pH-Sensitive Drug Delivery Systems by Radical Polymerization of Gelatin Derivatives

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ABSTRACT: The objective of this article was the synthesis and characterization of protein-based microspheres able to respond to pH changes using methacrylated gelatin hydrolyzed, methacrylic acid sodium salt and *N*,*N*′-methylenebisacrylamide as protein monomer, pH-responsive functional monomer, and cross-linking agent, respectively. Reverse-phase suspension polymerization as synthetic technique was adopted and, varying the molar ratios of the reagents in the polymerization feed, three different hydrogels were obtained. These were characterized by scanning electron microscopy and dimensional analyses to verify the spherical shape and the dimensional distribution. Then, Fourier transform infrared spectra and water uptake experiments at acidic and neutral pH were performed, in order to verify the copolymerization of all the components and the pH-responsivity, respectively. Finally, after loading of the microspheres with a common nonsteroidal anti-inflammatory drug, such as diclofenac sodium salt, drug release experiments in simulated gastric fluid (pH 1) and in simulated intestinal fluid (pH 7) were performed, confirming the suitability of the obtained materials as drug delivery devices. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 3006–3013, 2012

Key words: methacrylated gelatin hydrolyzed; pH-responsive microparticles; diclofenac sodium salt; hydrogels

INTRODUCTION

Hydrogels based on natural proteins, and in particular on gelatin (GL), have received particular attention due to their natural origin, low cost, good biocompatibility, and biodegradability.[1] Gelatin is a biopolymer with thermo-reversible properties and, at temperatures below 25°C, an aqueous gelatin solution solidifies due to the formation of triple helices and a rigid three-dimensional network. When the temperature is raised above approximately to 30°C, the conformation changes from a helix to the more flexible coil, with a consequent formation of the liquid form of the gel.[2,3] As the opposite thermal behavior is desired for biomedical applications, researchers have combined gelatin with other polymers showing thermal gelation close to body temperature.[4] In addition to its good biological properties, such as nontoxicity and nonimmunogenicity, gelatin has the advantage of allowing for easy modification on the amino acid level.

Drug carriers based on GL were successfully synthesized and employed as sustained delivery vehicles of therapeutic agents.[5–7] Gelatin networks have been prepared via physical (e.g., drying, heating,γ ray, electron beam, and UV light exposure) and chemical cross-linking methods (e.g., reaction involving formaldehyde, glutaraldehyde, polyepoxy compounds, tannic acid, dimethyl suberimidate, carbodiimide, acyl azide, transglutaminase, and genipin).[8,9] Biodegradable pH-sensitive hydrogels based on GL and acrylic acid were prepared using γ radiation as super clean source.[10] This study showed the dependence of the porous structure of the hydrogels on acidic units content and the pH of the swelling medium. *In vitro* release studies, performed using a nonsteroidal anti-inflammatory drugs (NSAID; ketoprofen) as a model drug, showed that the release profile depends on both hydrogel composition and pH of the releasing medium. Films of alginate and GL, cross-linked with Ca^{2+}, with ciprofloxacin hydrochloride, a common antibiotic, as model drug incorporated in different concentrations, were obtained by a casting/solvent evaporation method.[11] Chemical, morphological, and mechanical properties of the hydrogels influence the release profile of the drug and it was found that for pH 7.4, the drug release was faster compared to pH 3.6.

This work describes the synthesis of pH-sensitive microspheres, suitable for oral drug administration, by radical polymerization of hydrolyzed methacrylate gelatin (HGL-MA) with methacrylic acid sodium salt (NaMA), as pH-sensitive monomer, and

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N,*N*′-ethylenebisacrylamide, acting as additionally crosslinking agent. Our challenge was to design pH-responsive microspheres, acting as pharmaceutical device, able to modulate the drug release profile depending of the pH of the surrounding environment. The proposed materials should be usefully applied in the pharmaceutical field because of *undergo reversible volume* changes, by swelling-shrinking in *response* to an external stimuli and, at the same time, their oligopeptide structure and spherical shape are useful for a drug delivery device. The microspheres were synthesized by reverse-phase suspension polymerization, using ammonium persulfate/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as initiator system, and characterized by scanning electronic microscopy, Fourier transform infrared (FT-IR) spectrophotometry, particle size distribution, and swelling analyses. To verify the suitability of these materials as pH-responsive drug delivery system, a common anti-inflammatory drug, diclofenac sodium salt (DC), was loaded on the polymeric networks and the release profiles at pH 1.0 and 7.0 were evaluated. *In vitro* release studies in simulated gastrointestinal fluids have showed the influence of the environmental pH and the chemical nature of entrapped drug on release profiles and hydrogels crosslinking degree. Finally, to estimate the diffusional contribute on the delivery of the drug semi-empirical equations were employed.[12,13]

EXPERIMENTAL

Materials

Gelatin (Ph Eur, Bloom 160), methacrylic anhydride (MA), potassium hydroxide, sodium methacrylate (NaMA), hydrochloride acid, *N*,*N*′-methylenebisacrylamide (MBA), sorbitan trioleate (Span 85), polyoxyethylene sorbitan trioleate (Tween 85), TMEDA, ammonium persulfate, sodium hydrogen phosphate, disodium hydrogen phosphate, ammonium acetate, and diclofenac sodium salt were provided from Sigma–Aldrich (Sigma Chemical Co, St. Louis, MO). Acetonitrile, methanol, and water were from Carlo Erba Reagents (Milan, Italy) and all of high-pressure liquid chromatography (HPLC) grade. 2-propanol, ethanol, acetone, *n*-hexane, chloroform, glacial acetic acid, and diethyl ether were from Carlo Erba Reagents and all of analytical grade (Milan, Italy); *n*-hexane and chloroform were purified by standard procedures.[14]

Synthesis of methacrylated gelatin hydrolyzate (HGL-MA)

HGL-MA was prepared according to the literature with some modifications.[15,16] Briefly, a reaction mixture containing 40 g of gelatin were taken up in 60 g of water and, after the addition of 1.6 g of sodium hydroxide, the solution was heated for 16 h to 130°C. Then, after cooling to room temperature, 3 mL of MA was added to the reaction mixture. The pH value of the reaction mixture was kept at 10 by addition of dilute sodium hydroxide. After a reaction time of 5 h, the mixture was adjusted with dilute hydrochloride acid to a pH value of 7. The hydrolyzate was precipitated by adding the polymeric solutions to an excess volume of acetone under agitation at room temperature. The suspensions were filtered by sintered glass filter funnel (Pyrex, Ä30 mm; porosity 3) and washed with diethyl ether, and the recovered HGL-MA was dried in a vacuum oven at 40°C.

Microspheres preparation (standard procedure)

Microspheres based on HGL-MA, NaMA, and MBA were produced by reverse-phase suspension polymerization.[17] Briefly, a mixture of *n*-hexane and chloroform was placed in a round-bottomed cylindrical glass reaction vessel fitted with an anchor-type stirrer and thermostated at 30°C, then treated, after 30 min of N_2 bubbling, with an aqueous solution of HGL-MA, the comonomer (NaMA), the crosslinker (MBA), and ammonium persulfate as radical initiator. The density of the organic phase was adjusted by the addition of chloroform or *n*-hexane, so that the aqueous phase sank slowly when stirring stopped. Under stirring at 1000 rpm, the mixture was treated with Span 85 and Tween 85, then after 10 min, with TMEDA and stirring was continued for another 60 min. Table I reports on the experimental conditions of each polymerization reactions. The microspheres were filtered, washed with 50-mL portions of 2-propanol, ethanol, acetone and diethyl ether, and dried overnight under vacuum at 40°C.

Characterization of pH-responsive microspheres

FT-IR spectra of HGel-MA, NIPAAm, MBA, HG-1, HG-2, and HG-3 were measured as pellets in KBr with a FT-IR spectrophotometer (model Jasco FT-IR 4200) in the wavelength range of 4000–400 cm^{-1}. Signal averages were obtained for 100 scans at a resolution of 1 cm^{-1}.

The shape and surface morphology of the microspheres were studied using scanning electron microscopy. The sample was prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on aluminium stub. The stubs were then coated with gold to thickness of about 300 Å, using a sputter coater then viewed under scanning electron microscopy (Leo stereoscan 420) and shown in photomicrographs.

TABLE I
Experimental Conditions of the Synthesis of the pH-Responsive Microspheres

Aqueous dispersed phase			Organic continuous phase	Surfactants mixture,	Initator system,	Hydrogel	
HGL-MA (mg)	NaMA (mg/mmol)	MBA (mg/mmol)	$CHCl_3$/*n*-hexane (mL/mL)	Span 85/Tween 85 (μL/μL)	$(NH_4)_2S_2O_8$/ TMEDA (mg/μL)	Yield (mg) (mass %)	Code
100	300/2.77	100/0.59	22/22	240/50	200/150	440 (88%)	M-1
200	300/2.77	100/0.59	23/23	240/50	200/150	350 (59%)	M-2
300	300/2.77	100/0.59	23/23	240/50	200/150	470 (67%)	M-3

For all polymerizations, the amount of aqueous phase is 2.5 mL.

The particle size distribution was carried out using an image processing and analysis system, (Stereomicroscope Motic BA 300 Pol). This image processor calculates the particle area and converts it to an equivalent circle diameter.

The swelling characteristics were determined in order to test hydrophilic properties of the microspheres. Typically, aliquots (40–50 mg) of the microspheres dried to constant weight were placed in a tared 5-mL sintered glass filter (Ä10 mm; porosity, G3), weighted, and left to swell by immersing the filter plus support in a beaker containing the swelling media (PBS solution $10^{-3}M$, pH 7.0, and HCl 0.1*N* at 37°C). After 24 h, 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, and then centrifuged at 3500 rpm for 15 min and weighted. The filter tare was determined after centrifugation with only water. The weights recorded at the different times were averaged and used to give the water content percent (WR%) by eq. (1):

$$\mathrm{WR}(\%) = \frac{W_s - W_d}{W_s} \times 100 \quad (1)$$

where W_s and W_d are weights of swollen and dried microspheres, respectively. The WR(%) for all prepared materials are reported in Table II.

In vitro release experiments

Incorporation of DC into the microspheres was performed as follows: 200 mg of preformed empty microspheres were wetted with 2.0 mL in a concentrated drug solution (10 mg/mL). After 3 days, under slow stirring at 37°C, the microspheres were filtered and dried at reduced pressure in presence of P_2O_5 to constant weight. The loading efficiency percent (LE%) of all samples are determined by HPLC analysis of filtered solvent according to eq. (2):

$$\mathrm{LE}(\%) = \frac{C_i - C_0}{C_i} \times 100 \quad (2)$$

Here, C_i is the concentration of drug in solution before the loading study, C_0 the concentration of drug in solution after the loading study. The values of calculated LE percent and the drug loaded percent (DL%) in each matrix are listed in Table II, according to eq. (3):

$$\mathrm{DL}(\%) = \frac{\text{Amount of drug in the beads}}{\text{Amount of beads}} \times 100 \quad (3)$$

The calorimetric analyses of DC, empty microspheres and DC-loaded microspheres were performed using a Netzsch DSC200 PC. The analyses were performed on the dry samples from 70 to 290°C under an inert atmosphere with a flow rate of 25 mL/min and a heating rate of 10°C/min.

Drug stability was studied at 37°C and at different pH (1.0 and 7.0). Aliquots of drug (10 mg) were incubated at 37°C in HCl 0.1*M* (pH 1.0) and phosphate buffer solution (PBS) 10^{-3} M (pH 7.0). At scheduled time intervals, corresponding to the condition of the drug release experiments, the samples were withdrawn and assayed by HPLC, in order to determine the drug concentration. The HPLC conditions were a mixture of aqueous solution of

TABLE II
Swelling Behaviors, Dimensional Parameters, and Drug Loading Parameters of Hydrophilic pH-Responsive Microspheres

	Water content			Average	Drug loading parameters	
Hydrogel	pH 1.0	pH 7.0	S_r	diameter (μm)	LE (%)	DL (%)
M-1	62 ± 4	841 ± 2	13.6	87 ± 4	96.3 ± 0.3	9.8 ± 0.2
M-2	63 ± 2	779 ± 4	12.4	48 ± 3	92.1 ± 0.2	9.6 ± 0.1
M-3	67 ± 4	646 ± 3	9.6	21 ± 2	88.2 ± 0.4	9.5 ± 0.1

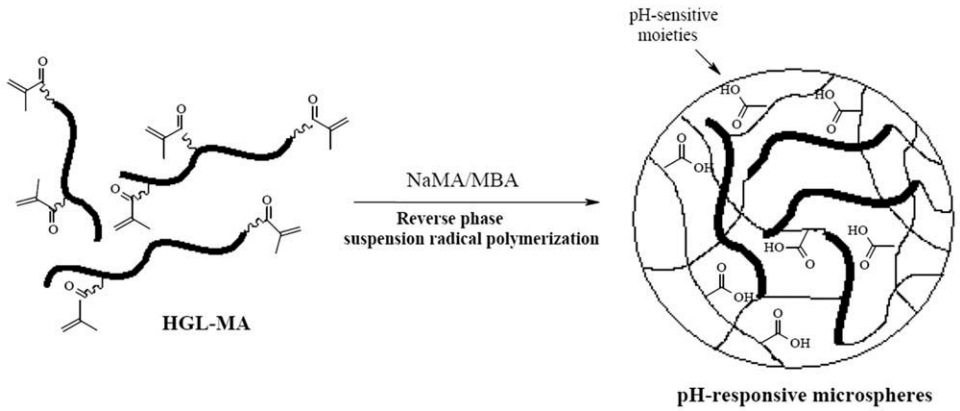

Figure 1 Reverse-phase suspension polymerization of HGL-MA macromers with NIPAAm and MBA.

ammonium acetate, methanol, and acetonitrile (40/30/30, v/v/v). The pH of the aqueous mobile phase portion of ammonium acetate buffer was adjusted with glacial acetic acid. The mobile phase was filtered, degassed, and pumped isocratically at a flow rate of 0.6 mL/min; UV detection at 284 nm.[18] The HPLC analyses were carried out using a Jasco PU-2080 liquid chromatography equipped with a Rheodyne 7725i injector (fitted with a 20-μL loop), a Jasco UV-2075 HPLC detector, and Jasco-Borwin integrator. A reversed-phase C18 column (μBondapak, 10 μm of 150 × 4.6 mm internal diameter obtained from Waters, Milford, MA) was used. Retention time 4.2 min; limit of detection 0.7 μM; limit of quantification 14 μM.

In vitro drug release profiles were obtained by HPLC analyses. Aliquots (10 mg) of drug-loaded microspheres were dispersed in flasks containing 10.0 mL of PBS solutions (pH 7.0, $10^{-3}M$), at 37.0°C ± 0.1°C, and sink conditions were maintained throughout the experiments. The samples, at suitable time intervals, were filtered and the solutions were analyzed by HPLC.

Additionally, *in vitro* studies were performed by initial dispersion of aliquots (10 mg) of drug-loaded microparticles in flasks containing HCl 0.1*M* (pH 1.0, simulating gastric fluid) and maintained at 37°C ± 0.1°C in a water bath for 2 h with magnetic stirring. After this time, a solution of 0.2*M* tribasic sodium phosphate was added to raise the pH to 7.0 (simulating intestinal fluid), according to the method reported in USP XXII (drug release test, method A, for enteric-coated particles), and the drug solution concentration was determined at suitable times until 24 h.[19] Sink conditions were maintained throughout the experiment. Then at suitable time intervals, samples were filtered and the solutions were analyzed by HPLC.

All the experiments were done in triplicate and the results were in agreement within ± 5% standard error. One-way analysis of variance was performed to assess the significance of the differences among data. Tukey–Kramer post-test was used to compare the means of different treatment data. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Synthesis of microspheres based on hydrolyzed gelatin

Chemical groups susceptible to radical polymerization were introduced onto hydrolyzed gelatin by acylation with MA to synthesized polymerizable polypeptide species (HGL-MA). Compared to the native protein, the hydrolyzates are characterized not only by enhanced water solubility but also by a greater number of nucleophilic groups disposable for the reaction with the acylating agent. Together with the thiolic groups of cysteine, hydroxyl groups of serine and tyrosine, and with the amino groups in the side chain of lysine residues, the terminal amino groups, derived from the alkaline hydrolysis of the peptide bonds, represent, indeed, reactive sites toward the derivatization with MA. Our goal was to obtain protein moieties bearing polymerizable functionalities to be directly copolymerized with a stimuli-responsive monomer, producing a network in which the polypeptide chains are linked by hydrocarbon bridge and randomly interrupted by growing chains of the functional monomer. To synthesize useful spherical polymeric materials, showing pH-responsive behavior, biocompatibility characteristics and hydrophilic properties, the HGL-MA was copolymerized with NaMA and MBA, acting as pH-sensitive and crosslinking agent, respectively, as reported in Figure 1.

On the other hand, the choice to obtain hydrogels characterized by spherical shape was dictated by the fact that this kind of materials are ideal vehicles for many controlled delivery applications, due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability, and sustained drug

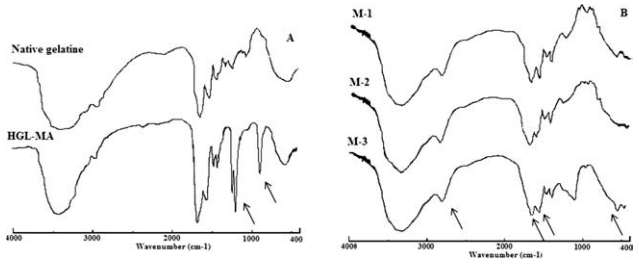

Figure 2 FT-IR spectra of native gelatin and HGL-MA (A) and of pH-responsive hydrogels P1, P2, and P3 (B).

release characteristics.[20] The microspheres were synthesized by free-radical suspension polymerization, in which TMEDA and ammonium persulfate was used as initiator system. The optimization of the polymerization method was performed, and it was observed that hydrophilic/lipophilic balance (HLB) of surfactants represents an important parameter to produce a water-in-oil emulsion consisting of water drops uniformly dispersed in the organic phase ($CHCl_3$/*n*-hexane) when stirring was stopped. Generally, water-in-oil emulsions are stabilized by surfactants in concentrations 0.5–1.5 (mass %) into water and literature data report that the best results were obtained using surfactant mixtures with different values of HLB.[21] In particular, the volume ratio of the surfactant mixture strictly depends on the dispersed phase. In our experiments, different tests were carried out, allowing to determine the correct ratio for Span85 (HLB = 1.8) and Tween85 (HLB = 11). A system with a total HLB equal to 3.4 was eventually found to be able in stabilizing the aqueous phase dispersed in the organic one. Varying the HGL-MA/NaMA/MBA molar ratio in the polymerization feed, three different hydrogels were prepared, as reported in Table I. In particular, NaMA/MBA molar ratio was 4.7, while the amount of hydrophilic crosslinker (HGL-MA) was 20.0% (w/w) for M-1 and was increased to 33.3 and 42.8% for M-2 and M-3, respectively. In the proposed polymerization protocol, we found that the change of both the crosslinking degree and the hydrophilic/hydrophobic balance of the polymeric networks seem to greatly influence the water affinity of the microspheres. The increased amount of pH-sensitive monomer in the hydrogel reduces the crosslinking degree of the network enhancing water-polymer affinity.

Characterization of gelatin microspheres

The materials were characterized by FT-IR spectrophotometry, swelling behavior, particle size distribution, and morphological analyses.

The FT-IR spectra of native and hydrolyzed methacrylate gelatin, reported in Figure 2(A), showed the appearance of the typical band of carbon–carbon double bond confirming the functionalization reaction. In addition, the FT-IR spectra of all samples [Fig. 2(B)] showed the disappearance of band at 917 cm^{-1} ascribable to carbon–carbon double bond of methacrylic functionalities of acidic monomer and HGL-MA, the appearance of the typical absorption bands of the reactive species involved in the polymerization process. In particular, the broad band at 3550–3350 cm^{-1} due to N—H stretching of the secondary amide and hydroxy group of protein and monomer, carbonyl groups stretching at 1655 cm^{-1}, N—H bending between 1570 and 1510 cm^{-1}, N—H out of plane wagging at 675 cm^{-1} and C—H stretching at 2925 and 2860 cm^{-1} are visible in the spectra of the hydrogels.

Using scanning electron microscopy, informations about the shape and the surface properties of the microparticles were obtained. In Figure 3(A) the spherical shape of sample M-2 was evident, while Figure 3(B) shows outside surface of M-1, characterized by a rough surface. Similar results were obtained for all of the spherically synthesized samples. The shape and the morphology of the microparticles suggest their potential use as drug delivery systems.

The dimensional distribution of the spherical microparticles was determined by using an optical stereomicroscope equipped with an image processor that calculates the particle area and converts it to an equivalent circle diameter. In our experiments, the particle diameter was in the dimensional range 60–80 μm for M-1, 40–60 μm for M-2, and 20–40 μm for M-3 and for each sample a distributional profile was recorded (Fig. 4). A broad particle size distribution was recorded for M-3, while the dimensional profile distributions of M-1 and M-2 were much narrower than M-3. The particle mean diameter of the hydrogels was 87 μm for M-1, 48 μm for M-2, and 21 μm for M-3. The microparticle diameters were strictly depending on the amount of crosslinkers (HGL-MA and MBA) and the ratio of the reactive species in the polymerization feed; the values of mean particle

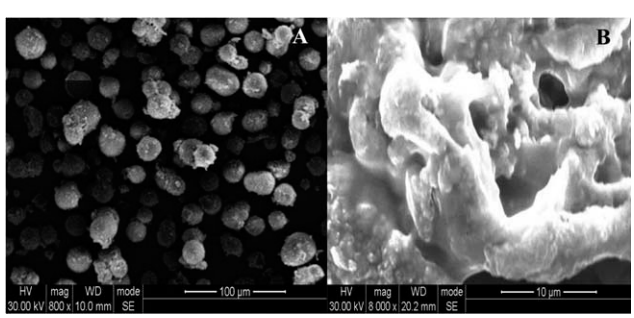

Figure 3 SEM micrographs of M-2 (A) and M-1 outside surface (B).

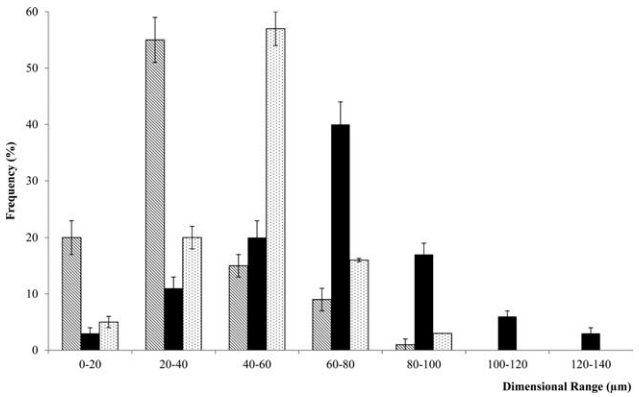

Figure 4 Size distribution profiles for M-1 (gray bars), M-2 (white bars), and M-3 (black bars).

diameter, in general, decrease as HGL-MA amount increases in the polymerization mixture composition.

Investigation of the applicability of these hydrogels in controlled release was done by studying their swelling behavior. The values of contained water percentage were determined in aqueous media that simulates biological fluids, such as gastric (pH 1.0) and intestinal (pH 7.0) at 37°C. The data reported in Table II illustrate the water uptake, in grams per grams of dry copolymer, for each composition and pH studied. We also reported the ratio between the swelling at pH 7.0 and pH 1.0 (S_r) for all samples. The prepared materials show different water affinity at pH 7.0 and acid pH due to pendant acidic groups in the polymeric chains. In particular, at pH 1.0 there is a considerable lowering of the water affinity due to acidic groups unionized at this pH value. When the pH is 7.0, the water content is greater than that found at pH 1.0 for all copolymers. It is possible to explain this behavior as a consequence of electrostatic repulsions between polymeric chains due to the increase of dissociated groups at pH 7.0. The hydrophilic/hydrophobic balance of the polymeric networks and the amount of the pH-sensitive monomer [ranging from 60% (w/w) for M-1 to 42% for M-3] justified the values recorded in the water uptake experiments. In particular, the hydrogel M-1 showed highest water affinity at neutral pH as consequence of the both increased acidic moieties in the polymeric backbone and lower crosslinking degree, while the S_r value increased as a function of the number of pH-sensitive monomeric units in the hydrogel ranging from 13.6 for M-1 to 9.6 for M-3.

In vitro release studies

The stimuli-responsive microgels were tested as site-specific delivery devices for DC, a well-known NSAID, selected for its pharmacokinetics properties and safety concerns. DC is indeed totally absorbed after oral administration throughout the intestinal tract with linear pharmacokinetics.[22,23] The absolute bioavailability of DC after oral administration did not differ significantly as a function of the pH, and the low solubility of drug at pH values of 4.5 and below does not pose a substantial risk for bioinequivalence. This may be the result of high permeability for DC, as well as the dynamic character of the uptake processes.[24] The complete absorption classifies DC as highly permeable as supported by several *in vitro* data.[25,26] Furthermore, as reported in the literature, the interest for alternative approaches to reduce gastrointestinal side-effects associated with NSAIDs has re-emerged.[27] There are two main components of gastric damaging properties of NSAIDs: the acute toxicity associated with the short-term intake of NSAIDs, which is principally caused by local irritation of the gastric mucosa and the chronic toxicity resulting mainly from systemic effects associated with prolonged administration of NSAIDs.[28] Sustained drug-delivery systems, designed for a site-specific delivery of drugs at predetermined rates for predefined periods of time, have been used to overcome the shortcomings of conventional drug formulations.[29]

The microgels were loaded by soaking procedure; the loading efficiency (LE%) and the DL% of all samples were determined by HPLC analysis (Table II). DC was loaded on the beads with a LE% > 88% for all copolymers. In our experiments, drug-loaded copolymers with DL% ranging from 9.5 to 9.8 were produced.

Considering the DSC analyses of drug, drug-loaded, and unloaded microparticles (Fig. 5), the

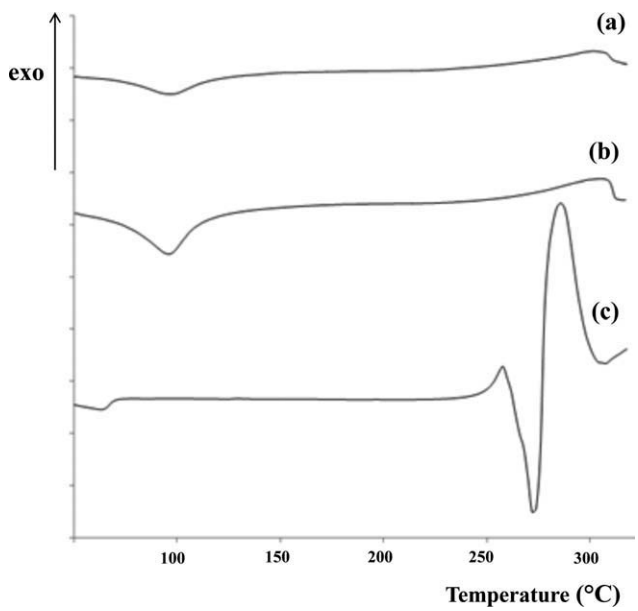

Figure 5 Differential scanning calorimetric thermograms of DC-unloaded M-1 microspheres (b) and DC-loaded M-1 microspheres (a) and pure DC (c). Analogous results have been found for all materials.

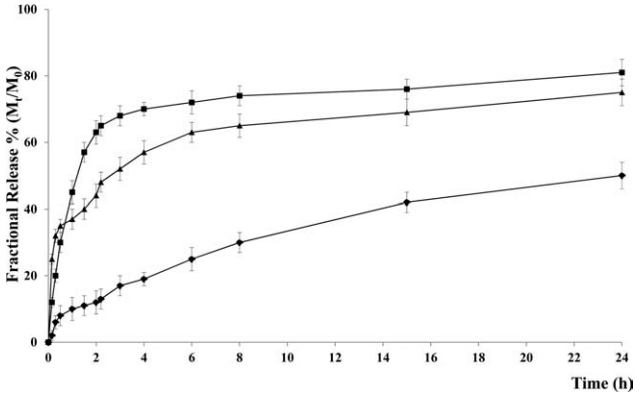

Figure 6 Drug release expressed as percent of DC delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for microspheres M-1 (■), M-2 (▲), and M-3 ([diaf2]) at 37°C and pH 7.0 (1 m*M*, PBS solution).

nature of the drug inside the polymer matrix can be assessed. This one may emerge in solid solution, metastable molecular dispersion or crystallization and may display relevant properties during *in vitro* release.[30] The onset melting peak of DC was observed at 288°C. However, no characteristic peak of DC was observed in the DSC curves of the drug-loaded microparticles, suggesting that the drug is molecularly dispersed in the polymer matrix.[31]

Drug release profiles were determined by HPLC analyses and the amount of drug released was expressed as percent of drug delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time at 37°C in PBS solution (pH 7.0, 1 m*M*) for all microgels (Fig. 6). Since the microparticles have a well-defined geometry and a narrow dimensional distribution, the mechanism of drug release (Fickian or non-Fickian) was determined. In particular, the kinetics of DC release at 37°C and pH 7.0 were analyzed by the semiempirical eq. (4) for $M_t/M_0 \leq 0.6$[12]:

$$\frac{M_t}{M_0} = Kt^n \tag{4}$$

where M_t/M_0 is the drug fraction released at time t, K and n are a constant and the kinetic exponent of drug release, respectively. Although the use of this equation requires detailed statistical analysis, the calculated exponent, n, gives an indication of the release kinetics. If $n = 0.43$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion, while a Case II transport occurs if $n = 0.85$. With $0.43 \leq n \leq 0.85$, transport lies between Fickian and Case II. With $n < 0.43$, pseudo-Fickian diffusion behavior occurs, where sorption curves resemble Fickian curves, but with a slower approach to equilibrium. Finally, $n > 0.85$ implies that solvent (or drug) transport rate accelerates as equilibrium is approached (Super-Case II transport). The least-squares estimations of the fractional release data along with the estimated correlation coefficient values, r, are presented in Table III. As shown in the results, the exponents n in the release experiments at 37°C and pH 7.0 for the M-1 and M-2 were 0.32 and 0.31, respectively, which were meant the polymers at this temperature and pH mainly followed a pseudo-Fickian diffusion way. For the hydrogel M-3 the n value was 0.47, which meant that the transport lies between Fickian and Case II with a more pronounced Fickian behavior.

A more informative analysis can be obtained by fitting the data with the model proposed by Peppas and Sahlin.[13] The equation for this model is:

$$\frac{M_t}{M_0} = K_1 \cdot t^{1/2} + K_2 \cdot t \tag{5}$$

with $M_t/M_0 \leq 0.95$. In this equation, the first term is the Fickian contribution and the second term is the Case II relaxational contribution. Table III reports K_1 and K_2 values according to eq. (5). For all samples the term $K_1t^{1/2}$ is greater than the term K_2t, indicating that the predominant release mechanism of DC is the Fickian diffusion through the swollen microparticles. Thus, the drug release was determined by two factors: the swelling rate of polymer and the diffusivity of the drug through the network. Because, at the same temperature, there are no marked differences of the diffusivity of drug in each polymer, the swelling rate of the polymer was the dominating factor. When the dried gels are placed in the release media, water molecules begin to diffuse into the gel network and the matrix swelled. At the same time, drug molecules start to diffuse through the gel layer and to the medium.

TABLE III
Release Kinetics Parameters of Different Formulations

	$M_t/M_0 = Kt^n$			$M_t/M_0 = K_1.t^{1/2} + K_2.t$		
Sample	$K \times 10^3$ (min^{-n})	n	r	$K_1 \times 10^3$ ($min^{-1/2}$)	$K_2 \times 10^3$ (min^{-1})	r
M-1	45.24 ± 1.65	0.32 ± 0.04	0.97	48.77 ± 1.16	−4.90 ± 0.13	0.94
M-2	39.96 ± 0.98	0.31 ± 0.02	0.97	39.45 ± 1.14	−5.17 ± 0.11	0.96
M-3	9.29 ± 0.05	0.47 ± 0.03	0.98	8.05 ± 0.70	0.51 ± 0.09	0.98

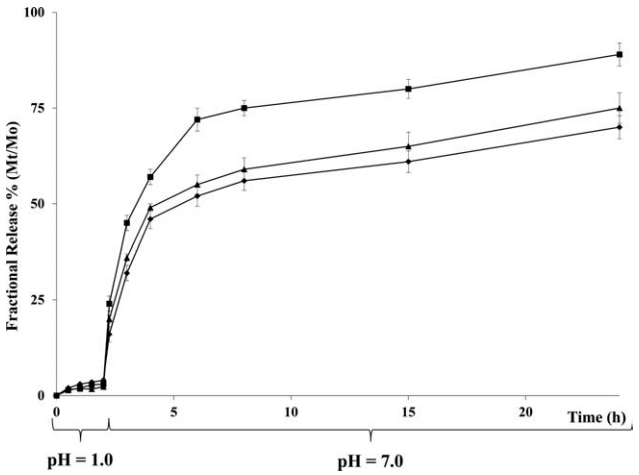

Figure 7 Drug release expressed as the percent of DC delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time using the pH change method for microspheres M-1 (■), M-2 (▲), and M-3 ([diaf2]) at 37°C.

In addition, in order to simulate gastrointestinal tract, *in vitro* release studies at 37°C and at pH 1.0 for 2 h, and then at pH 7.0 for 22 h using the pH change method, were performed. Because of the presence of strong ionizable groups in the polymeric network, pH of the swelling medium induces a change in the degree of ionization of the polyelectrolyte and therefore in the swelling capacity of the microgel. The experimental data showed an increase of DC release for all samples at pH 7.0 due to the repulsion of negative charges of carboxyl groups in the polymeric backbone. The acidic groups are undissociated after 2 h at pH 1.0 and low amounts of drug (M_t/M_0 percent < 10.0) are released. When the pH is 7.0, the swelling of the network increases and the drug molecules easily diffuse through the polymeric structure (Fig. 7).

CONCLUSIONS

In this work, pH-responsive microspheres (M-1, M-2, and M-3) based on methacrylated gelatin hydrolyzed, sodium methacrylate and *N,N′*-ethylenebisacrylamide in different molar ratios were prepared and tested as site-specific delivery devices of diclofenac sodium salt. Reverse-phase suspension polymerization was chosen as synthetic methodology to obtain microparticles with spherical shape, porous surface, and narrow size distribution. The copolymerization of all components was verified by FT-IR spectra, while water uptake experiments at pH 1 and 7 confirmed the pH-responsivity of all hydrogels; in particular, it was observed that, increasing the amount of pH-sensitive monomer in the polymeric feed, and thus reducing the crosslinking degree, the water affinity enhances, raising the highest value for M-1 matrix.

After drug loading, DSC analyses were performed to demonstrate the homogeneous distribution of DC in the polymer matrices, and release experiments in simulated gastric fluid (pH 1) and in simulated intestinal fluid (pH 7) were performed.

Finally, in order to estimate the diffusional contribute on the drug delivery, semiempirical equations were employed showing, for each sample, the predominant role of the diffusional component in the release mechanism.

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