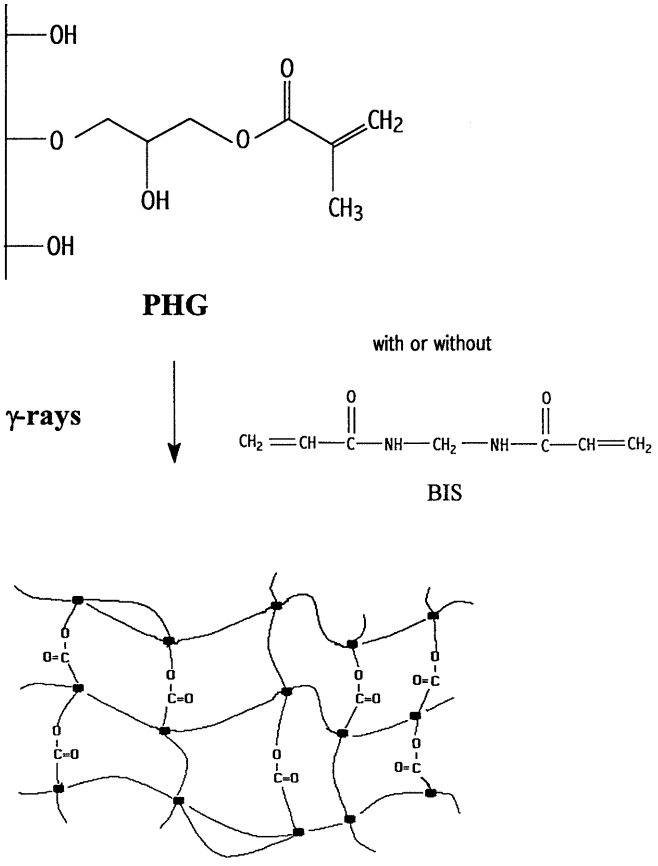

Fig. 1 Schematic representation of γ-ray effects on PHG in the presence or in the absence of BIS

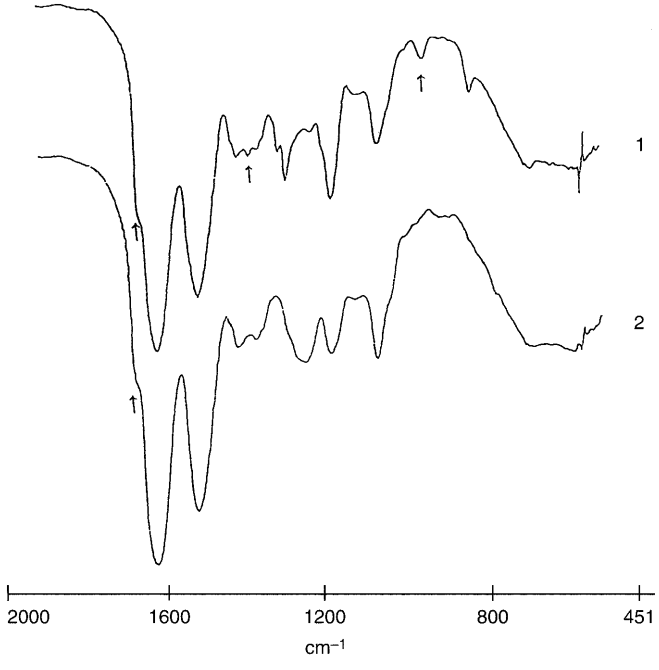

Fig. 2 FT-IR spectra of (1) uncrosslinked PHG and (2) PHG crosslinked by γ-rays

Table 1 Yield % values of microparticle samples obtained after γ-irradiation of PHG or PHG + BIS aqueous solutions at different γ-ray doses

Sample	γ-Ray dose (KGy)	Yield % (± 2%)
PHG (a)	2	78
PHG (b)	2.5	91
PHG + BIS (a′)	2	90
PHG + BIB (b′)	2.5	99

Table 2 Weight swelling ratio (q) values of microparticle samples obtained after γ-irradiation of PHG or PHG + BIS aqueous solutions at 2 KGy and 2.5 KGy (mean ± S.E.)

Sample	Weight swelling ratio (q)			
	H_2O	pH 7.4	pH 6.8	pH 1
a	15.2 ± 0.4	11.9 ± 0.3	12.3 ± 0.3	7.8 ± 0.2
a′	7.0 ± 0.2	6.2 ± 0.1	6.4 ± 0.1	4.8 ± 0.1
b	10.2 ± 0.3	9.6 ± 0.2	10.0 ± 0.3	5.8 ± 0.1
b′	5.6 ± 0.1	4.2 ± 0.1	4.6 ± 0.1	3.4 ± 0.1

smaller swelling values are observed in all the media considered. This behavior can probably be ascribed to the more compact network obtained in the presence of BIS. As far as the effect of pH medium and ionic strength on 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 pH 7.4 these values are smaller due to the osmotic pressure and ionic strength of the swelling medium. The lowest swelling occurs at acidic pH and this is probably because of the presence of ionizable groups eventually formed during the irradiation process [11].

Since the irradiation process was carried out at 0 °C and at low absorbed doses, it is possible to think of incorporating drug molecules into a network structure, during irradiation, without alteration of their chemical structure. With this aim, 5-Fluorouracil, a known anti-tumor drug was chosen as a model drug and, in order to verify the possibility of introducing it into a polymeric network during an irradiation process, 5-FU solutions were exposed to γ-irradiation under the same experimental conditions as those used to obtain PHG networks (see Experimental). Since all analytical and spectral data confirmed the absence of altering effects on 5-FU by γ-irradiation, under evaluated experimental conditions, solutions of PHG copolymer and 5-FU with or without BIS were irradiated with a total dose of 2 kGy to obtain, respectively, *5-FU-a′* and *5-FU-a* samples, and with a total dose of 2.5 kGy to obtain *5-FU-b′* and *5-FU-b* samples, respectively. In Table 3, yield % values of these samples are reported.

Like drug unloaded samples, yield values increase in the presence of BIS and with increasing total irradiation dose.

The drug content of dried *5-FU-a*, *5-FU-a′*, *5-FU-b*, and *5-FU-b′* samples, determined by extensive extraction with distilled water followed by HPLC analysis, was equal to 15 wt% for *5-FU-a′* and *5-FU-b′* samples and to 10 wt% for *5-FU-a* and *5-FU-b* samples.

The determination of the drug dispersion state in PHG microparticles was performed by X-ray analysis. Figure 3 reports, for example, the X-ray diffraction patterns of pure 5-Fluorouracil, *5-FU-a*, and sample *a* (drug unloaded).

It is evident that pure 5-FU is in the crystalline state; on the other hand, when dispersed in PHG network it is in the amorphous state like the unloaded sample.

In order to have some preliminary information on the potential of these hydrogels as drug delivery systems, in vitro release studies were performed in simulated gastric (pH 1) and extracellular (pH 7.4) fluids at 37 °C. Results of release experiments are reported in Fig. 4a, b.

As can be seen at pH 7.4, all samples are able to release the total drug content within a time interval ranging from 20 min to 60 min. In particular BIS free samples, in accordance with a less compact crosslinked structure, already indicated by swelling behavior, showed a faster drug release rate than that shown by samples containing this crosslinking agent. In addition, drug release rate decreases as total irradiation dose increases in accordance with a higher degree of cross-linking. Analogous behavior was shown at pH 1 where the complete drug release occurs within a time interval ranging from 20 min to 150 min. (see Fig. 4b). The higher release rate at pH 7.4 in comparison with pH 1 can be related to the higher swelling degree of micro-particles in the first medium.

Table 3 Yield % values of microparticle samples obtained after γ-irradiation of PHG or PHG + BIS aqueous solutions in the presence of 5-Fluorouracil at different γ-ray doses

Sample	γ-Ray dose (KGy)	Yield % (±2%)
5-FU-a	2	80
5-FU-b	2.5	93
5-FU-a′	2	92
5-FU-b′	2.5	99

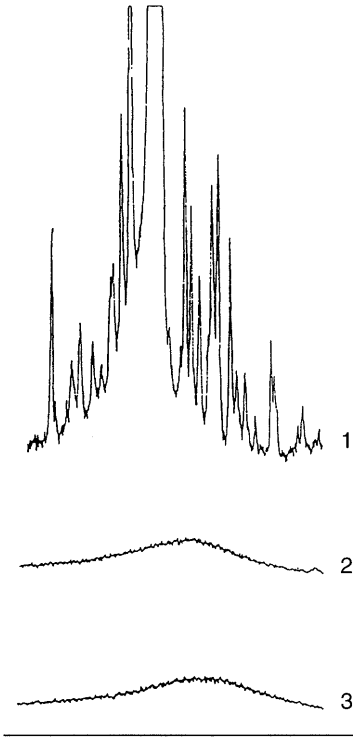

Fig. 3 X-ray diffraction patterns of (1) pure 5-Fluorouracil, (2) unloaded PHG microparticles (sample a), and (3) PHG microparticles loaded with 5-Fluorouracil (sample 5-FU-a)

Gels

In recent years a great interest in gel systems has been developed. In fact, gel-forming hydrophilic polymers are

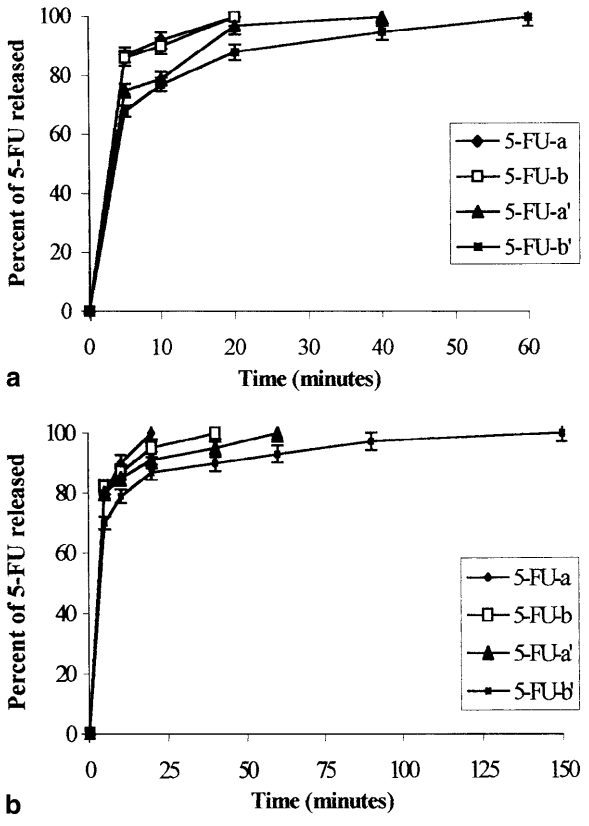

Fig. 4 **a** 5-Fluorouracil release at pH 7.4 from PHG microparticles as a function of time. **b** 5-Fluorouracil release at pH 1 from PHG microparticles as a function of time

commonly used to prepare semisolid dosage forms which are especially useful for application of therapeutic agents to mucous membranes and ulcerated tissues because their high water content reduces irritation. For this reason, besides preparing polymeric networks shaped as microparticles, gel systems have also been prepared through irradiation of PHG aqueous solutions (50 mg/ml) under nitrogen and at 0 °C, with a total irradiation dose of 2 kGy or 2.5 kGy (see experimental, gels *d* and *e* respectively). The same procedure in the presence of BIS gives rise to gels *d′* and *e′*, respectively.

All gels so obtained were subjected to viscosity measurements using an angular speed ranging from 10 rpm to 260 rpm. Also, in order to verify the possibility of storing these gels in the dry state, i.e., lyophilized and after swelling in water to regain quickly gels with the same rheological behavior as the starting gels, samples *d*, *d′*, *e*, and *e′* were lyophilized and then analyzed again by viscosity measurements after swelling in an appropriate (see Experimental) amount of water (samples *d, d′, e*, and *e′* "swelled"). Values of viscosity as a function of angular speed of all these samples are reported in Fig. 5.

As can be observed in all the rheograms, viscosity decreases as angular speed increases, thus showing the non-Newtonian pseudoplastic behavior of prepared gels.

The trend of *d* and *e* samples as well as that of *d′* and *e′* samples showed a little increase of viscosity values on increasing the total irradiation dose from 2 kGy to 2.5 kGy. In addition, gels obtained in the presence of

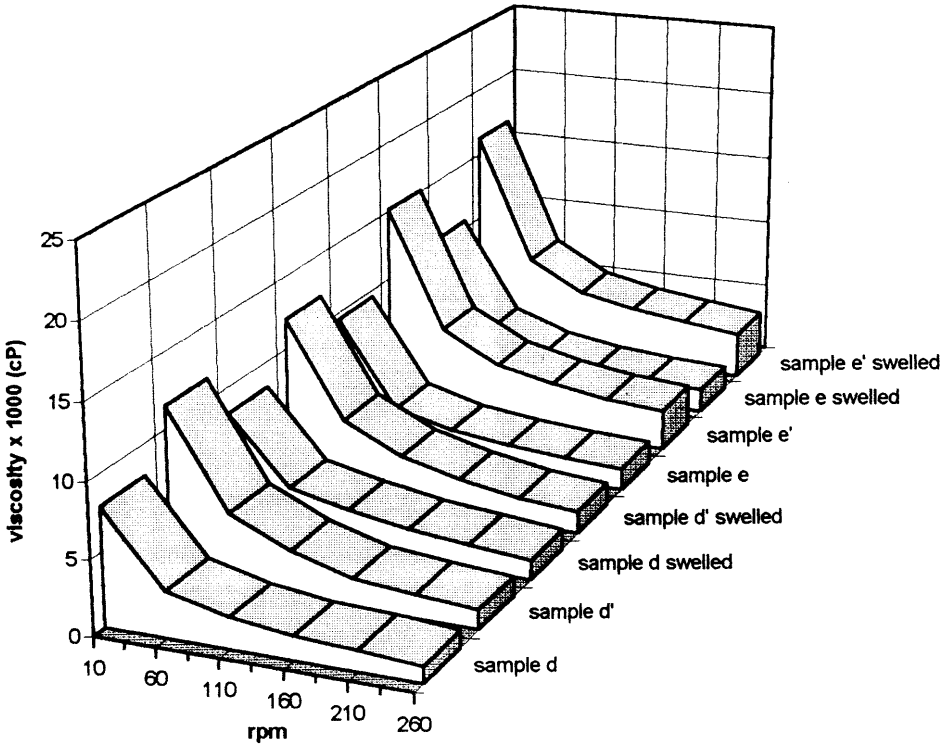

Fig. 5 Plot of viscosity vs angular rate for gels of PHG obtained in the presence or in the absence of BIS and for "swelled" samples

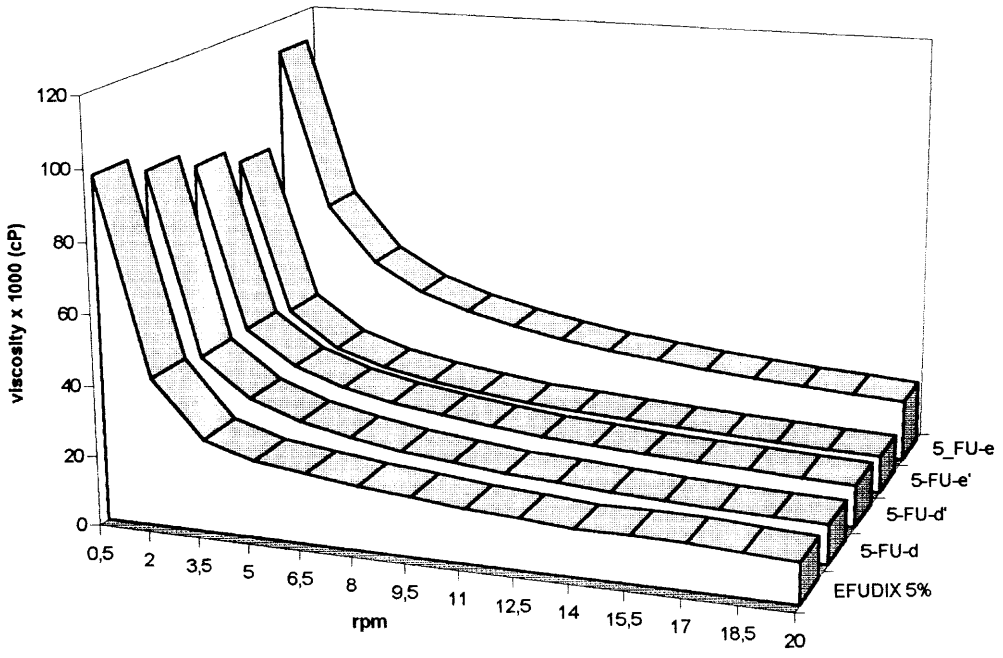

Fig. 6 Plot of viscosity vs angular rate for EFUDIX and gels of PHG containing 5-Fluorouracil obtained in the presence or in the absence of BIS

BIS show viscosity values greater than those obtained without BIS over the whole angular speed interval investigated (samples *d′* and *e′* in comparison with samples *d* and *e*); this probably occurs because gels containing BIS residues have a greater crosslinking degree than those obtained without this molecule. This gives them a greater rigidity. Finally, samples called "swelled" show viscosity values and trends quite similar to those obtained from starting gels, before lyophilization and re-swelling. This strongly supports the possibility of storing these gels in the dry state and regaining gels immediately after swelling in water.

When irradiation was performed under the same experimental conditions but in the presence of 5-FU, gels loaded with drug were obtained, respectively *5-FU-d* and *5-FU-e* samples in the absence of BIS at 2 kGy and 2.5 kGy respectively, and *5-FU-d′* and *5-FU-e′* samples in the presence of BIS at 2 kGy and 2.5 kGy respectively. The amount of loaded drug in gel samples, determined by extensive extraction of these materials at room temperature with distilled water, followed by HPLC analysis, was evaluated as equal to 1.1 wt%. Also, these gels were analyzed by viscosity measurements and in Fig. 6 the viscosity values as a function of angular speed (range 0.5–20 rpm, reported as example) are shown. As a comparison in the same figure, viscosity values vs angular speed of a commercial ointment containing 5-FU at 5 wt% (EFUDIX) are also reported.

As can be seen, the viscosity of prepared gels appears to be similar to that of EFUDIX. The small difference observed can be ascribed to the greater rigidity of prepared networks in comparison with the commercial ointment.

The evaluation of the ability of drug molecules physically entrapped into the gel structure to diffuse out (releasing process) is important as an estimate of their potential use as systems for drug delivery. For this reason, we considered it interesting to study the release rate of 5-Fluorouracil from the prepared gels. In particular, drug release was determined through a cellulose acetate membrane using Franz diffusion cells [23] and buffer solutions at pH 5.5 or pH 7.4 as a receptor phase. In order to compare the release data obtained from prepared gels with those of EFUDIX, the aliquots

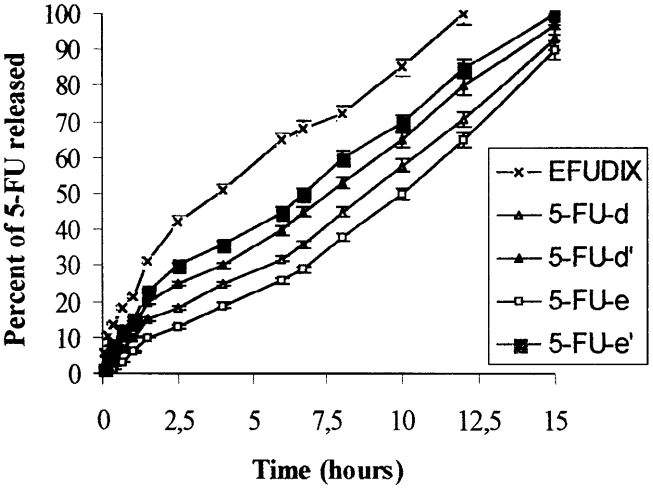

Fig. 7 Release of 5-Fluorouracil at pH 5.5 from EFUDIX and gels of PHG containing 5-Fluorouracil obtained in the presence or in the absence of BIS

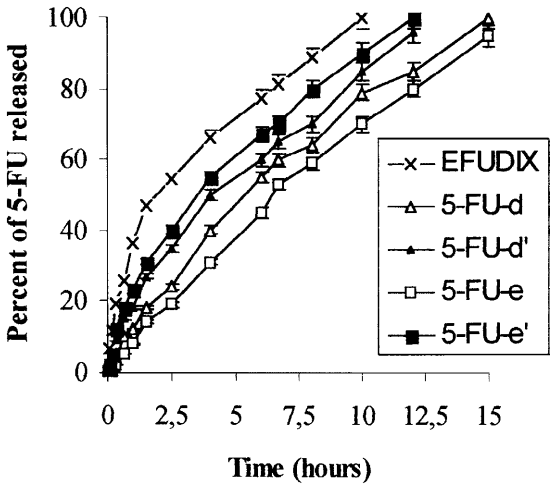

Fig. 8 Release of 5-Fluorouracil at pH 7.4 from EFUDIX and gels of PHG containing 5-Fluorouracil obtained in the presence or in the absence of BIS

of gels or ointment placed on the synthetic membrane contained the same amount of 5-Fluorouracil.

The amounts of released drug from *5-FU-d*, *5-FU-e*, *5-FU-d′*, and *5-FU-e′* samples at pH 5.5 as a function of time are reported in Fig. 7; as a comparison the amount of released drug from EFUDIX is also reported in the same figure.

As can be seen, all gels considered showed a slower release than EFUDIX. A similar trend was found when the receptor phase was a buffer solution at pH 7.4 (Fig. 8).

The values of the initial release rate calculated for each sample, reported in Table 4, show that from the very beginning of each experiment, 5-Fluorouracil was released from gels with a rate lower than EFUDIX in both the receptor phases.

The results obtained suggest the potential use of these gels in the treatment of superficial basal cell epithelioma and multiple actinic keratoses.

Table 4 Starting rate of 5-Fluorouracil release in different media from EFUDIX and gels of PHG obtained in the presence or in the absence of BIS

Sample	Starting rate of release (mg% h^{-1})	
	pH 7.4	pH 5.5
EFUDIX	71.40	63.86
5-FU-e′	24.10	12.05
5-FU-d′	12.05	6.02
5-FU-d	2.41	1.08
5-FU-e	0.94	0.24

Conclusion

Water-swellable microparticles and gels have been prepared by γ-irradiation of aqueous solutions of PHG alone or in the presence of BIS at different γ-ray doses. The high efficiency of crosslinking process triggered by γ-rays is confirmed by high yield % values which increase further in the presence of BIS. The low absorbed doses and the low temperature employed in our experiments make it possible to incorporate into these networks, during the irradiation, drug molecules without alteration of their structure. In particular, 5-Fluorouracil, chosen as model drug, has been entrapped into PHG hydrogels during the crosslinking process. Microparticles of PHG, obtained with or without the presence of BIS, containing this drug are able to release it in simulated biological fluids (pH 1 and pH 7.4). Finally, the comparison between gels based on PHG with or without BIS, containing 5-Fluorouracil and a commercial ointment containing the same drug, has been performed. In vitro release studies showed that the prepared gels are able to release 5-Fluorouracil and appear potentially useful for a topical application.

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