Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 312 (2006) 151–157
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

pH-Sensitive hydrogels based on bovine serum albumin for oral drug delivery

Francesca Iemma *, U. Gianfranco Spizzirri, Francesco Puoci, Rita Muzzalupo, Sonia Trombino, Roberta Cassano, Sonia Leta, Nevio Picci

Dipartimento di Scienze Farmaceutiche, Università della Calabria, 87036 Rende (CS), Italy

Received 27 October 2005; received in revised form 2 November 2005; accepted 9 January 2006
Available online 21 February 2006

Abstract

pH-Sensitive bovine serum albumin (BSA) hydrophilic microspheres were prepared by free radical polymerization of methacrylate derivatized BSA and methacrylic acid sodium salt. Incorporation of both monomers in hydrogels was confirmed by Fourier transform infrared spectroscopy. Morphological analysis by scanning electron microscopy showed spherical shape and porous surface of all prepared samples. The microspheres showed high water affinity at neutral pH values and a narrow dimensional distribution. Network density of hydrogels depends on derivatization degree (DD%) of BSA and/or concentration of modified BSA in the polymerization feed. In order to employ the prepared samples such as pH-sensitive hydrogels, in vitro release studies, in media simulating biological fluids, were performed using diflunisal (DF) and β-propranolol (PR) as model drugs. Experimental data showed that the release profiles depend on drug–matrix interactions and diffusional limitation awardable to crosslinking degree of microparticles. β-Propranolol is quickly released at pH 1.0 and a complete release after 1 h at pH 6.8 was observed. In the case of diflunisal pH-sensitive release was observed. At pH 1.0 low amounts of drug were released (w/w < 10% after 2 h). When the pH is 6.8, the diflunisal release increased in the amount (w/w > 75% after 24 h).

Keywords: BSA; Radical polymerization; Hydrogel; Microspheres; pH-Controlled release

1. Introduction

Controlled drug delivery systems, designed to deliver drugs at predetermined rates for predefined periods of time, have been used to overcome the shortcomings of conventional drug formulations (Griffith, 2000; Vasir et al., 2003; Stubbe et al., 2004).

Hydrogels have emerged as a promising option in this regard (Peppas et al., 2000; Byrne et al., 2002; Gupta et al., 2002; Hoffman, 2002; Freiberg and Zhu, 2004; Kashyap et al., 2005; Omidian et al., 2005). Hydrogels are crosslinked, hydrophilic polymeric structures that can imbibe large amounts of water or biological fluids. Lately, stimuli-responsive hydrogels have been studied since they exhibit reversible swelling behavior in response to external stimuli such as pH, temperature or magnetic and electric field (Kost and Langer, 2001; Qiu and Park, 2001; Miyata et al., 2002; Morishita et al., 2004; Murdan, 2003). In particular, hydrogel pH-sensitive are widely used because of variations in pH that are known to occur at several body sites such as the gastrointestinal tract, vagina and blood vessels (Guyton and Hall, 1998). All pH-sensitive polymers contain pendant acidic (e.g. carboxylic and sulfonic acids) or basic (e.g. ammonium salts) groups that either accept or release protons in response to changes in environmental pH. Hydrogels bearing acid groups were used to develop formulations that release drugs in a neutral pH environment (Brannon-Peppas and Peppas, 1990; Khare and Peppas, 1993; Chiu et al., 2001; Alvarez-Lorenzo and Concheiro, 2002).

The design of a new biodegradable and biocompatible stimuli-sensitive polymeric systems play a key role in the development of stimuli-responsive biomaterials and represents an interesting incentive for several researchers (Gil and Hudson, 2004; Leonard et al., 2004; Sinha. et al., 2004).

Considerable interest in recent years has been shown in the use of natural proteins as a carrier for drug delivery. In particular, albumin is an attractive macromolecular carrier used to prepare microspheres for the sustained delivery of therapeutic agents

* Corresponding author. Tel.: +39 0984 493011; fax: +39 0984 493163.
E-mail address: francesca.iemma@unical.it (F. Iemma).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.01.010

and as drug carriers. (Arnedo et al., 2002; Sahin et al., 2002; Langer et al., 2003; Kao et al., 2003; Patil, 2003; Wunder et al., 2003). The albumin carriers extensively studied in previous works are suitable for drug delivery since they are biodegradable, biocompatible and relatively easy to prepare over a wide range of particle sizes. They are reported to be relatively non-toxic and non-immunogenic (Chuo et al., 1996; Ghassabian et al., 1996; Park et al., 1998).

In previous works we reported design and synthesis of bovine serum albumin (BSA) microspheres by radical copolymerization of methacrylate BSA (BSA_m) and *N*,*N*-dimethylacrylamide (Iemma et al., 2004; Iemma et al., 2005). This polymerization technique allows to obtain spherical microparticles of albumin with a narrow size distribution (Muzzalupo et al., 2001; Pitarresi et al., 2004). Spherical shape should be advisable in order to avoid swelling anisotropic behaviour associated with other geometries (Park, 1988).

The hydrophilicity of beads allowed to incorporate high concentrations of water-soluble drugs into the spheres after synthesis. Employing different model drugs releases profile from microspheres were studied. In particular, the drug release features depended principally on crosslinking degree of polymers, ratio among functionalized protein and comonomer, and drug–matrix interactions.

In order to prepare pH-sensitive microspheres, suitable for oral drug administration, the present work describes the synthesis of materials by reverse phase suspension copolymerization of methacrylic acid sodium salt (NaM) and BSA_m. The beads obtained were characterized by scanning electronic microscopy (SEM), Fourier Transform IR spectrophotometry, particle size distribution analysis and swelling analysis. The acidic groups in the polymeric network gave at prepared microsphers a pH-dependent behaviour. Finally, to obtain information about drug release profile, microparticles into a solution of diflunisal (DF) and β-propranolol (PR), used such as model drugs, were soaked. 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.

2. Materials and methods

2.1. Apparatus

The used dialysis tubes are 6–27/32", Medicell International LTD. "Freezing-drying apparatus" Micro Modulyo, Edwards. Ultraviolet spectra were recorded with a U-2000 Hitachi spectrophotometer using 1 cm quartz cells. The number of scans was 100. Particle size distribution was carried out using an image processing and analysis system, Leica DMRB equipped with a Leica Wild 3D stereomicroscope. This image processor calculates the particle area and converts it to an equivalent circle diameter. Scanning electron microscopy photographs were obtained with a Leo stereoscan 420; the sample surface was made conductive by the deposition of a layer of gold on the samples in a vacuum chamber. X-ray diffraction analysis was performed using a diffractometer Philips PW 1729 X-ray generator. The experimental parameters were: 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. High-pressure liquid chromatography (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-Borwin1 integrator. A reversed-phase C18 column (μBondapak, 10 μm of 250 mm × 4.6 mm internal diameter obtained from Waters) was used.

Table 1
Reaction between BSA and MA

BSA (g)	MA (mmol/mg)	DD%	Sample
2.00	2.14/330	63	A
2.00	5.37/828	100	B

All reactions were carried out in H_2O (15 ml) at pH 6.5–7.5 (HCO_3^{2-}/CO_3^{2-} buffer 0.2 M) and $T = 0\,^{\circ}C$.

2.2. Materials

All the reagents used were of analytical grade, unless otherwise stated *n*-hexane and carbon tetrachloride, purchased from Carlo Erba Reagents (Milan, Italy), were purified by standard procedures. BSA fraction V (MW 68.000; pH 7.0 ± 0.2; grade ≥98%) was from Roche Diagnostics GmbH. Methacrylic anhydride (MA), methacrylic acid sodium salt, sorbitan trioleate (Span 85), polyoxyethylene sorbitan trioleate (Tween 85), *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TMEDA) and ammonium persulfate, diflunisal, β-propranolol, phosphoric acid, acetic acid, ammoniac 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 HPLC grade.

2.3. Derivatization of BSA

Functionalized BSA (labelled A and B in Table 1) according to a procedure elsewhere reported were prepared (Iemma et al., 2004; Iemma et al., 2005). Derivatization of BSA with MA was carried out in distilled aqueous phase, under conditions of controlled pH and temperature (pH 7 and 0 °C), using a suitable amount of MA and stirred for 1 h at 0 °C). The aqueous solution obtained was introduced into dialysis tubes and dipped into a glass vessel containing distilled water at 20° C for 48 h with four changes of water. The resulting solution was frozen and dried with "freezing-drying apparatus" to afford a vaporous solid.

The derivatization degree (DD%) of BSA_m in agreement with a procedure reported in literature was determined (Snyder and Sobocinski, 1975).

2.4. Microspheres preparation (standard procedure)

BSA_m and NaM microspheres based by radical copolymerization technique were produced. Briefly a mixture of *n*-hexane and carbon tetrachloride was placed in a round-bottomed cylin-

Table 2
Copolymerization of BSA_m with NaM

Aqueous dispersed phase	Organic continuous phase	Hydrogel	
Reagents (mg)	CCl_4/esano (ml/ml)	mg (conv. %)	Sample
A (350) NaM (194)	17/23	430 (79.0)	AM_1
A (450) NaM (40.0)	17/23	350 (71.4)	AM_2
B (350) NaM (194)	17/23	370 (68.5)	BM_1
B (450) NaM (40.0)	17/23	390 (79.6)	BM_2

For all polymerisations, the amount of aqueous phase is 2.5 ml; initiator system is $(NH_4)_2S_2O_8$/TMEDA (150 mg/150 μl); surfactants are Span 85/Tween 85 (100 μl/50 μl).

drical glass reaction vessel fitted with an anchor-type stirrer and thermostated at 40 °C, then treated, after 30 min of N_2 bubbling, with a solution of BSA_m, comonomer (NaM) and ammonium persulfate in water such as radical initiator. The density of the organic phase was adjusted by the addition of CCl_4 or *n*-hexane so that the aqueous phase sank slowly when stirring stopped. Under stirring at 1000 rpm, the mixture was treated with Span85 and Tween85, then after 10 min with TMEDA and stirring was continued for another 60 min. The amounts of all reagents used in these experiments are reported in Table 2. Each matrix so obtained was filtered, washed with 50 ml portions of 2-propanol, ethanol, acetone and diethyl ether and dried overnight under vacuum at 40 °C.

2.5. Water content of microspheres

The swelling characteristics of microspheres were determined in order to check hydrophilic affinity of spherical microparticles. Typically aliquots (40–50 mg) of the microparticles dried to constant weight were placed in a tared 5-ml sintered glass filter (Ø10 mm; porosity, G3), weighted, and left to swell by immersing the filter plus support in a beaker containing the swelling media, i.e. HCl 0.1 N (pH 1, simulated gastric fluid) and phosphate buffer (pH 6.8, simulated intestinal fluid). At a predetermined 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 3500 rpm for 15 min and weighted. This operation was repeated at the different times (1, 4 and 24 h). 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 the following 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 spherical microparticles, respectively. Each experiment was carried out in triplicate and the results were in agreement within ±4% standard error. The WR (%) for all prepared materials are reported on Table 3.

Table 3
Water content (%) of microparticles in various media and ratio between the swelling at pH 6.8 and 1.0 (S_r)

Sample	Water content (%)		S_r
	pH 1	pH 6.8	
AM_1	83	265	3.2
AM_2	58	286	4.9
BM_1	60	593	9.9
BM_2	83	340	4.1

2.6. Incorporation of drug into preformed microspheres

Incorporation of drugs into microspheres was performed as follows: 150 mg of preformed empty microspheres (prepared as described above) were wetted with 2 ml in a concentrated drug solution (15 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 in according to Eq. (2):

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

Here C_i was the concentration of drug in solution before the loading study, C_0 the concentration of drug in solution after the loading study and M_i was the mass of drug available. The calculated LE (%) of different copolymers are listed on Table 4.

2.7. Drug stability at pH 1.0 and 6.8

The stability of each drug was studied at pH 1.0 and 6.8. Aliquots of drug (10 mg) were incubated at 37 °C in HCl 0.1 N (pH 1) or phosphate buffer solution pH 6.8. At scheduled time intervals, samples were withdrawn and assayed by HPLC, in order to determine the drug concentration.

HPLC conditions were:

- for diflunisal: H_3PO_4 (0.1%, v/v)/methanol (5/95), 1.0 ml/min flow, UV detection at λ = 254 nm;
- for β-propranolol: acetonitrile/metanol/CH_3COOH/NH_3 (25%) (95/4.6/0.3/0.1), 0.7 ml/min flow, UV detection at λ = 254 nm.

Table 4
Loading efficiency of microspheres (LE, %) after 72 h at 37 °C

Drug	Sample			
	AM_1	AM_2	BM_1	BM_2
Diflunisal	96.3	97.5	96.5	92.4
β-Propranolol	67.4	43.0	59.1	47.0

2.8. In vitro drug release at pH 1.0 and 6.8 from microparticles

In vitro drug release profiles were obtained by HPLC. Aliquots (10 mg) of drug-loaded microparticles were dispersed in flasks containing HCl 0.1 N (pH 1.0, simulated gastric fluid) and maintained at 37 ± 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 6.8 (simulated intestinal fluid), according to the method reported in USP XXII (drug release test, method A, for enteric-coated particles). Sink condition were maintained throughout the experiment. Then at suitable time intervals, samples were filtered and the solutions were analysed by HPLC. Each experiment was carried out in triplicate and the results were in agreement vithin ±5% standard error.

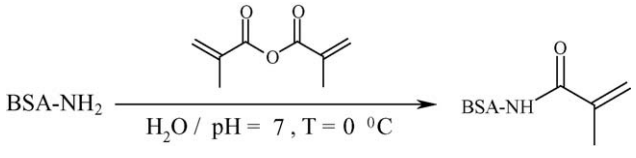
3. Results and discussion

Chemical groups susceptible of radical polymerization were introduced onto BSA by acylation with MA in water at 0 °C and neutral pH (Scheme 1). Under mild reaction conditions only sterically accessible amino groups in the side chain of lysine react with acylation agent (Iemma et al., 2004). BSA_m samples (Table 1) with various derivatization degree, using different amount of MA, were obtained. In the present work we realized BSA_m with 63% (A) and 100% (B) of the available amino groups acylated.

pH-Responsive microspheres of albumin can be synthesized by copolymerization of BSA_m with an acidic monomer such as acrylic or methacrylic acid. Nevertheless organic acid in the polymerization feed brought about BSA_m precipitation. In order to overcome this trouble we selected NaM to introduce pH-sensitive behaviour into materials. Samples A and B were copolymerized with NaM by reverse phase suspension copolymerization to prepare spherical microparticles for oral drug delivery (Table 2). Varying the amount of crosslinker (BSA_m) and the derivatizion degree of BSA_m, hydrogels with different crosslinking degree were created.

The reaction was started using TMEDA and ammonium persulfate as initatior system. Optimization of the polymerization method required several attempts. It was observed that hydrophilic/lypophilic balance (HLB) of surfactants is very important. Many tests were carried out to determine the correct ratio for Span85 (HLB = 1.8) and Tween85 (HLB = 11). Finally, we observed that a system with HLB = 4.8 is able to stabilize aqueous dispersed phase.

The polymerisation reaction, owing to steric and geometric constraints, involves only the methacrylic functions of BSA_m which are accessible to the growing chains. The microparticle structure obtained is characterised by a network where the BSA chains are linked by some hydrocarbon bridges. The obtained materials were characterized by Fourier Transform IR spectrophotometry, swelling behaviour, particle size distribution analysis and morphological analysis.



Scheme 1. Derivation reaction of BSA with MA.

The FT-IR spectra of all samples shows the disappearance of bands at 1307 and 934 cm^{-1} awardable to BSA_m methacrylic groups and at 944 and 921 cm^{-1} awardable to C–C double bond of methacrylic acid sodium salt. Furthermore, a absorption band at 617 cm^{-1} (a typical band of BSA_m homopolymers) in all samples was observed.

Investigation of the applicability of these hydrogels in controlled release was done by studying their swelling behaviour. The value of contained water percentage was determined in aqueous media which simulates some biological fluids, such as gastric (pH 1) and intestinal (pH 6.8) at 37 °C. The data reported in Table 3 illustrate the water uptake, in grams per grams dry copolymer, for each composition and pH studied. We reported also the ratio between the swelling at pH 6.8 and pH 1 (S_r) for all samples. The prepared materials shows different water affinity at pH 6.8 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 6.8, the water content is greater than that found at pH 1 for all copolymers. It is possible to explain this behaviour as a consequence of electrostatic repulsions between polymeric chains due to the increase of dissociated groups at pH 6.8. The BM_1 sample showed highest S_r value. We suppose this behaviour can be due to greater NaM amount in the polymeric network.

Using scanning electron microscopy we obtained information about the surface properties of the microparticles, and we were able to check that the microparticles had a spherical shape. In Fig. 1(a and b), the spherical shapes of sample AM_1 and BM_1, respectively, are evident. Fig. 1(c) shows outside surface of BM_1, characterized by a high degree of porosity. Similar results were obtained for all of the spherical created samples.

In our experiments a mean particle diameter of around 10 μm for AM_1 and AM_2 (Fig. 2(a)) and 15 μm for BM_1 and BM_2 (Fig. 2(b)) was obtained.

In order to estimate the ability of prepared matrices to release drug molecules, the beads were loaded with various drugs by soaking procedure. Diflunisal and β-propranolol were chosen such as model drugs and the loading efficiency of all samples (LE, %) are determined by HPLC analysis such as reported in experimental part.

The experimental data, reported in Table 4, are interesting. The diflunisal was almost completely loaded on the polymeric beads (LE (%) > 90 for all hydrogels), whereas β-propranolol was poorly uptaken on the beads (LE (%) < 70% for all macromolecular systems). It is possible to explain this different behaviour as consequence of strong interactions between BSA and acidic drugs, such as DF.

The determination of the drug dispersion state in all preformed hydrogel was performed by X-ray analysis. Figs. 3 and 4 report the X-ray diffraction patterns of pure drugs (curves A),

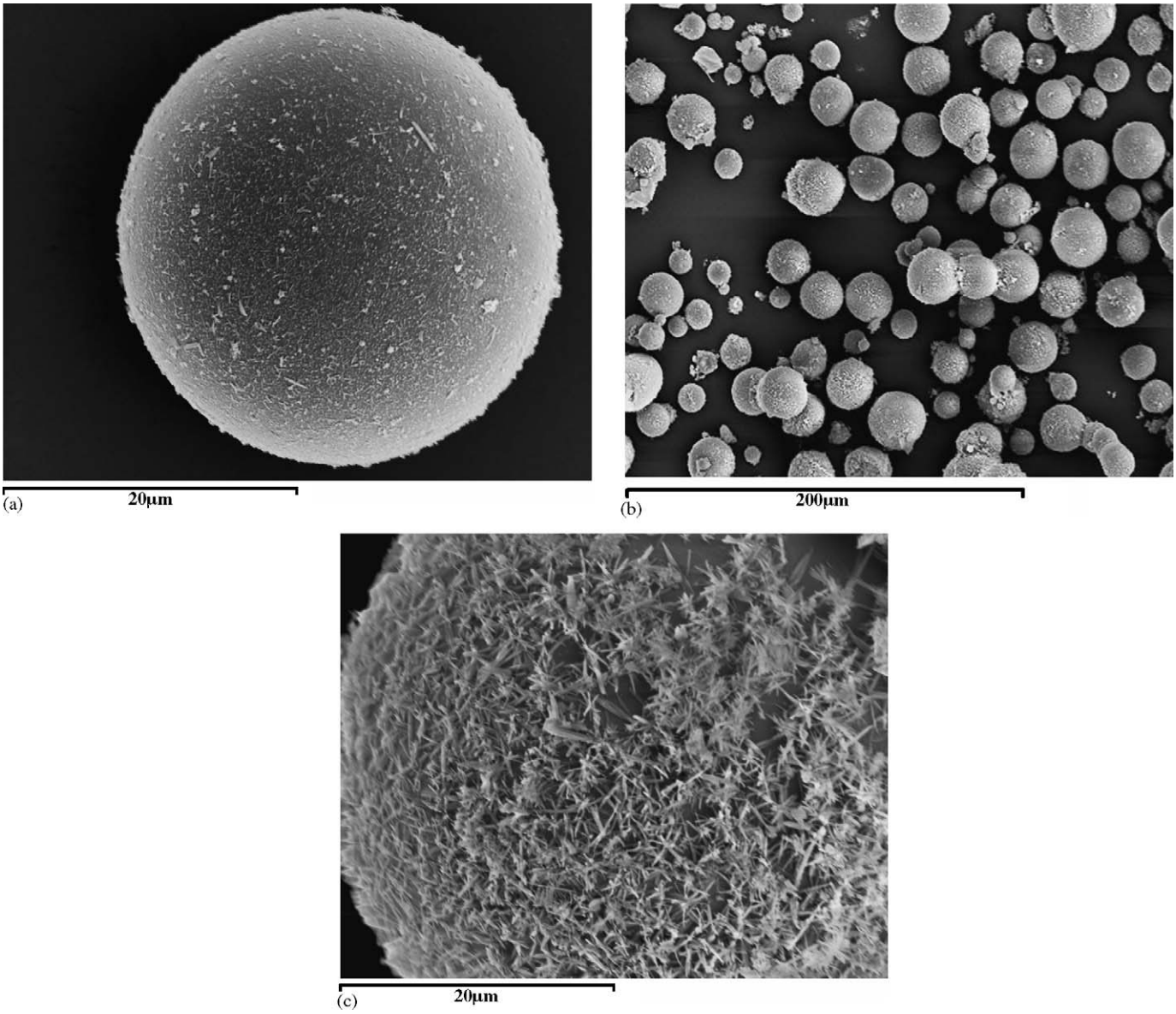


Fig. 1. SEM micrographs for AM_1 (a), BM_1 (b) and BM_1 outside surface (c).

unloaded (curves B) and drugs-loaded AM_1 hydrogels (curves C). It is evident that pure drugs are in the crystalline state; on the contrary, both the drugs unloaded and loaded microparticles are in the amorphous state. The obtained results demonstrate that during the polymerization/crosslinking reaction no crystalline region was formed and that the drug is molecularly entrapped inside the network. Analogous results have been found for other spherical microparticles.

Drugs release profile was determined by HPLC analysis. The drug release was expressed as the percent of drug delivered, related to the effectively entrapped total dose, as a function of time.

We have carried out the in vitro release studies at 37 °C and at pH 1 for 2 h, and then at pH 6.8 using the pH change method.

The experimental data showed an increase of DF release for all samples at pH 6.8. DF, containing acidic groups, is undissociated at pH 1.0 after 2 h and low amounts (w/w < 10%) are released. When the pH is 6.8, the swelling of the network increases and the DF ionized form prevails; both these factors cause a significant increase in the amount of released drug (w/w > 75%), (Fig. 5(a and b)).

The influence of hydrogel crosslinking degree on drug release is very important. Poorly crosslinked materials (AM_1 and BM_1) showed high DF release (w/w > 90% after 24 h) as a result of poor diffusional limitations on drug transport. The diffusional limitations become significant (w/w ≈ 75% after 24 h) increasing the amount of crosslinking agent in the polymeric structures (AM_2 and BM_2).

On the contrary, for PR a different behaviour was observed. In particular, drug release was significant already at acidic pH values (w/w > 60%) and complete after 1 h at pH 6.8 for all polymeric samples (Fig. 6(a and b)). In this case diffusional limitations do not have effect on the release profile.

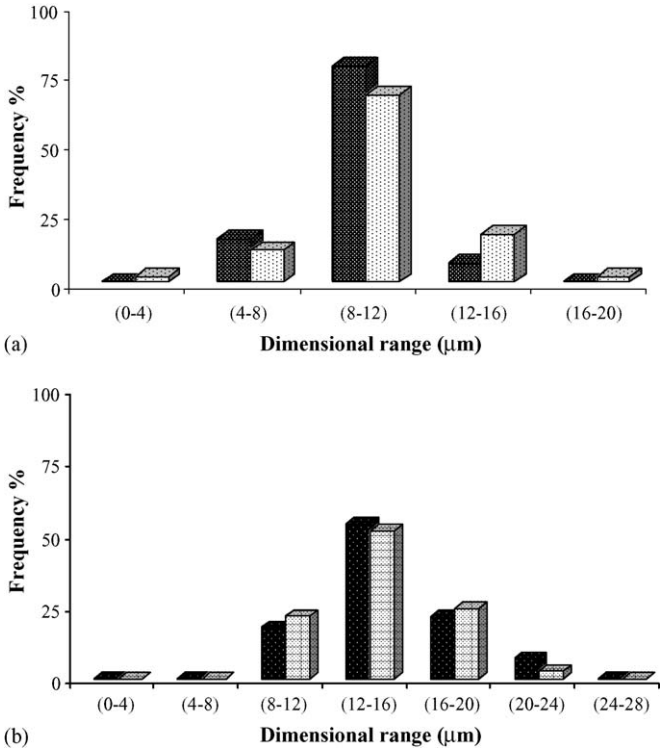


Fig. 2. Size distribution profiles for AM_1 (▩) and AM_2 (▨) (a); BM_1 (▩) and BM_2 (▨) (b).

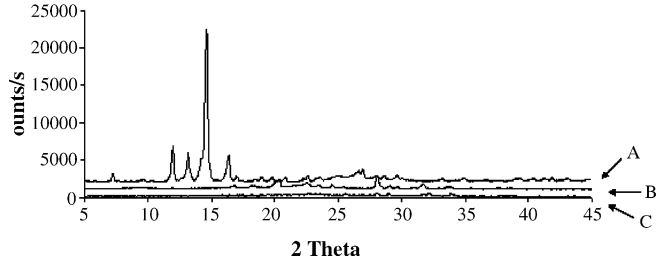


Fig. 3. X-ray diffraction patterns of pure drug diflunisal (A), diflunisal-unloaded AM_1 microspheres (B) and diflunisal-loaded microspheres samples (C). Analogous results have been found for all materials.

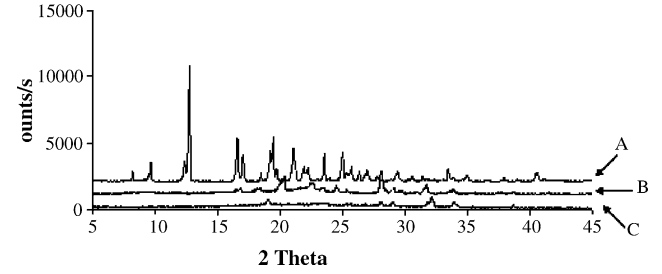


Fig. 4. X-ray diffraction patterns of pure β-propranolol (A), drug unloaded AM_1 microspheres (B) and β-propranolol-loaded microspheres samples (C). Analogous results have been found for all materials.

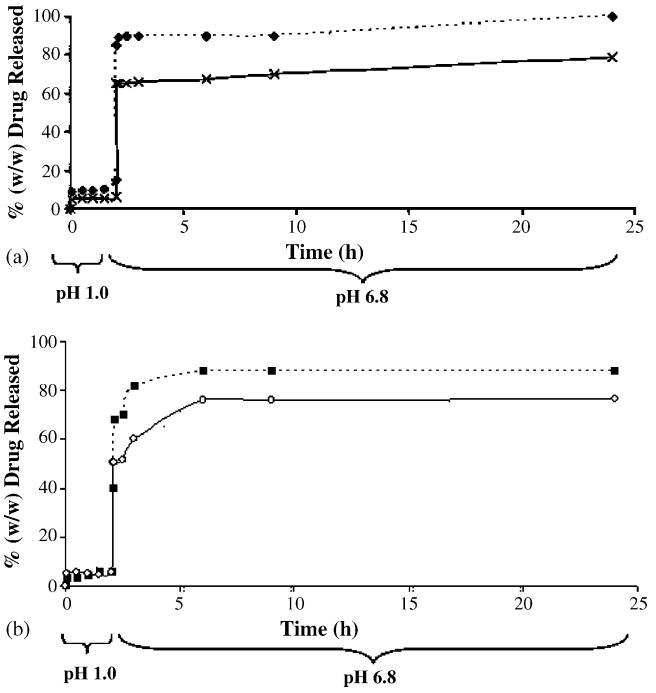


Fig. 5. Drug release expressed as the percent of diflunisal delivered as a function of time for beads AM_1(♦) and AM_2(×) (a), BM_1(■) and BM_2(◯) (b).

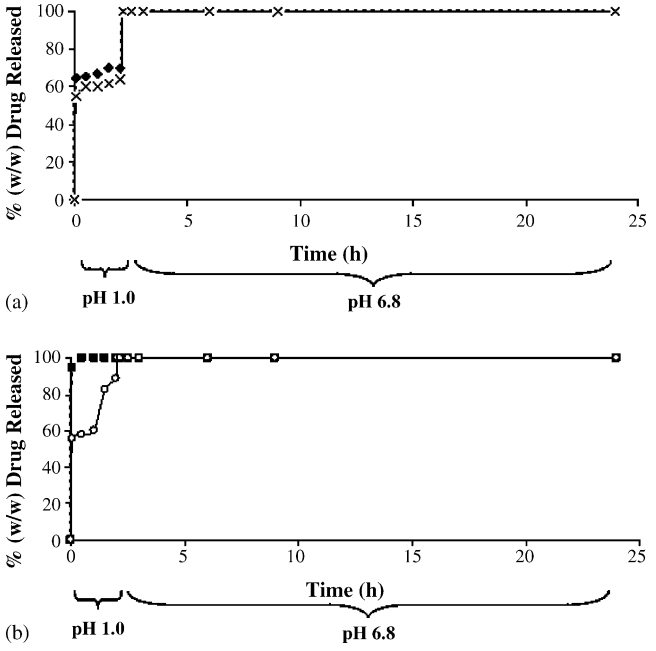


Fig. 6. Drug release expressed as the percent of β-propranolol delivered as a function of time for beads AM_1(♦) and AM_2(×) (a), BM_1(■) and BM_2(◯) (b).

4. Conclusion

BSA was successfully derivatized by reaction with MA, under mild conditions, in order to obtain a protein which contains chemical groups able to undergo radical polymerisation. Using different amounts of MA, several materials were prepared and the derivatization degree was spectrophotometrically determined. The beads obtained, by radical copolymerization with

NaM showed a narrow size distribution profiles, spherical shape and a porous surfaces. The elevated water affinity and the high degree of swelling at pH 6.8 suggests that these materials can be used such as oral drug carriers.

In order to test preformed microspheres as oral drug carriers DF and PR, such as model drug, were chosen and drug entrapment percentual was determined. The drug release profiles, in media which simulate gastrointestinal fluids, depend on hydrogels crosslinking degree and the interaction loaded drug-beads. Poor PR-beads affinity produced fast drug release in about 3 h. In the case of DF was recorded a pH-dependent release almost complete in 24 h. These results suggest the potential use of the prepared hydrogels for intestinal release of acidic drugs.

Acknowledgments

This work was financially supported by MIUR (Programma di ricerca di rilevante interesse nazionale 2005) and the University funds.

References

Alvarez-Lorenzo, C., Concheiro, A., 2002. Reversible adsorption by a pH- and temperature-sensitive acrylic hydrogel. J. Control Release 80, 247–257.

Arnedo, A., Espuelas, S., Hirache, J.M., 2002. Albumin nanoparticles as carriers for a phosphodiester oligonucleotide. Int. J. Pharm. 244, 59–72.

Brannon-Peppas, L., Peppas, N.A., 1990. Dynamic and equilibrium swelling behaviour of pH-sensitive hydrogels containing 2-transhydroxyethyl methacrylate. Biomaterials 11, 635–644.

Byrne, M.E., Park, K., Peppas, N.A., 2002. Molecular imprinting within hydrogels. Adv. Drug Deliv. Rev. 54, 149–161.

Chiu, H.C., Wu, A.T., Lin, Y.F., 2001. Synthesis and characterization of acrylic acid-containing dextran hydrogels. Polymer 42, 1471–1479.

Chuo, W.H., Tsai, T.R., Hsu, S.H., Cham, T.M., 1996. Preparation and in-vitro evaluation of nifedipine loaded albumin microspheres cross-linked by different glutaraldehyde concentrations. Int. J. Pharm. 144, 241–245.

Freiberg, S., Zhu, X.X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.

Ghassabian, S., Ehtezazi, T., Forutan, S.M., Martazavi, S.A., 1996. Dexamethasone-loaded magnetic albumin microspheres: preparation and in vitro release. Int. J. Pharm. 130, 49–55.

Gil, E.S., Hudson, S.M., 2004. Stimuli-responsive polymers and their bioconjugates. Prog. Polym. Sci. 29, 1173–1222.

Griffith, L.G., 2000. Polymeric biomaterials. Acta Mater. 48, 263–277.

Gupta, P., Vermani, K., Garg, S., 2002. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Deliv. Tod. 7, 569–579.

Guyton, A.C., Hall, J.E., 1998. Secretory functions of the alimentary tract. In: Guyton, A.C., Hall, J.E. (Eds.), Textbook of Medical Physiology. W.B. Saunders Co., pp. 815–832.

Hoffman, A.S., 2002. Hydrogels for biomedical applications. Adv. Drug Deliv. Rev. 43, 3–12.

Iemma, F., Spizzirri, U.G., Muzzalupo, R., Puoci, F., Trombino, S., Picci, N., 2004. Spherical hydrophilic microparticles by radical copolymerization of functionalized bovine serum albumin. Colloid Polym. Sci. 283, 250–256.

Iemma, F., Spizzirri, U.G., Muzzalupo, R., Puoci, F., Trombino, S., Picci, N., 2005. Radical cross-linked albumin microspheres as potential drug delivery systems: preparation and in vitro studies. Drug Deliv. 12, 179–184.

Kao, W.J., Burmania, J., Li, J., Einerson, N., Witte, R., Stevens, S., Nelson, D., Martinez-Diaz, G., 2003. Multifunctional protein-based matrix for drug delivery and wound management. Mater. Sci. Forum 426, 3145–3150.

Kashyap, N., Kumar, N., Ravi Kumar, E.M.N.V., 2005. Hydrogel for pharmaceutical and biomedical applications. Crit. Rev. Ther. Carrier Syst. 22, 107–149.

Khare, A.R., Peppas, N.A., 1993. Release behavior of bioactive agents from pH-sensitive hydrogels. J. Biomater. Sci. Polym. 4, 275–289.

Kost, J., Langer, R., 2001. Responsive polymeric delivery systems. Adv. Drug Deliv. Rev. 46, 125–148.

Langer, K., Balthasar, S., Vogel, V., Dinaner, N., von Briesen, H., Schubert, D., 2003. Optimization of the preparation process for human serum albumin (HSA) nanoparticles. Int. J. Pharm. 257, 169–180.

Leonard, M., Rastello De Boisseson, M., Hubert, P., Dalençon, F., Dellacherie, E., 2004. Hydrophobically modified alginate hydrogels as protein carriers with specific controlled release properties. J. Control Release 98, 395–405.

Miyata, T., Uragami, T., Nakamae, K., 2002. Biomolecule-sensitive hydrogels. Adv. Drug Deliv. Rev. 54, 79–98.

Morishita, M., Goto, T., Peppas, N.A., Joseph, J.I., Torjman, M.C., Munsick, C., Nakamura, K., Yamagata, T., Takayama, K., Lowman, A.M., 2004. Mucosal insulin delivery systems based on complexation polymer hydrogels: effect of particle size on insulin enteral absorption. J. Control Release 97, 115–124.

Murdan, S., 2003. Electro-responsive drug delivery from hydrogels. J. Control Release 92, 1–17.

Muzzalupo, R., Iemma, F., Picci, N., Pitarresi, G., Cavallaio, G., Giammona, G., 2001. Novel water-swellable beads based on an acryloylated polyaspartamide. Colloid Polym. Sci. 279, 688–695.

Omidian, H., Rocca, J.G., Park, K., 2005. Advances in superporous hydrogels. J. Control Release 102, 3–12.

Park, H.Y., Song, I.H., Kim, J.H., Kim, W.S., 1998. Preparation of thermally denatured albumin gel and its pH-sensitive swelling. Int. J. Pharm. 175, 231–236.

Park, K., 1988. Enzyme-digestible swelling hydrogels as platforms for long-term oral drug delivery: synthesis and characterization. Biomaterials 9, 435–442.

Patil, G.V., 2003. Biopolymer albumin for diagnosis and in drug delivery. Drug Dev. Res. 58, 219–247.

Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.

Pitarresi, G., Pierro, P., Giammona, G., Iemma, F., Muzzalupo, R., Picci, N., 2004. Drug release from α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide-based microparticles. Biomaterials 25, 4333–4343.

Qiu, Y., Park, K., 2001. Environment-sensitive hydrogels for drug delivery. Adv. Drug Deliv. Rev. 53, 321–339.

Sahin, S., Selek, H., Pronchel, G., Ercan, M.T., Sargon, M., Hincal, A.A., Kas, H.S., 2002. Preparation, characterization and in vivo distribution of terbutaline sulfate loaded albumin microspheres. J. Control Release 82, 345–358.

Sinha, V.R., Singla, A.K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., Dhawan, S., 2004. Chitosan microspheres as a potential carrier for drugs. Int. J. Pharm. 274, 1–33.

Snyder, S.L., Sobocinski, P.Z., 1975. An improved 2,4,6-trinitrobenzenesulfonic acid method for the determination of amines. Anal. Biochem. 64, 284–288.

Stubbe, B.G., De Smedt, S.C., Demeester, J., 2004. Programmed Polymeric Devices for Pulsed Drug Delivery. Pharm. Res. 21, 1732–1740.

Vasir, J.K., Tambwekar, K., Garg, S., 2003. Bioadhesive microspheres as a controlled drug delivery system. Int. J. Pharm. 255, 13–32.

Wunder, A., Müller-Ladner, U., Stelzer, E.H., Funk, J., Neumann, E., Stehle, G., Pap, T., Sinn, H., Gay, S., Fiehn, C., 2003. Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis. J. Immunol. 170, 4793–4801.