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Novel water-swellable beads based on an acryloylated polyaspartamide

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Abstract Spherical polymeric microparticles have been prepared by a reverse-phase suspension polymerization technique. The starting polymer was α,β-poly (*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) partially functionalised with glycidylmethacrylate (GMA) in order to introduce reactive vinyl groups in the side chain. The PHEA–GMA copolymer obtained (PHG) was cross-linked in a mixture of water/hexane–carbon tetrachloride in the presence of sorbitan trioleate (Span 85) as surfactant and ammonium persulfate/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine as initiator system. The reaction was also carried out in the presence of *N*,*N*′-dimethylacrylamide as comonomer or *N*,*N*′-ethylenebisacrylamide as a cross-linking agent. The beads obtained were characterized by Fourier transformIR spectrophotometry, particle size distribution analysis and scanning electron microscopy, which revealed their microporous structure. X-ray diffractometry and differential scanning calorimetry showed the amorphous state and the dependence of the glass-transition temperature on the chemical structure of the samples prepared, respectively. Finally, in order to have information on the water affinity of the networks obtained, swelling measurements were performed. The water regain values and the high rate of swelling demonstrate a remarkable ability of the samples investigated to entrap water molecules. The dependence of aqueous swelling on the degree of cross-linking and the chemical structure of the samples is also shown.

Key words Acryloylated α,β-poly-(*N*-2-hydroxyethyl)-DL-aspartamide · Glycidylmethacrylate · Reverse-phase suspension polymerization · Spherical microparticles · Hydrogels

Introduction

Recently intensive studies have been performed in order to develop new materials for biomedical and pharmaceutical applications [1]. In this context, α,β-poly(*N*-2 hydroxyethyl)-DL-aspartamide (PHEA) is a polymer already proposed as a plasma expander [2], a drug carrier for a macromolecular prodrug [3, 4] and a starting macromolecule to prepare hydrogels [5–7]. The latter are an interesting class of cross-linked-polymeric systems, swollen in water or biological fluids, extensively studied and used for a variety of applications in biomedical and pharmaceutical fields. The research of new drug delivery systems, in particular, represents a fertile area for the investigation and application of hydrogels, since they are tissue-compatible and able to release bioactive molecules physically entrapped in their cross-linked structure [8–10]. The drug release rate depends on various parameters, such as the shape and size of the system, the nature of the polymer, the drug solubility, the degree of swelling, the drug loading procedure [11–13] and generally is affected by two

phenomena: matrix swelling owing to the penetrant medium and drug diffusion into the swollen polymer [14–17]. For biodegradable hydrogels, i.e. systems which in vivo undergo a chemical or enzymatic hydrolysis, obviously, drug release also depends on the degradation rate of the polymeric network [18, 19]. Preparation of polymeric networks may be performed by various techniques, for example, a hydrophilic macromolecule can be cross-linked using a small amount of cross-linking agent able to form cross-linked bonds by inter- and intrapolymeric chains. This reaction is usually carried out in aqueous solution, although suspension polymerization may be used for the production of hydrogels as microparticles [20]. Cross-linking reactions may also occur in the absence of cross-linking agents. Free radicals in the backbone chain are formed by means of a free-radical initiator [21, 22] or by irradiation with β rays, UV rays or γ rays [23–26]. Depending on the polymer chemical structure [27] these radicals can evolve and produce chain degradation and they can be cross-linked to give rise to a macromolecular network. In the hydrogels formed by a radical mechanism, the macromolecules are connected to each other by covalent bonds. This makes them unable to dissolve even at a high temperature, unlike the "physical" hydrogels formed as a result of weak interactions among the macromolecular chains [28, 29]. In this context, recently our interest has focused on the preparation of hydrogels via radical reaction using PHEA as a starting macromolecule. First, PHEA was cross-linked in aqueous solutions by gamma irradiation, but the formation of compact and homogeneous hydrogels occurred at a high dose of irradiation (about 550 kGy) [30], whereas when PHEA was irradiated by UV rays at 254 and 313 nm hydrogel formation did not occur. Then, in order to improve the reactivity of PHEA towards the radical reactions, its structure was partially modified by introducing groups bearing unsaturation, i.e. by functionalization of the polymer with glycidylmethacrylate (GMA), thus obtaining the copolymer PHEA–GMA (PHG) [31].

In particular, a series of PHG copolymers with various GMA contents were obtained by varying some parameters of the derivatization reaction, such as solvent, catalyst, pH, GMA concentration and reaction time [31].

PHG turned out to be particularly reactive towards γ or UV rays. In fact, the exposure of aqueous solutions of PHG to a ^{60}Co source (at 0 °C and low absorbed doses) or to a UV ray source (at 254 or 313 nm) gives rise to hydrogel systems [32–34]. These hydrogels, prepared either in the presence or in the absence of a biodegradable cross-linker, such as *N*,*N*′-methylenebisacrylamide, exhibit a high affinity towards aqueous media mimicking biological fluids. In addition, owing to the presence of ester groups, which are maintained after the cross-linking process, these hydrogels undergo a partial degradation by chemical or enzymatic hydrolysis.

Now, our attention is focused on the preparation of new hydrogels based on PHG using a reverse-phase suspension polymerization technique [35, 36]. The procedure adopted allows spherical particles to be obtained with a narrow size distribution which are generally more preferred than other geometries, for example, planar or cylindrical, especially for applications involving multiparticulate dosage forms. On the other hand, the spherical geometry eliminates the anisotropic swelling behaviour normally associated with sheet samples [21]. Beads were also prepared starting from PHG in combination with a cross-linking agent, such as *N*,*N*′-ethylenebisacrylamide (EBA), or a comonomer, such as *N*,*N*′-dimethylacrylamide (DMAA), in order to synthesize particles with a different chemical structure and cross-linking density. Physicochemical characterization, with regard to morphological investigation, particle size distribution, swelling behaviour, thermal analysis and X-ray diffraction measurement of all the samples is reported here.

Experimental

Materials

All the reagents used were of analytical grade, unless otherwise stated. Anhydrous *N*,*N*-dimethylacetamide (DMA) was obtained from Fluka. GMA, EBA, 4-dimethylaminopyridine (4-DMAP) 99.9%, Span 85, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and ammonium persulfate were purchased from Aldrich Chemical Co. *n*-Hexane, CCl_4 and DMAA were provided by Fluka Chemie and freshly distilled.

PHEA was prepared according to a procedure reported elsewhere [37]. The batch of PHEA used in the present study had a weight-average molecular weight of 56900 ($M_w/M_n = 1.79$).

Derivatization of PHEA with GMA to obtain PHG copolymer was carried out in an organic phase (anhydrous DMA), using 4-DMAP as a catalyst, purified and characterized according to a procedure reported elsewhere [31]. The degree of derivatization of the PHG prepared, determined by ^{1}H NMR gave a result of 28 ± 1 mol%. The weight-average molecular weight of PHG copolymer determined by light scattering measurements was 71000 ($M_w/M_n = 1.86$). Fourier transform (FT) IR analysis: 3293 br, 3078 m (ν_{as} OH + ν_{as} NH + ν_{as} NH_2); 1720 m (ν_{as} COO); 1651 vs (amide I); 1542 s (amide II), 1437 m (δ C–H), 1405 m–w (scissoring –C = C–), 1180 m (ν_s COO + ether COC), 951 m-w (wagging –C = C–) cm^{-1}.

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 using 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 FT spectrophotometer. The resolution was 1 cm^{-1}. The number of scans was 100.

Particle size distribution and aqueous dynamic swelling measurements were carried out using an image processing and analysis system, a Leica Quantimet Q 500, equipped with a Leica Wild 3D stereomicroscope. This image processor calculates the particle area and converts it to an equivalent circle diameter.

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 differential scanning calorimetry (DSC) was performed on a Mettler TA 3000 calorimeter equipped with a DCS-30 cell and TC-10 processor. Samples were heated from 5 to 180 °C; the heating rate was 2 °C/min. Before each test the samples were carefully dried for 72 h under vacuum in the presence of P_2O_5 and then ground in a mortar in order to ensure a good contact with the aluminium pan. The glass-transition temperature, T_g, was determined as the temperature corresponding to the slope change in the specific heat–temperature plot.

The scanning electron microscopy photographs were obtained with a Leo stereoscan 420; the surface of the samples was made conductive by the deposition of a layer of gold on the samples in a vacuum chamber.

Microscopy investigation were performed with a Leitz Laborlux microscope.

Bead preparation

In a typical experiment, a mixture of *n*-hexane and carbon tetrachloride was placed in a round-bottomed cylindrical glass reaction vessel fitted with an anchor-type stirrer and thermostatted at 35 °C, then treated, after 30 min of N_2 bubbling, with a solution of PHG, comonomer (DMAA) or cross-linker (EBA) and ammonium persulfate in H_2O.

The density of the organic phase was adjusted by the addition of CCl_4 so that the aqueous phase sank slowly when stirring stopped. With the stirrer at 1000 rpm, the mixture was treated with Span 85, then after 10 min with TMEDA and stirring was continued for another 60 min. All reagents and the amounts used in these experiments are reported in Table 1.

Each matrix so obtained was filtered, washed with 50-ml portions of 2-propanol, chloroform, ethanol, chloroform, 2-propanol and acetone.

These samples, having a microparticulate shape, were called M1, M2 and M3 with the cross-linking PHG, PHG/DMAA and PHG/EBA, respectively.

FT-IR analysis of all the samples showed principal bands at 3389 br, 3102 m (ν_{as} OH + ν_{as} NH + ν_{as} NH_2); 1730 m (ν_{as} COO); 1656 vs (amide I); 1545 s (amide II), 1437 m (δ C–H) and 1193 m (ν_s COO + ether COC) cm^{-1}.

Determination of water regain

Aliquots (80–100 mg) of the microparticles (M1, M2, M3) dried to constant weight by heating to 60 °C/0.01 torr were placed in a tared 5-ml sintered glass filter (diameter 10 mm; porosity, G3), weighed, and left to swell by immersing the filter plus support in a beaker containing distilled water. At a predeterminated time, the excess water was removed by percolation at atmospheric pressure. Then, the filter was placed in a properly sized centrifuge test tube by fixing it with the help of a bored silicone stopper, then centrifuged at 350 rpm for 15 min and weighed. This operation was repeated at different immersion times (1, 4 and 24 h). The filter tare was determined after centrifugation with water alone. The weights recorded at the different times were averaged and used to give the water regain by the following equation:

$$\text{Water regain} = (W_S - W_d)/W_d \times 100 \ ,$$

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

Each experiment was carried out in triplicate and the results were in agreement within ±4% standard error.

Dynamic swelling

Aqueous dynamic swelling was determined by observing, through an optical stereomicroscope equipped with an image processor (see Apparatus), the variation of the microparticle diameter in distilled water at room temperature until the microparticles achieved the full swollen equilibrium with a diameter d_∞. The values of the normalized diameter, d_t/d_0, were determined, d_t being the diameter of the swollen microparticle at time t and d_0 the diameter of the dry microparticle.

The experiment was carried out by analysing 20 microparticles of each sample and the results were in agreement within ±3% standard error.

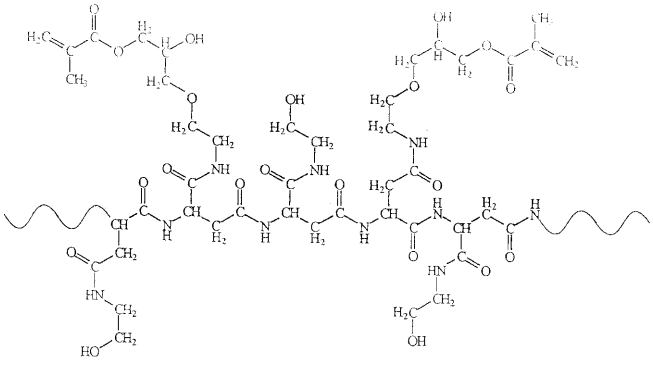
Results and discussion

PHEA partially modified by reaction with GMA gives rise to a copolymer PHEA–GMA (PHG) having pendant methacrylate groups. The chemical structure of PHG is shown in Fig. 1.

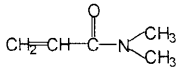
Aqueous solutions of PHG were cross-linked by radical polymerization through a reverse-phase suspension polymerization technique. During the PHG cross-linking reaction, an initiator system of TMEDA and

Table 1 Amounts of reagents [α,β-poly (*N*-2-hydroxyethyl)-DL-aspartamide–glycidylmethacrylate (*PHG*), *N*,*N*′-dimethylacrylamide (*DMAA*), *N*,*N*′-ethylenebisacrylamide (*EBA*)] solvents and initiators employed in the cross-linking reactions and yield values of the microparticles obtained

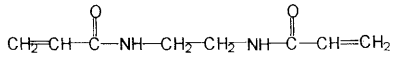
Dispersed phase		Continuous phase		Initiator system		Sample name mg (yield %)
Reagents (mg)	Solvent (ml)	Solvents (ml)	Surfactant (μl)	$(NH_4)_2S_2O_8$ (mg)	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (μl)	
PHG (370)	H_2O (3)	CCl_4/hexane (17/22)	Span 85 (50)	(22)	(110)	M1 300 (81%)
PHG (350) DMAA (147)	H_2O (3)	CCl_4/hexane (22/29)	Span 85 (70)	(30)	(80)	M2 388 (78%)
PHG (350) EBA (125)	H_2O (3)	CCl_4/hexane (23/26)	Span 85 (75)	(26)	(80)	M3 400 (84%)



PHEA - GMA (PHG)



DMAA



EBA

Fig. 1 Chemical structures of α,β-poly (*N*-2-hydroxyethyl)-DL-aspartamide (*PHEA*)–glycidylmethacrylate (GMA) (*PHG*), *N*,*N*′-dimethylacrylamide (*DMAA*) and *N*,*N*′-ethylenebisacrylamide (*EBA*)

ammonium persulfate was employed to initiate the formation of methacrylic functions randomly arranged in the chain. Because of steric and geometric constraints, such polymerization involves only the methacrylic functions which are accessible to the growing chains. The microparticle structure obtained is formed by the network where the PHG chains are linked by some hydrocarbon bridges. The PHG cross-linking was also performed in the presence of a comonomer (DMAA) or a cross-linking agent (EBA) to study the effects of a different chemical structure and degree of cross-linking on the physical properties of the microparticles. The chemical structures of DMAA and EBA are also shown in Fig. 1. It can be supposed that in the PHG polymerization with DMAA the chains formed consisted of many DMAA units randomly interrupted by methacrylic PHG functions, thus permitting the growing chains to reach also those methacrylic PHG functions which are sterically and geometrically hindered. When the reaction is carried out with a bifunctional monomer, such as EBA, the formation of a network based principally of EBA units in which the methacrylic PHG functions are randomly inserted can be supposed. However, the disappearance of FT-IR bands at 1405 and 951 cm^{-1}, attributable to methacrylic bonds, suggests the opening of double bonds, which gives rise to the occurrence of inter and intrapolymeric chain cross-linking. Probably this opening does not involve the polymerization of all methacrylic functions when the reaction is performed in the presence of PHG alone, owing to steric and conformational hindrances as mentioned previously, whereas in the presence of DMAA or EBA the polymerization/cross-linking process is facilitated. In addition, the reverse-phase suspension polymerization technique represents a simple method to obtain spherical microparticles whose size can be conveniently varied, according to special needs, by changing the reaction conditions, such as, for example, the speed of stirring. Obviously, under the same experimental conditions (stirring at 1000 rpm), a different size of microparticles can be ascribed to a different chemical structure and, in this case, also to a different degree of cross-linking . In effect, in our experiments, a mean diameter of particles of around 40 μm was obtained for sample M1, 20 μm for sample M2 and 10 μm for sample M3 as shown in Fig. 2, where size distribution profiles of the samples are reported.

The results of the dimensional analysis agree with the hypothesis that the degree of cross-linking should increase in the following order M1 < M2 < M3.

The photomicrography reported in Fig. 3 shows, as an example, the dried M1 microparticles; it is observed that their spherical shape and their sizes are rather uniform according to the narrow size distribution also shown in Fig. 2.

In addition, scanning electron microscopy allowed information to be obtained about the inside and outside surface properties as well as the confirmation of the spherical shape of the microparticles. It is evident in Fig. 4 that the presence of pores, especially in the inside surface, of sample M1 is in accord with the presence of a polymeric network formed during the cross-linking reaction. Similar results were obtained for samples M2 and M3.

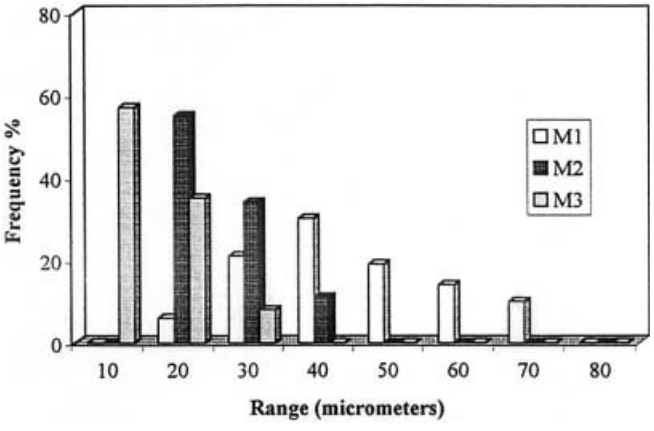


Fig. 2 Size distribution profiles of M1, M2 and M3 microparticles

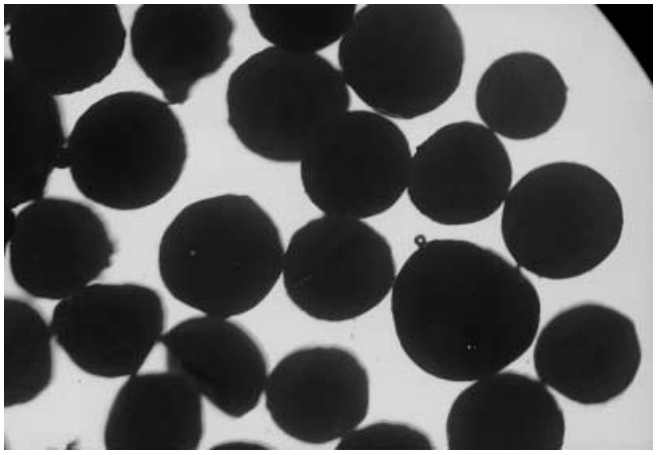
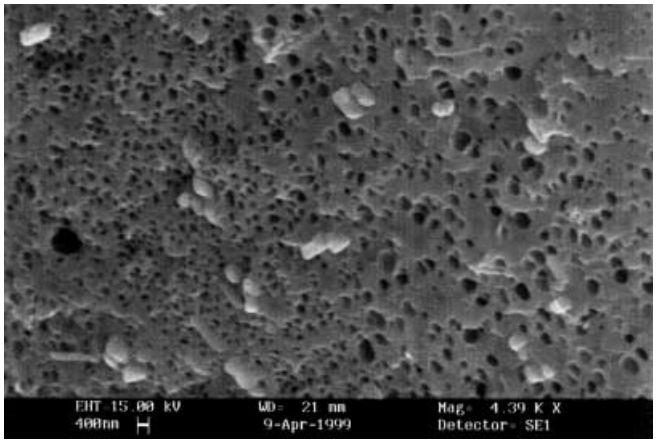


Fig. 3 Photomicrography of dried M1 microparticles: 1 cm is about 30 μm



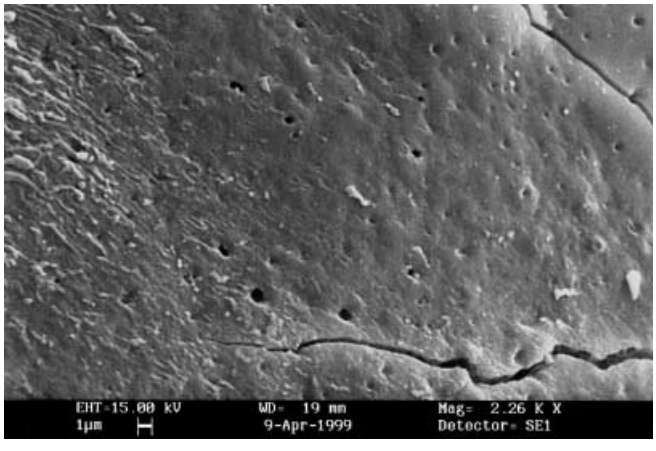


Fig. 4 Scanning electron micrographs. *A* Inside surface of sample M1: 1.5 mm is about 400 nm. *B* Outside surface of sample M1: 2.0 mm is about 1 μm

The determination of the physical state of the microparticles was performed by the X-ray diffraction method. In particular, the X-ray diffraction patterns reported in Fig. 5 show the amorphous state of the microparticles, like non-cross-linked PHG; this means that during the polymerization/cross-linking reaction no crystalline region was formed.

On the other hand, the absence of melting peaks in the DSC thermograms confirms that samples M1, M2 and M3 are in the amorphous state. Significant effects were observed on the T_g values, depending on the nature of the sample. In fact, the T_g values of all the samples are greater than that of non-cross-linked PHG (Table 2), thus confirming the increase in the rigidity of the material after the cross-linking reaction. In addition, the T_g values increase with the following trend M1 < M2 < M3 according to the degree of cross-linking, which probably increases in the same order.

Aqueous swelling measurements reported later, provide evidence that the degree of cross-linking follows the trend reported earlier. In effect, it is well known that the parameters affecting the swelling of hydrophilic polymeric networks include factors depending on the structure of the material, such as the degree of cross-linking, the hydrophilic/hydrophobic balance, the shape and dimension of the system, etc. Then, in order to evaluate the affinity of the networks towards the aqueous medium and to evaluate how the different structure of the microparticles influences their swelling behaviour, the water regain was determined after immersion of samples M1, M2 and M3 in distilled water (see Experimental). The values of the water regain reported in Table 3 show that the swelling ability decreases in the order M1 > M2 > M3 in accord with the increase in the degree of cross-linking as well as to a less hydrophilic structure when DMAA and EBA are used in the polymerization/cross-linking reaction.

In addition, Fig. 6 shows, as an example, the photomicrography of an isolated M1 microparticle before and after the swelling experiment. As can be observed, after the water penetration, the microparticle maintains its spherical shape but appears translucent and has a greater dimension (analogous results were obtained for samples M2 and M3). Therefore, considering that the swelling process is reflected in the change of sample dimension as a function of time, we performed a dynamic swelling study, evaluating through an optical stereomicroscope equipped with an image processor the variation of the microparticle diameter as a function of time when samples are immersed in distilled water. The results of the dynamic swelling measurements are reported in Fig. 7 as normalized diameter values (d_t/d_0) as a function of time for all the samples investigated. As can be observed, the diameter of the microparticles increases monotonically towards the equilibrium swollen value (d_∞) according to the different degree of

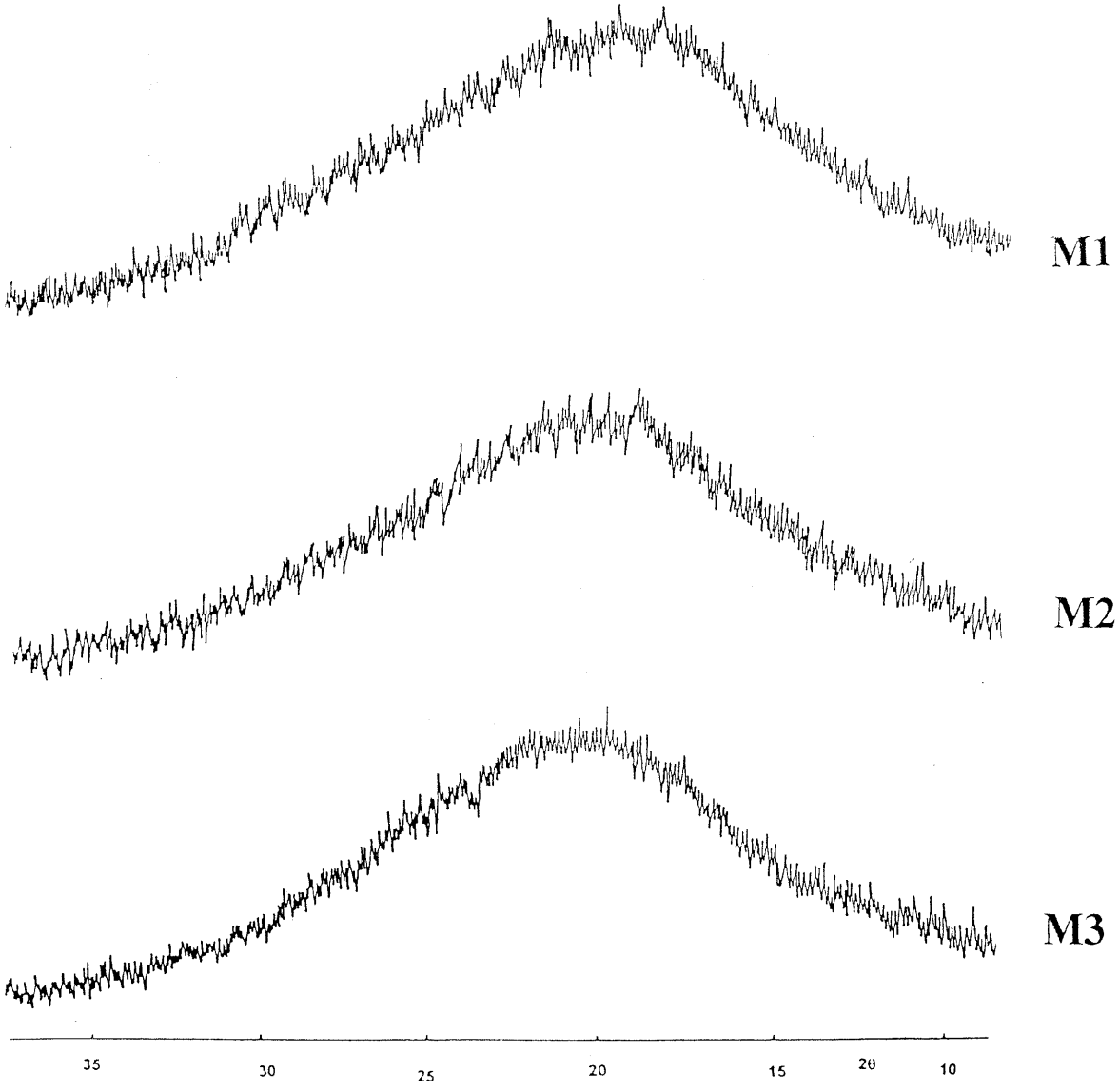


Fig. 5 X-ray diffraction patterns of M1, M2 and M3 microparticles

Table 2 Values of the glass-transition temperature (T_g) of microparticles M1, M2 and M3

Sample	T_g (°C)
Starting non-cross-linked PHG	54.6
M1	64.3
M2	72.8
M3	82.7

Table 3 Water regain and equilibrium normalized diameter values of M1, M2 and M3 microparticles

Sample	Water regain (%)	Equilibrium normalized diameter, d_∞/d_0
M1	742	1.98
M2	375	1.84
M3	239	1.62

cross-linking and hydrophilic/hydrophobic balance of the microparticles investigated. In particular, the values of the equilibrium normalized diameter (d_∞/d_0) reported in Table 3 decrease following the same trend of water regain as the values discussed previously. In addition, the samples investigated swell with a different rate, in particular, M1 microparticles in order to reach the greatest d_∞ value swell for a longer time (up to about 35 min), whereas for M2 and M3 microparticles, aqueous swelling is complete in about 10 min.

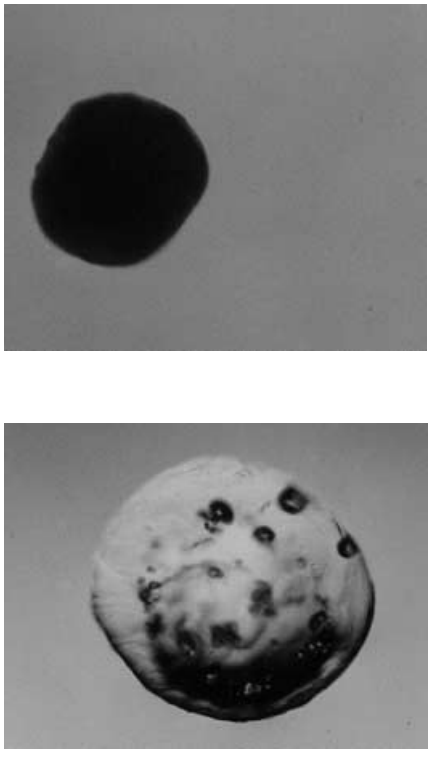


Fig. 6 Photomicrographs of *A* isolated dried M1 microparticle and *B* isolated swollen M1 microparticle. In these photomicrographs 1 cm is about 30 μm

In any case, the fast swelling and the high values of the water regain demonstrate a remarkable affinity of the networks obtained towards an aqueous medium. This result is a peculiarity of hydrogel systems which grants them potential biocompatibility and the ability to release drug molecules in a biological medium.

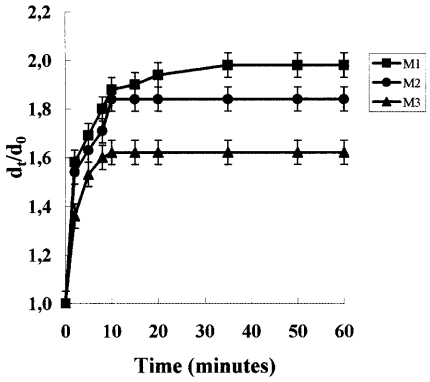


Fig. 7 Normalized diameter values (d_t/d_0) versus time for M1, M2 and M3 microparticles

Conclusions

The reverse-phase suspension polymerization represents a profitable method to obtain matrices of PHG cross-linked alone or in the presence of DMAA or EBA. These matrices are characterized by a spherical shape and a narrow size distribution profile. Their structure was shown to be porous as evidenced by scanning electron microscopy studies, whereas X-ray diffractometry revealed their amorphous state.

The microparticles obtained exhibit an outstanding aqueous swelling behaviour depending on their structural properties, such as the hydrophilic/hydrophobic balance and the degree of cross-linking. The high water affinity and the fast swelling suggest the suitability of these new matrices as drug delivery systems.

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