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ORIGINAL CONTRIBUTION

Synthesis and release profile analysis of thermo-sensitive albumin hydrogels

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Abstract Novel thermo-responsive hydrophilic microspheres were prepared by free radical polymerization of methacrylate bovine serum albumin and *N*-isopropylacrylamide, as cross-linker and functional monomer, respectively. The incorporation of monomers in the network was confirmed by infrared spectroscopy, while the network density and shape of hydrogels strictly depend on concentration of monomers in the polymerization feed. The thermal analyses showed negative thermo-responsive behavior with pronounced water affinity of microspheres at temperature lower than lower critical solution temperature (LCST). The in vitro release studies of drug-loaded thermo-sensitive hydrogels were performed. Experimental data showed, for the copolymers with functional monomer/cross-linker ratio≤ 1, a predominant drug release in the collapsed state, while the copolymers with functional monomer/cross-linker ratio> 1 showed prominent drug release in the swollen state. Below the hydrogel LCST, drug release through the swollen polymeric networks was observed, while a squeezing-out effect at temperature above the LCST was predominant.

Keywords Albumin microparticle · Controlled drug delivery · Thermo-responsive · Hydrogels

Introduction

Hydrogels consist of elastic networks that can uptake as much as 90–99% *w*/*w* of water in their interstitial space [1, 2]. In a physical point of view, hydrogels resemble living tissues because they have high water content and a soft and rubbery consistency. Such systems have been especially focused in the biomedical area as they provide adequate semi-wet three-dimensional environment for cells and tissue interaction, and they can be combined, through covalent links or physical entrapments, with biological or therapeutic molecules. They can be also chemically controlled and designed to tailor their mechanical and functional properties. Therefore, hydrogels have been proposed for a series of biomedical and biological applications, including tissue engineering, drug release systems, biological sensors, microarrays, imaging, and actuators. Some kinds of hydrogels can change their shape and volume reversibly following changes in external physical and chemical conditions such as temperature, solvent composition, ionic strength, pH, electric field, and light. Recently, stimuli-responsive polymer hydrogels have been attracting the attention of many researchers and are playing a part in a variety of fields, such as chemical engineering, medicine and pharmacy, life sciences, biotechnology, etc. [3–7]

Among the family of temperature responsive hydrogels, poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel is one of the most widely studied. It exhibits a lower critical solution temperature (LCST) or transition temperature at 30 °C in aqueous solution and shows an abrupt thermo-reversible change in volume as external temperature cycles around this critical temperature. PNIPAAm hydrogels are usually formed by the covalent cross-linking of PNIPAAm chains with a commercial cross-linking agent as *N*,*N*'-methylenebisacrylamide, to modify physicochemical properties of the materials (e.g., LCST, cross-linking degree, hydrophilic/hydrophobic balance, biodegradability, biocompatibility) [8–11]. The hydrogels based on PNIPAAm exhibit negative thermal response which means that below

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its LCST, PNIPAAm chains hydrate to form an expanded structure with a large mesh size enabling the water diffusion, while above its LCST, these chains dehydrate to form a shrunken structure with a small mesh size. The change in the hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intra- and intermolecular hydrogen bonding of the polymer molecules are favored compared to interaction with water molecules. Thermodynamics can explain this behavior with a balance between entropic effects due to the dissolution process itself and to the ordered state of water molecules around the polymer. Enthalpic effects depend on the balance between intra- and intermolecular forces and on the solvation, e.g., hydrogen bonding and hydrophobic interactions. The transition is then accompanied by coil-to-globule transition [8].

PNIPAAm hydrogels are nonbiodegradable, which may restrict their applications as biomaterials. The importance of the biodegradability for a biomaterial is self-evident due to the absence of a chronic foreign body reaction, which is usually accompanied with the permanent presence of the nonbiodegradable materials [12]. Furthermore, the devices obtained from biodegradable materials do not require additional surgery for their removal. So, there is a need to develop nontoxic, biodegradable hydrogels for the biomedical applications without losing their desirable properties, as temperature sensitivity. Hence, the bonding of the gel is designed to be labile, and it can be degraded by enzyme or chemisorptions in the physiological phenomenon. The most of the binding is degraded in the organism by the way of hydrolysis producing the little member, showing a low toxicity.

In view of these aspects, a promising strategy for designing novel hydrogel drug delivery systems is to combine the merits of both bioresponsive and biodegradable hydrogels. Recently, great attention has been paid especially for biomedical applications to the development of stimuli-responsive polymeric gels with unique properties such as biocompatibility, biodegradability, and biological functionality. They may be prepared by combining thermoresponsive polymers with natural based polymeric component, to form smart hydrogels. Polymeric systems composed of NIPAAm and acrylic acid were prepared by redox polymerization with peptide cross-linkers to create an enzymatically degradable matrix [13]. Peptide degradable cross-linkers were synthesized by the acrylation of the amine groups of lysine residues within peptide sequences potentially cleavable by matrix metalloproteinases. In addition, Yu et al. proposed the synthesis of an hydrogel based on P(NIPAAm-co-acrylic acid) and a polyaspartic acid derivatives (acryloyloxyethylamino polysuccinimide) as cross-linkers [14]. Thus, the covalent conjugation of a biodegradable macromolecule, as a protein (gelatin) or polysaccharides (chitosan, dextran, xyloglucan), to a thermo-responsive monomer represents a versatile strategy to produce intelligent biodegradable hydrogels, suitable for pharmaceutical and biomedical applications [15–18].

The albumin is a biodegradable protein, which is susceptible to enzymatic digestion in human body, and considerable interest has been shown in the use of protein microspheres as starting materials for active drug targeting, as well as for producing a sustained and controlled rate of drug release, and microspheres can be used in the treatment of many diseases that require controlled release of the drug into plasma, cells, or organs. The albumin microspheres are suitable for drug delivery, since they are biodegradable, biocompatible, and relatively easy to prepare over a wide range of particle sizes. By virtue of their ability to interact with a wide variety of drugs and their simple and low cost preparation, albumin microspheres represent very interesting materials for therapeutic applications [19]. A considerable number of strategies have been developed to obtain albumin microspheres; they can be achieved by thermal denaturation either by direct reaction between functional groups (usually carboxyl and amino goups) in the polypeptide side chains and also by chemically cross-linking agents as bifunctional carbonyl reagents [20]. By these techniques, hydrophobic materials, able to release the drug through an erosion mechanism, were prepared. In our previous works, the synthesis of bovine serum albumin (BSA) microspheres by radical copolymerization of methacrylate BSA (BSA-MA) with *N*,*N*-dimethylacrylamide [21] or methacrylic acid sodium salt [22] was reported. This polymerization technique allows obtaining albumin microparticles with a narrow size distribution, a spherical shape, and a high water affinity. Furthermore, this proteic hydrogels were tested as carriers for water-soluble drugs in media simulating biological fluids.

In this paper, a novel class of biodegradable hydrogels with proteic structure and thermo-responsive behavior was synthesized. In order to prepare thermo-sensitive microspheres, the present work describes the synthesis of materials by reverse phase suspension copolymerization of *N*-isopropylacrylamide (NIPAAm) and BSA-MA, as functional monomer and proteic cross-linker, respectively. This research proposes a new synthetic approach by radical polymerization of functionalized BSA and thermo-responsive monomer because this synthetic procedure permits to modify polymeric network composition producing hydrogels with appropriate and mouldable physico-chemical properties.

The beads were characterized by scanning electronic microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), particle size distribution, calorimetric and swelling analyses. The FT-IR spectra confirms the insertion of

functional monomers and proteic cross-linker in the polymeric network. The functional monomer/cross-linker ratio in the polymerization feed strictly influences morphological and chemical properties of hydrogels, and all samples showed high water affinity and a significant volume change in response to temperature variation across their LCST values.

Finally, to evaluate thermo-responsive microparticles as smart drug carrier, the hydrogels into a solution of caffeine (CF) and theophylline (TH) were soaked. Media release temperature, around material LCST values, hydrogel cross-linking degree, and chemical structure of entrapped drug strictly influence release profiles. Depending on ratio between functionalized protein and NIPAAm in the hydrogels, different release mechanisms were hypothesized.

Materials and methods

Reagents and standards

All the reagents were of analytical-reagent grade, and used without further purification unless otherwise stated. *n*-Hexane and chloroform, 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), 2,4,6-trinitrobenzensulphonic acid (TNBS), *N*-isopropylacrylamide (NIPAAm), sorbitan trioleate (Span 85), polyoxyethylene sorbitan trioleate (Tween 85), *N*,*N*,*N*',*N*'-tetramethylethylendiamine (TMEDA), ammonium persulfate, CF, TH were provided from Sigma-Aldrich (Sigma Chemical, St. Louis, MO, USA). Acetonitrile and water were from Carlo Erba Reagents (Milan, Italy), and all of high-performance liquid chromatography (HPLC) grade. 2-Propanol, ethanol, acetone, and diethyl ether were from Carlo Erba Reagents (Milan, Italy) and all were of analytical grade.

Derivatization of BSA

Functionalized BSA (BSA-MA), according to a procedure elsewhere reported, was prepared [19]. 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 a freezing-drier apparatus to afford a vaporous solid. The derivatization degree (DD%) of BSA-MA in agreement with a procedure reported in literature was determined [23].

Microspheres preparation (standard procedure)

Microspheres based on BSA-MA and NIPAAm were produced by radical copolymerization technique. 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 a solution of BSA-MA, comonomer (NIPAAm) and ammonium persulfate in water 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 1,000 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 1 reports the experimental conditions of each polymerization reactions. The microparticles were filtered, washed with 50 ml portions of 2-propanol, ethanol, acetone, and diethyl ether and dried overnight under vacuum at 40 °C.

Water content of microspheres

The swelling characteristics were determined in order to check hydrophilic affinity of 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 (PBS solution, pH=6.8, at 25 °C and 40 °C). 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 3,500 rpm for 15 min and weighted. This operation was repeated at 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 microparticles, respectively. Each experiment was carried out in quintuplicate, and the results were in agreement within ±4% SE. The WR (%) for all prepared materials is reported on Table 1.

Thermo-behavior of BSA-MA/NIPAAm hydrogels

The LCST property of the hydrogel samples was determined by using a DSC. The LCST value for all polymers are reported in Table 1. In a standard procedure, the sample was

Table 1 Copolymerization of BSA-MA with NIPAAm

Dispersed aqueous phase		Continuous organic phase	Hydrogel				WR (%)	
BSA-MA (mg)	NIPAAm (mg)	CCl_4/*n*-hexane (ml/ml)	mg (conv.%)	Code	LCST (°C)	Shape	25 °C 40 °C	S_r
200	400	15/24	474 (79.0)	I	30.6	ISM	408 225	1.8
250	350	16/23	429 (71.4)	II	30.0	SM	367 220	1.6
300	300	17/23	411 (68.5)	III	31.1	SM	325 210	1.6
350	250	17/23	478 (79.6)	IV	32.0	SM	315 200	1.4
400	200	16/24	475 (79.2)	V	32.2	ISM	266 193	1.3

For all polymerizations, the amount of aqueous phase is 2.5 ml; initiator system is $(NH_4)_2S_2O_8$/TMEDA (100 mg/150 µl); surfactants are Span 85/Tween 85 (140 µl/30 µl)

SM spherical micropartcles, *ISM* irregular and spherical microparticles, *WR (%)* water content percent, S_r ratio between the swelling at 25 °C and 40 °C

immersed in distilled water at room temperature for at least 2 days to reach a swollen state. About 10 mg swollen sample was placed inside a hermetic aluminum pan and then sealed tightly by a hermetic aluminum lid. The thermal analyses were performed from 25 °C to 55 °C on the swollen hydrogel samples under a dry nitrogen atmosphere with a flow rate of 25 ml min^{-1} and heating rate 3 °C min^{-1}.

Incorporation of drug into preformed microspheres

Incorporation of drugs (caffeine and theophilline) into microspheres was performed as follows: 200 mg of preformed empty microspheres (prepared as described above) 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 the presence of P_2O_5 to constant weight. The loading efficiency percent (LE %) of all samples is determined by HPLC analysis of filtered solvent according to Eq. 2:

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

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 calculated LE (%) of different copolymers is listed in Table 2.

In vitro drug release at 25 °C and 40 °C from microparticles

In vitro drug release profiles were obtained by HPLC. Aliquots (10 mg) of drug-loaded microparticles were dispersed in flasks containing PBS solution (pH 6.8) and maintained at 25.0±0.1 and 40.0±0.1 °C in a water bath., The samples, at suitable time intervals, were filtered, and the solutions were analyzed by HPLC. Each experiment was carried out in quintuplicate, and the results were in agreement within ±5% SE. For caffeine and theophylline, HPLC conditions were acetonitrile/water 6:4 (*v*/*v*); 0.5 ml/min flow; UV detection at 280 nm.

Instruments

The dialysis membranes of 6–27/32 in. Medicell International LTD (MWCO, 12–14,000) were employed. The freeze drier Micro Modulyo, Edwards was utilized. The ultraviolet spectra with a U-2000 Hitachi spectrophotometer using 1-cm quartz cells were recorded. The FT-IR spectra were recorded as pellets in KBr in the range 4,000–400 cm^{-1} using a Jasco FT-IR 4200 spectrophotometer (resolution 1 cm^{-1}). The particle size distribution was carried out using

Table 2 Microparticles loading efficiency percent (LE %) of CF and TH after 72 h at room temperature

Hydrogel	LE (%)	
	CF	TH
I	71±3	46±2
II	76±1	42±4
III	66±4	53±2
IV	67±2	51±5
V	69±1	73±3

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. The 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. The X-ray diffraction analyses were 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. Calorimetric analyses were performed using a Netzsch DSC200 PC. The 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.

Results and discussion

Chemical groups susceptible to radical polymerization were introduced onto BSA by acylation with methacrylic anhydride (MA) in water at 0 °C and neutral pH. Under mild reaction conditions, only sterically accessible amino groups in the side chain of lysine react with acylation agent [20]. The nucleophilic chemical groups in BSA that could react with MA are the thyolic groups of cysteine, hydroxilic groups of serine and tyrosine, and amino groups in the side chain of lysine residues. The former groups are involved in disulfide bridges, except cys-34; the latter are the least nucleophilic, and do not react in the mild experimental conditions. The amino groups of lysine, sterically accessible, react chiefly with acylant agent at controlled pH and temperature to produce water-soluble BSA-MA. If the reaction is carried out without pH and temperature control, denaturation of BSA was observed, and its water solubility is lost.

In this work, BSA-MA with all the available amino groups acylated was prepared (DD%=100%). The derivatization degree was determined by a spectrophotometric method, using 2,4,6-trinitrobenzensulphonic acid (TNBS) as the chromophore group. In this procedure, TNBS was employed as a reagent for measuring the free amino groups of BSA. The amino content is related to the increase in absorbance at 420 nm that is ascribable to the trinitrophenylsulfonic group bounded to BSA-MA, after a relatively short incubation period.

Thermo-responsive microspheres with proteic structure were synthesized by reverse phase suspension copolymerization of BSA-MA with a monomer showing a thermal behavior, such as NIPAAm.

Varying cross-linker/comonomer ratio, hydrogels with different cross-linking degree were prepared (Table 1). The polymerization reaction, owing to steric and geometric constraints, involves only the methacrylic functions of BSA-MA which are accessible to the growing chains. The obtained microparticle structure is characterized by a network where the BSA chains are linked by hydrocarbon bridges. It can be presumed that in the copolymerization reaction, the chains obtained consist of NIPAAm units randomly interrupted by methacrylic BSA-MA functions which are sterically and geometrically attainable (Fig. 1).

The reaction was started using TMEDA and ammonium persulfate as initiator system. Optimization of the polymerization method required several attempts. It was observed that hydrophilic/lipophilic balance (HLB) of surfactants is very important. Many tests were carried out to determine the correct ratio of Span 85 (HLB=1.8) and Tween 85 (HLB=11). Finally, a system with a total HLB=3.4 was able to stabilize aqueous dispersed phase. The polymerizations produced materials with different shapes; in particular, the ratio (*w*/*w*) between BSA-MA and NIPAAm is significant for the preparation of spherical microparticles (Table 1). When BSA-MA/NIPAAm ratio in the copolymerization feed was in the range 0.7–1.4, spherical shape was observed (copolymers II, III, and IV). A mixture of

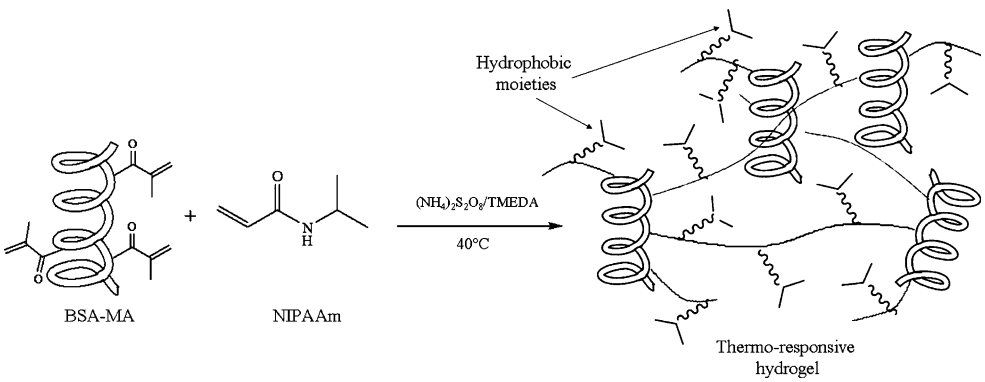

Fig. 1 Synthesis of thermo-responsive hydrogels poly (BSA-MA-*co*-NIPAAm)

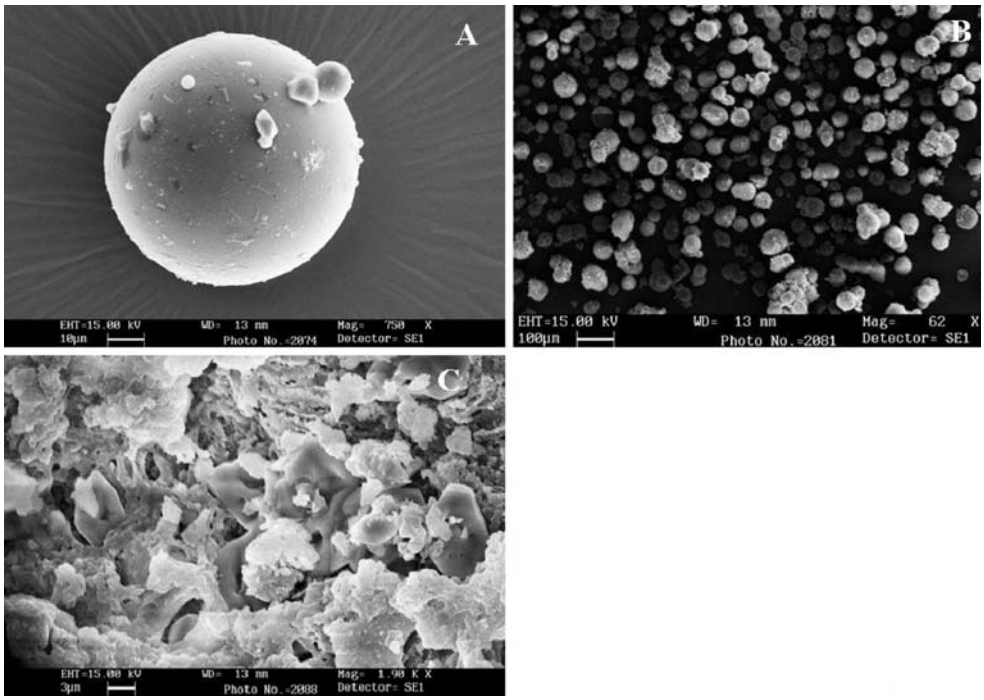

Fig. 2 SEM micrographs for II (**a** and **b**) and II outside surface (**c**)

irregular and spherical particles was recorded outside this range (copolymers I and V).

The materials were characterized by FT-IR spectrophotometry, swelling behavior, particle size distribution, morphological and calorimetric analyses. The FT-IR spectra of all samples show the disappearance of bands at 1,307 and 934 cm^{-1} awardable to BSA-MA methacrylic groups and at 944 and 921 cm^{-1} ascribable to C–C double bond of NIPAAm. Furthermore, an absorption band at 617 cm^{-1} (a typical band of BSA-MA homopolymer) in all samples was observed. Investigation of the applicability of these hydrogels in controlled release was done by studying their swelling behavior. The value of contained water percentage was determined in aqueous media (PBS solution pH=6.8; 0.01 M) at 25 °C and 40 °C respectively. The data, reported in Table 1, illustrate the water uptake at different temperatures, in grams per gram of dry copolymer, for each studied composition, and the ratio between the swelling at 25 °C and 40 °C (S_r) was reported for all samples. The prepared materials showed different water affinity at 25 °C and 40 °C due to pendant hydrophobic groups in the polymeric chains. In particular, at 40 °C, there is a considerable lowering of the water content, due to solvent diffusion outside the polymeric network and to resultant hydrophobic interactions between hydrocarbon moiety on polymeric chains. When the temperature rises to 25 °C, the water content is greater than that found at 40 °C for all copolymers. Due to greater NIPAAm amount in the polymeric network, sample I showed the highest S_r value. At both temperatures, the water uptake decreases from copolymer I to V, according to network cross-linking degree and in contrast with the copolymer hydrophilicity. This finding can be explained assuming that the effect due to increased cross-linking

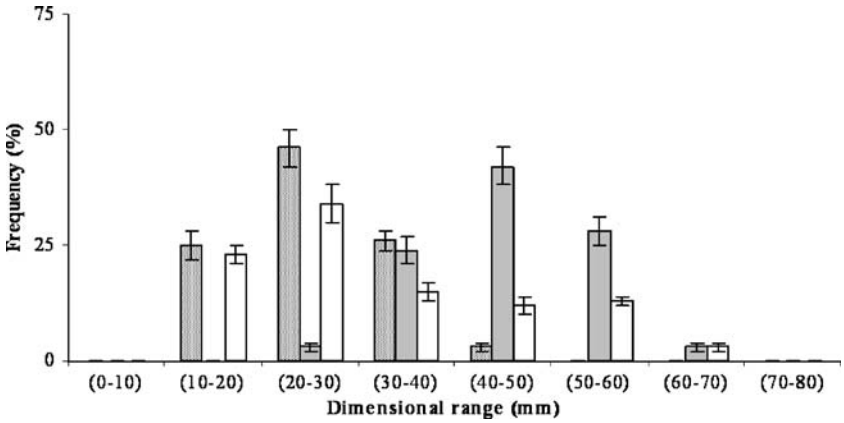

Fig. 3 Size distribution profiles for II (*filled bars*), III (*dotted bars*) and IV (*empty bars*)

degree is predominant with respect to increased hydrophilic moieties in the network.

Using scanning electron microscopy, the surface properties of the microparticles were evaluated, and spherical shape of the microparticles was confirmed. In Fig. 2 (a and b), the spherical shape of sample II is evident, while Fig. 2 (c) shows the high porosity of the outside surface of the microparticles. Similar results were obtained for all the spherical samples. In our experiments, the particle diameter was in the dimensional range 20–30 μm for III and IV and 40–50 μm for II, and a narrow distributional profile for the samples II and III was recorded (Fig. 3) The microparticle diameters were strictly depending on the cross-linker amount in the polymeric networks; the values of mean particle diameter, in general, decrease as the cross-link density increases.

Thermal analyses were performed from 25 °C to 55 °C on the swollen hydrogel samples, and the range of LCST values was 30–32.2 °C (Table 1). The LCST values were strictly dependent on functional monomer/cross-linker ratio in the polymerization feed. The data indicate that all copolymers had a higher LCST than the pure PNIPAAm hydrogel, a polymer exhibiting LCST value equal to 30 °C [24]. The increase in the LCST recorded in BSA-MA-co-NIPAAm copolymers can be attributed to the increased hydrophilic content with respect to hydrophobic moiety, from I to V (Fig. 4). Thermo-responsive behavior of PNIPAAm hydrogel is strongly influenced by polymer–water affinity. At a temperature below its LCST, the hydrophilic groups (amide groups) in the side chains of the PNIPAAm hydrogel bond the water molecules through hydrogen bonds. However, as the external temperature increases, the copolymer–water hydrogen bonds are broken. When the temperature raises the LCST, the water molecules, rigidly structured around the polymer chains, gain more freedom degrees, and they diffuse in their bulk phase. As a result, hydrogen bonds between solvent molecules in the continuous phase are formed; while inside the polymeric network, hydrophobic interactions among the isopropyl groups become dominant [9].

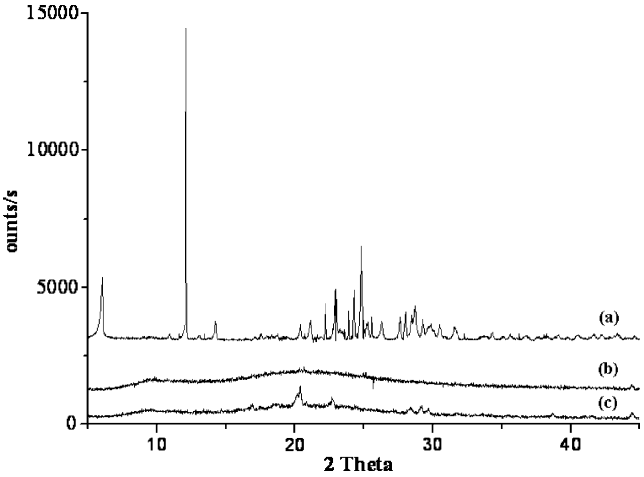

Fig. 5 X-ray diffraction patterns of pure TH (*a*), TH-unloaded III microspheres (*b*), and TH-loaded microspheres samples (*c*). Analogous results have been found for all materials

In order to estimate the ability of the matrices to release drug molecules, the beads were loaded with various drugs by soaking procedure. Theophylline and caffeine were chosen as model drugs, and the loading efficiency of all samples (LE %) was determined by HPLC analysis as reported in the experimental part (Table 2). The CF was loaded on the polymeric beads with a LE (%) >65% for all hydrogels, whereas TH was poorly uptaken on the beads [LE (%) approximate to 50% for all macromolecular systems except for V, where LE %=73%]. Different drug–polymer hydrophobic interactions explain these results, the interaction of

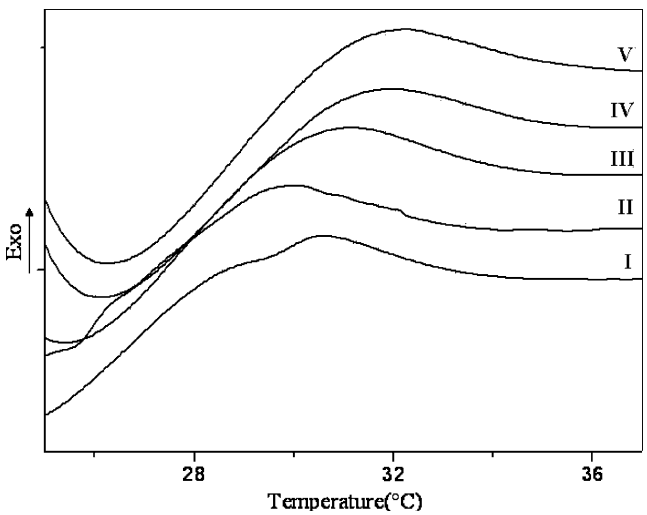

Fig. 4 DSC thermograms at a heating rate of 3 °C of BSA-NIPAAm hydrogels

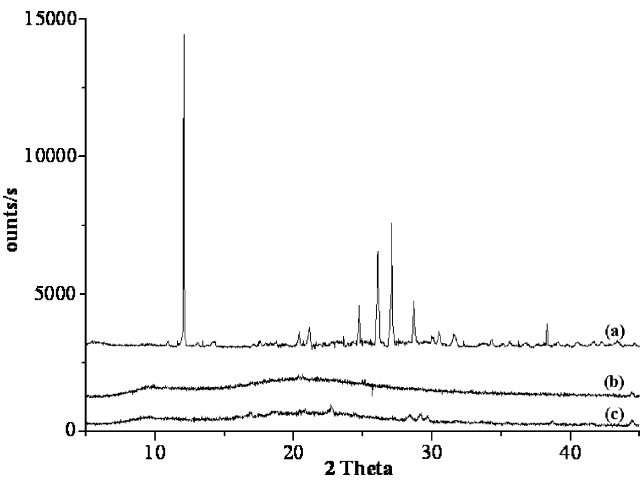

Fig. 6 X-ray diffraction patterns of pure CF (*a*), CF-unloaded III microspheres (*b*), and CF-loaded microspheres samples (*c*). Analogous results have been found for all materials

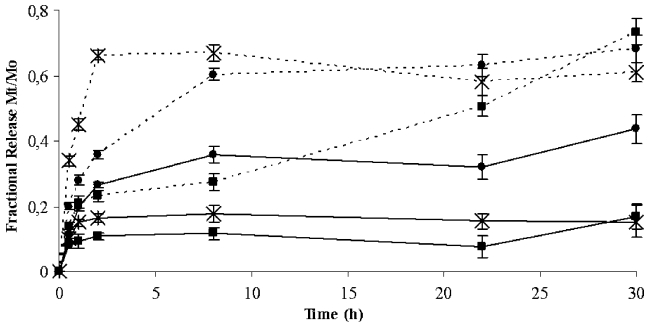

Fig. 7 Drug release expressed as theophylline delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads I (*filled squares*), II (*exes*), III (*filled circles*) at 25 °C (*solid lines*), and 40 °C (*dashed lines*)

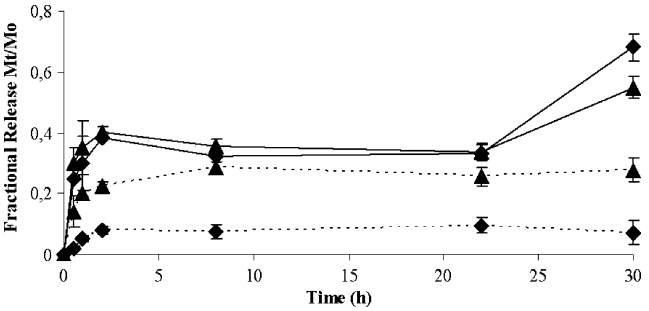

Fig. 9 Drug release was expressed as theophylline delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads IV (*filled diamonds*) and V (*filled triangles*) at 25 °C (*solid lines*) and 40 °C (*dashed lines*)

CF, indeed, is particularly strong with the more hydrophobic samples (copolymers I, II). On the contrary, these samples showed a weak interaction with the less hydrophobic drug, TH, while decreasing the hydrophobicity of the hydrogel (copolymer V), highest TH amount was uptaken. The determination of the drug dispersion state in all preformed hydrogels was performed by X-ray analysis. Analyzing X-ray diffraction patterns of pure drug, unloaded and drug-loaded hydrogels, it is evident that pure drugs are in the crystalline state; on the contrary, both the drug-unloaded and loaded microparticles are in the amorphous state (Figs. 5 and 6). The results demonstrate that during the polymerization/cross-linking reaction, no crystalline region was formed and that the drug is molecularly entrapped inside the network. Analogous results have been found for all samples.

Drug release profile was determined by HPLC analysis. The drug release was expressed as drug delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time at 25 °C and 40 °C. All synthesized copolymers showed a thermo-responsive behavior. Experimental data showed, for copolymers I, II, and III [functional monomer/cross-linker (*w*/*w*) ratio≤1], a predominant drug release in the collapsed state (Figs. 7 and 8), at a temperature above the LCST, while the copolymers IV and V [functional monomer/cross-linker (*w*/*w*) ratio>1] showed prominent drug release in the swollen state, below the LCST of the materials (Figs. 9 and 10).

Drug–polymer interactions, bead cross-linking degree, and temperature of release media determine release profiles. At 40 °C, squeezing-out effect controls the drug release. This effect is pronounced in the polymer I (M_t/M_0>0.70 after 30 h for TH), while it is irrelevant in V (M_t/M_0<0.10 after 30 h for TH), where only a poor burst effect was observed. According to S_r values in Table 1, copolymers I, II, and III, bearing more hydrophobic groups in the side chain and low-density cross-link points, underwent a drastic volume transition phase. On the contrary, squeezing-out effect is less evident increasing cross-linking degree (copolymers IV and V), due to the constraints imposed by the cross-links.

The data at 25 °C suggest a release mechanism due to cooperation of different factors for all materials in the swollen state. In particular, it is important to consider the diffusion of drugs through a complex polymeric network containing proteic moieties.

Drug–polymer interactions were found to be important on TH and CF release profiles. A relationship between the release profiles and the substituent on xanthine ring at 7 position can be invoked. Drug release is strictly associated

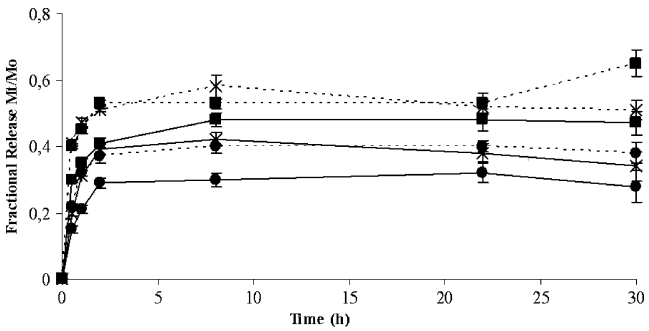

Fig. 8 Drug release expressed as Caffeine delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads I (*filled squares*), II (*exes*), III (*filled circle*) at 25 °C (*solid lines*) and 40 °C (*dashed lines*)

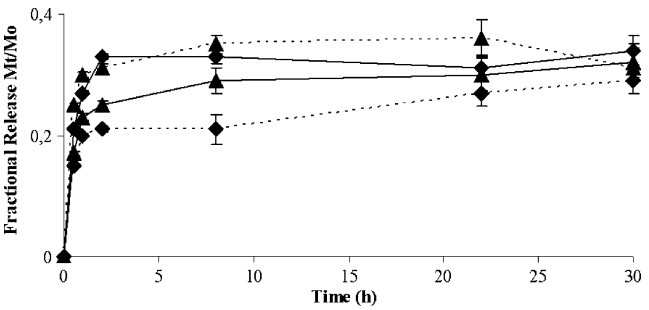

Fig. 10 Drug release expressed as caffeine delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads IV (*filled diamonds*) and V (*filled triangles*) at 25 °C (*solid lines*) and 40 °C (*dashed lines*)

to hydration state of the copolymers; at 25 °C, evident differences between TH and CF were observed, and more hydrophilic drug easily interacts with swelling media when density of cross-link points increased.

Conclusion

Thermo-responsive hydrogels were designed and synthesized by reverse suspension radical polymerization starting from BSA-derivative and temperature-sensitive monomer (NIPAAm). With respect to the thermoresponsive hydrogels reported in literature, the polymerization technique proposed in this paper allows to synthesize systems with spherical shape suitable to prevent anisotropic swelling of materials in the media release. The mild and controlled reaction conditions in the functionalization of BSA with methacrylic anhydride permit to preserve water solubility of native protein. Thus, derivatized BSA was covalently inserted in a polymeric network by water/oil emulsion polymerization, using a water-soluble radical initiator system.

Varying the feed composition, a novel class of temperature-responsive polymers with different physico-chemical properties was obtained. The insertion of a biomacromolecules in a cross-linked structure provides a biocompatible and biodegradable network, suitable as drug delivery systems. In this paper, extensive studies to achieve a spherical shape were performed, and the functional monomer/cross-linker ratio was found to greatly influence the geometric and morphological properties of microparticles. On the other hand, materials with different hydrophobic/hydrophilic balance were synthesized; these ones showed changed affinity to aqueous media depending on swelling temperature. Negative thermo-responsive behavior for all samples was observed.

In order to test the materials as drug carriers, TH and CF were loaded on the microspheres, and drug entrapment percent was determined. Depending on media temperature and loaded drug–bead interactions, drug release across the proteinous hydrogels takes place by modification of volume hydrogels.

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