Thermo-Responsive Albumin Hydrogels with LCST Near the Physiological Temperature

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ABSTRACT: This paper deals with the synthesis of thermoresponsive microspheres with proteic structure exhibiting a transition temperature close to the body temperature. The hydrogels were synthesized by free radical polymerization of methacrylate Bovine Serum Albumin (BSA-MA) as crosslinker, and 2-hydroxyethyl methacrylate (HEMA) and/or *N*-isopropylacrylamide (NIPAAm), as hydrophilic and thermoresponsive monomers, respectively. The modification of the hydrophilic/hydrophobic balance in the polymerization feed allows to modulate the volume phase transition temperature of the macromolecular network. The hydrogels were characterized by infrared spectroscopy and thermal analyses, which showed negative thermoresponsive behavior for all compositions and, by increasing the content of the hydrophilic moieties in the network, the transition temperature was ranged from 34.2 to 36.8°C. To test the preformed materials as drug carriers, diclofenac diethyl ammonium salt was chosen and drug entrapment percent was determined. Drug release profiles, in media at different temperature, depend on the crosslinking degree and on the composition of the hydrogels. By using semiempirical equations, the release mechanism was extensively studied and the diffusional contribute evaluated. The physic-chemical characteristics of thermoresponsive materials confirm the applicability of the microspheres as drug delivery device.
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Key words: hydrogel; drug delivery system; radical polymerization; stimuli-sensitive polymers

INTRODUCTION

Stimuli-sensitive hydrogels are a particular class of hydrogels which can modify their structural network according to variations in the local environment. In the last years, most of the research work in this area has concentrated on the fabrication of novel materials able to respond to different stimuli such as temperature, electric field, pH, glucose levels, or other chemical stimuli.[1–5] To date, one of the most exciting applications of these materials is the drug delivery, and in particular the targeted delivery of drugs at controlled rates.[6] Considering the temperature sensitive hydrogels, it should be noted that the swelling/collapse transition due to the temperature, alters the permeability of the gel network; therefore, these systems can be used to switch the drug release on and off.[6–11] Among the family of temperature sensitive hydrogels, poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel is one of the most widely studied.[12,13] It exhibits a lower critical solution temperature (LCST) at 30°C in aqueous solution, changing from a hydrophilic state below this temperature to a hydrophobic state above it, resulting in a shrinking of the hydrogel and in the release of the solvent (water) into the surrounding medium.[14]

Thermoresponsive behavior of the hydrogel can be explained as a balance of the enthalpyc and entropic contributes to the swelling-shrinking process. As the temperature increases above a critical point, the entropic gain is due to the release of the water molecules, originally associated with the isopropyl moieties in the side chains, into the bulk aqueous phase. To be more precisely, the LCST is associated with the region of the phase diagram at which the enthalphic contributions of the water hydrogen-bonded to the polymer chain become lower than the entropic gain of the system as a whole (water disassociated from the polymer) and thus it is largely dependent on the hydrogen-bonding capabilities of the constituent monomer units.

As a consequence, the LCST of a given polymer can be tuned as desired by varying the ratio between

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the hydrophilic and the hydrophobic comonomer(when using materials based on PNIPAM copolymers).[15] In this way, the LCST of a thermoresponsive hydrogel can be adjusted to become close to the body human temperature by the copolymerization or the interpenetration of a themo-sensitive monomer with other monomers.

In our previous works, the synthesis of microspheres based on functionalized Bovine Serum Albumin (BSA) by radical copolymerization of methacrylate BSA (BSA-MA) with *N*,*N*-dimethylacrylamide,[16] methacrylic acid sodium salt,[5] and *N*-isopropylacrylamide[17] was reported. This polymerization technique allows to obtain albumin microparticles with a narrow size distribution, a spherical shape and a high water affinity. In this paper, a novel class of thermoresponsive hydrogels based on derivative Bovine Serum Albumin (BSA-MA) acting as intelligent hydrogel owing a LCST close to the physiological temperature was synthesized. Furthermore, the influence of the hydrophilic/hydrophobic balance of the monomers in the polymerization feed on the transition temperature of the macromolecular device was investigated. The thermoresponsive microspheres were synthesized by reverse phase suspension copolymerization of 2-hydroxyethylmethacrylate (HEMA) and/or *N*-isopropylacrylamide (NIPAAm), as hydrophilic and thermoresponsive monomers respectively, in presence of aproteic crosslinker, such as BSA-MA. The proposed new synthetic approach allows to modify the polymeric network composition producing hydrogels with appropriate and modulable physicochemical properties. The beads were characterized by Scanning Electronic Microscopy (SEM), Fourier Transform infrared spectroscopy, calorimetric and swelling analyses. The FT-IR spectra confirm the insertion of both the functional monomers and the proteic crosslinker in the polymeric network. All samples showed high water affinity and a significant volume change in response to temperature variation across their LCST. Finally, to evaluate stimuli-responsive microparticles as smart drug carrier, Diclofenac diethyl ammonium (DDA) was loaded onto the synthesized hydrogels. The temperature of the releasing media, together with the hydrogel crosslinking degree and the chemical structure of the entrapped drug, strictly influence the release profiles and the release rates of the drug. To optimize the performance of the stimuli-sensitive systems, a sound understanding of the effects of hydrophilic and hydrophobic interactions of hydrogel on both the swelling/deswelling and the temperature-sensitive properties, as well as of the drug loading and release behaviors of different model drugs is essential. To estimate the diffusional contribute on the drug delivery, semiempirical equations were employed.[18,19]

EXPERIMENTAL

Materials

All the reagents were of analytical-reagent grade, and used without further purification unless otherwise stated. BSA fraction V (MW 68.000; pH 7.0 ± 0.2; grade ≥98%) was from Roche Diagnostics GmbH. *N*-isopropylacrilamide (NIPAAm), 2-hydroxyethylmethacrylate (HEMA), 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 and Diclofenac diethyl ammonium salt (DDA) were provided from Sigma-Aldrich (Sigma Chemical Co, St. Louis, MO). Acetonitrile and water were from Carlo Erba Reagents (Milano, Italy) and all of HPLC grade. *n*-hexane, chloroform 2-propanol, ethanol, acetone and diethyl ether were from Carlo Erba Reagents and all of analytical grade. *n*-hexane and chloroform were purified by standard procedures.

Instruments

Dialysis membranes of 6-27/32" Medicell International LTD (MWCO: 12-14000) were employed. Freeze drier Micro Modulyo, Edwards was utilized. Uultraviolet spectra were recorded with a U-2000 Hitachi spectrophotometer using 1 cm quartz cells. FT-IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Jasco FT-IR 4200 spectrophotometer (resolution 1 cm^{-1}). 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 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. 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, 5 μm of 250 mm × 4.6 mm internal diameter, obtained from Waters) was used.

Microspheres preparation (standard procedure)

Functionalized BSA (BSA-MA) was prepared according to a procedure reported elsewhere.[20] The derivatization degree (DD%) of BSA-MA was

TABLE I
Experimental Conditions for Stimuli Responsive Microspheres Synthesis

Aqueous dispersed phase			Organic continuous phase			Hydrogel	
NIPAAm mg/mmol	BSA-MA mg	HEMA mL/mmol	$CHCl_3$/n-hexane mL/mL	Surfactants mixture Span 85/Tween 85 μL/μL	Initator system $(NH_4)_2S_2O_8$/TMEDA mg/μL	mg (conv.%)	Code
350/3.10	250	–	16/23	140/30	100/150	429 (71.4)	I
300/2.65	300	–	16/23	140/30	100/150	411 (68.5)	II
250/2.20	350	–	16/23	140/30	100/150	478 (79.6)	III
175/1.50	250	175/1.34	20/23	100/50	120/160	310 (52.0)	IV
150/1.30	300	150/1.15	20/23	100/50	120/160	296 (49.0)	V
125/1.10	350	125/0.96	20/23	100/50	120/160	327 (54.0)	VI

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

determined in agreement with a procedure reported in literature.[21]

Microspheres based on BSA-MA and stimuli responsive monomers were produced by radical copolymerization technique.[22] Briefly, a mixture of *n*-hexane and chloroform was placed into a round-bottomed cylindrical glass reaction vessel fitted with an anchor-type stirrer and thermostated at 30°C, and then treated, after 30 min of N_2 bubbling, with a solution of BSA-MA, comonomers 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 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 the experimental conditions for 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.

Equilibrium swelling of microspheres

Swelling characteristics were determined to check the 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 10^{-3} mol L^{-1}, pH 7.0, at 25 and 40°C). At a predetermined time, the excess of 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 time intervals were averaged and used to give the water content percent (WR %) by the following eq. (1):

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

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

Thermobehavior of BSA-MA/Nipaam hydrogels

LCST of the hydrogel samples was determined by using a DSC and the obtained values are reported in

TABLE II
Thermal Analysis, Swelling Behaviors, and Drug-Loading Parameters of Hydrophilic Thermo-Sensitive Microspheres

	Calorimetric analysis	Swelling behavior			Drug-loading parameters	
Hydrogel	LCST (°C)	WR_{25}	WR_{40}	S_r	LE%	DL%
I	30.0	365 ± 4	222 ± 4	1.6	92.3 ± 0.2	7.94 ± 0.04
II	31.1	320 ± 3	212 ± 2	1.6	93.8 ± 0.2	8.08 ± 0.02
III	32.0	317 ± 3	200 ± 3	1.4	95.8 ± 0.3	8.66 ± 0.02
IV	36.8	393 ± 2	206 ± 4	1.9	91.6 ± 0.9	8.24 ± 0.03
V	36.2	370 ± 1	205 ± 2	1.8	97.1 ± 0.7	8.69 ± 0.02
VI	34.8	342 ± 3	190 ± 1	1.8	83.8 ± 0.3	7.53 ± 0.01

Table II. In a standard procedure the sample was immersed in distilled water at room temperature for at least two days to reach a swollen state. About 10 mg of 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 to 55°C on the swollen hydrogel samples under a dry nitrogen atmosphere with a flow rate of 25 mL min^{-1} and a heating rate of 3°C min^{-1}.

Incorporation of drug into preformed microspheres

Incorporation of DDA into 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^{-1}). After three days, under slow stirring at room temperature, 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 was determined by HPLC analysis of filtered solvent according to eq. (2):

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

Here, C_i was the concentration of drug in solution before the loading step, C_0 the concentration of drug in solution after the loading step, considering the change of the volume of solution due to the water uptake of the microparticles. The calculated LE (%) for all the different copolymers are listed on Table II. In addition, the drug loaded percent (DL%) in each matrix was calculated and the values were listed on Table II, according to eq. (3):

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

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

The DDA stability was studied at pH 7.0 and at different temperatures (25 and 40°C). Aliquots of drug (10 mg) were incubated at 25 and 40°C in phosphate buffer solution (PBS) 10^{-3} mol L^{-1} at pH 7.0. At scheduled time intervals, the samples were withdrawn and assayed by HPLC, to determine the drug concentration. HPLC conditions were acetonitrile/PBS (10^{-3} mol L^{-1}, pH 7.0) (70/30), 0.5 mL min^{-1} flow, UV detection at $\lambda = 284$ nm.[23]

In vitro drug release profiles were obtained by HPLC. Aliquots (10 mg) of drug-loaded microparticles were dispersed in flasks containing PBS solution (pH 7.0) and maintained at 25.0 ± 0.1 and 40.0 ± 0.1°C in a water bath. At Suitable time intervals, the samples were filtered and the solutions were analyzed by HPLC.

In vitro pulsatile drug release from 25 to 40°C

Oscillatory drug release profile of the semisynthetic gel beads was investigated by immersing the beads in a solution at pH 7.0 (0.001 mol L^{-1}, PBS), and alternating the temperature between 25 and 40°C at suitable time intervals. At each time, the samples were filtered and the solutions were analyzed by HPLC. The release period was extended over several cycles until no further drug was released. Two different experiments were performed: the first starting from 25°C and the second from 40°C. The larger temperature difference was used to help increase the speed of collapse, since DDA is a small molecule.

Statistical analysis

All of the experiments were done in triplicate. 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 and characterization of stimuli-responsive microspheres

Reverse phase suspension copolymerization was performed to synthesize thermoresponsive microspheres based on derivative Bovine Serum Albumin (BSA-MA) and a monomer showing a thermal behavior, such as NIPAAm (copolymer I, II, and III). In addition, the introduction in the polymerization feed of a hydrophilic monomer, 2-hydroxyethyl methacrylate (HEMA), allowed to modulate the transition temperature of the hydrogels and the resulting macromolecular systems (copolymer IV, V, and VI) are able to undergo abrupt volume changes at temperatures close to the body temperature. As reported in literature,[20] BSA was modified by the introduction of methacrylic functionalities susceptible to radical polymerization. Varying both the polymerization feed composition and the crosslinker/comonomer ratio, hydrogels with different chemical structure, and crosslinking degree were prepared (Table I). The polymerization reaction, owing to steric and geometric constraints, involves only the methacrylic functions of BSA-MA which are accessible to the growing chains. It can be presumed that, in the copolymerization reaction, the obtained chains consist of HEMA and/or NIPAAm units randomly interrupted by methacrylic BSA-MA functions which are sterically and geometrically attainable (Fig. 1). The reaction was initiated using TMEDA and ammonium persulfate as initiator system. Different attempts were performed to optimize the

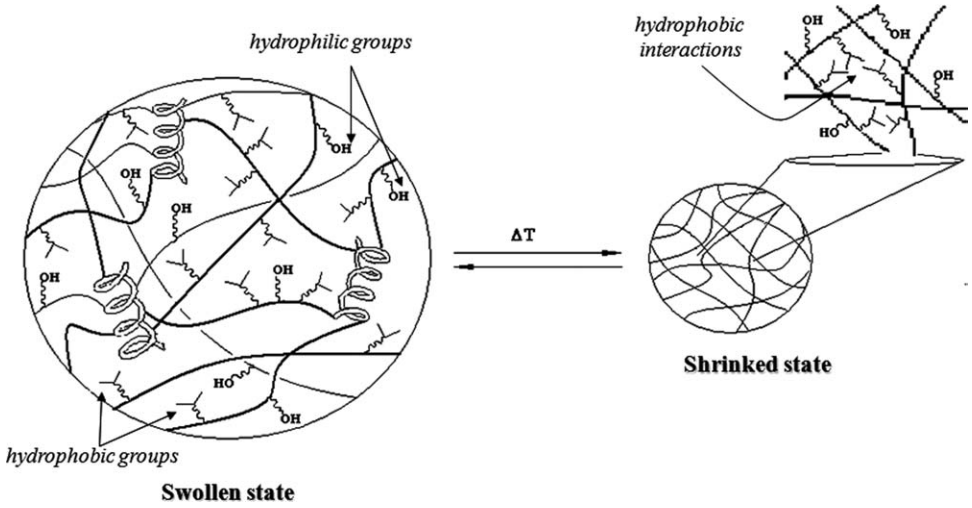

Figure 1 Schematic representation of thermoresponsive microspheres.

polymerization process, and in particular it was observed that the hydrophilic/lipophilic balance (HLB) of surfactants is very important. The correct ratio of Span 85 (HLB = 1.8) and Tween 85 (HLB = 11) was determined and a system with a total HLB = 3.4 was able to stabilize aqueous dispersed phase in the thermoresponsive hydrogels, while for the hydrophilic thermo- sensitive microspheres the optimum HLB value was 4.9.

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

The FT-IR spectra of all samples showed the disappearance of bands at 1307 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. Moreover, in the FT-IR spectra of the polymers IV, V, and VI the disappearance of the bands at 947 and 902 cm^{-1}, imputable to polymerizable functionalities of HEMA, was observed. The insertion of the monomers in the polymeric network was unambiguously confirmed by the presence of the bands at 3270 (hydroxyl and aminic groups), 2950 (hydrocarburic moieties), 1655 cm^{-1} (amidic and carboxylate groups).

Using scanning electron microscopy, information about the surface properties of the microparticles were obtained, and the spherical shape of microparticles confirmed. Figures 2(A,B), clearly show the spherical shape of Samples II and V, respectively. Figure 2(C) shows the outside surface of VI, characterized by a high degree of porosity. Similar results were obtained for all the spherical samples. The shape and the morphology of the prepared microparticles suggest their potential use as drug delivery systems. The spherical shape, indeed, allows to eliminate the anisotropic swelling normally associated with others geometries, while the presence of micropores could facilitate the drug diffusion through the polymeric network.

Thermal analyses were performed on the swollen samples from 25 to 55°C and the LCST values were collected on Table II. These values were strictly dependent on the hydrophobic/hydrophilic balance in the polymerization feed and on the chemical and structural properties of hydrophilic monomer/crosslinker. The data indicate that all the copolymers are characterized by a LCST value higher than the pure PNIPAAm hydrogel (30°C).[14] The increase in the LCST of crosslinked copolymers can be attributed to the increased hydrophilic/hydrophobic balance in the polymeric structure. Thermoresponsive behavior of PNIPAAm hydrogel is strongly influenced by polymer-water affinity; at temperature below its LCST, the hydrophilic groups (amide groups) in the side chains of the PNIPAAm hydrogel interact with the water molecules by hydrogen bonds. However, as the external temperature increases, the copolymer-water hydrogen bonds are broken and the water molecules, rigidly structured around the polymer chains, gain more freedom degrees and can rapidly diffuse across the 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. When hydrophilic groups are randomly inserted in the polymeric chains, polymer-water interactions significantly increase and more energy is required to destroy hydrogen bond, allowing solvent diffusion. The increase in the LCST recorded in BSA-MA-*co*-NIPAAm copolymers can be attributed to the increased hydrophilic content with respect to the hydrophobic moiety, from I to III (Fig. 3). The insertion in the polymeric network of HEMA determines, in all the hydrogels, a steep increase in the transition

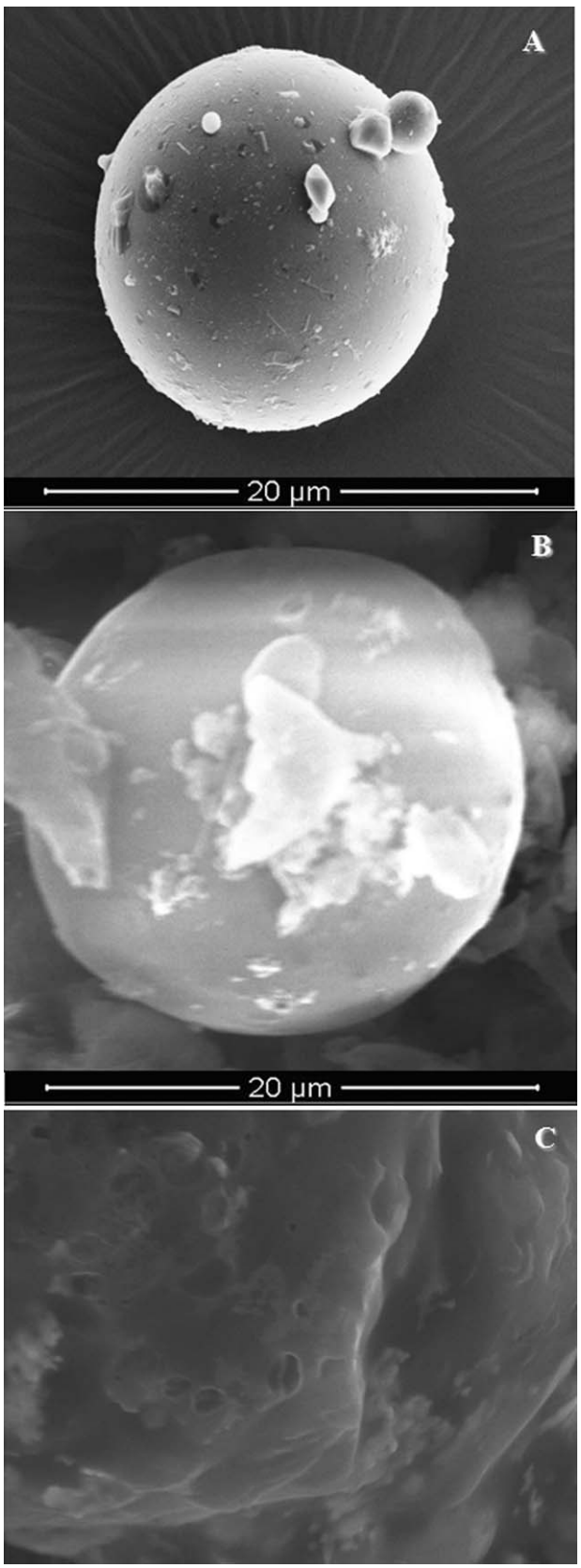

Figure 2 SEM micrographs of II (A), IV (B), and V outside surface (C).

temperature of the macromolecular system, strictly correlated to the HEMA amount in the polymerization feed. For IV, V, and VI the LCST values were recorded in the 36.8–34.8°C range close to the body temperature. These data confirmed that the hydrophilic contribution of BSA-MA and HEMA appears quite different, and a modification of the hydrophobic/hydrophilic balance in the polymerization feed produces a temperature variation ranged from 6.8°C for I–IV to 2.8°C for hydrogels III–VI, according to the crosslinking degree of microspheres.

Investigation of the applicability of the hydrogels I–VI in controlled release was carried out by studying their swelling behavior at different temperature and the results are collected in Table II. For each studied composition, the water uptake was reported in grams per gram of dry copolymer, and the ratio between the swelling at 25°C and 40°C at fixed pH values (Sr) was determined. The materials showed different water affinity at 25 and 40°C due to the presence of pendant hydrophobic groups in the polymeric chains. In particular, at 40°C, there is a considerable lowering of the equilibrium swelling percent, because of the solvent diffusion outside the polymeric network and the resultant hydrophobic interactions between hydrocarbon moieties on the polymeric chains as illustrated in the Figure 1. When the temperature decreases to 25°C, below the transition temperature of the hydrogels, the swelling equilibrium percent is greater than that found at 40°C for all copolymers and the Sr values ranged from 1.6 to 1.4 for I–III and from 1.9 to 1.8 for IV–VI. At both temperatures, the water uptake decreases from copolymer I to III and from IV to VI, according to the crosslinking degree of the network and in contrast with the hydrophilic content of the copolymer.

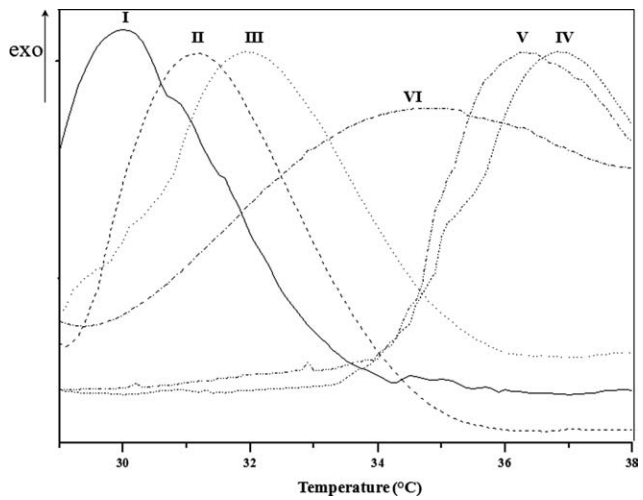

Figure 3 DSC thermograms of the swollen stimuli responsive hydrogels at a heating rate of 3°C (the temperatures at the maximum points of the exotherms were referred as volume phase transition temperature of the hydrogels).

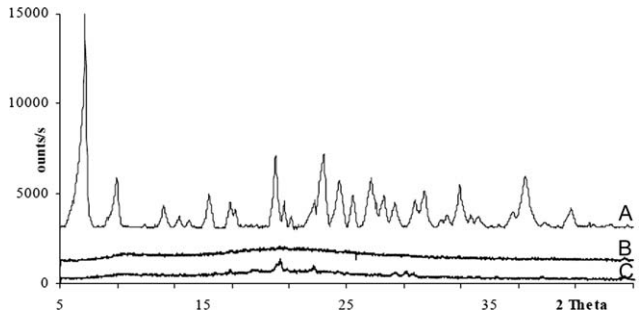

Figure 4 X-ray diffraction patterns of pure DDA (A), DDA-unloaded V microspheres (B), and DDA-loaded IV microspheres (C). Analogous results have been found for all materials.

This finding can be explained assuming that the effect of the increased crosslinking degree is predominant with respect to the increased hydrophilic moieties in the macromolecular network. In addition, for compositions IV–VI, the insertion of the hydrophilic monomer in the polymeric backbone produces an increase of the water affinity.

In vitro release studies

To estimate the ability of the matrices to release drug molecules, the beads were loaded with DDA by soaking procedure and LE(%) and DL(%) were dermined for each composition (Table II). Diclofenac salt, a nonsteroid anti-inflammatory drug, shows therapeutic efficacy by means of pain relief, antifebrile and anti-inflammation and is widely used in the treatment of rheumatic arthritis, osteoarthritis, spastic spondylitis, acute gout, and inflammation or gout of lesion after operation. When DDA is orally administered for a long period of time, remarkable side effects are observed. To reduce such side effects, studies on the method of formulating DDA into a site-specific matrix have actively been in progress, and stimuli responsive hydrogels, because of their capability to modulate the release of a therapeutic, are of great interest in this regard. The DDA was loaded on the beads with a LE % > 90% for copolymers I–V, whereas it was uptaken on the beads VI with LE % approximate to 84%. In our experiments drug-loaded copolymers with DL % ranging from 7.53 and 8.69 were produced.

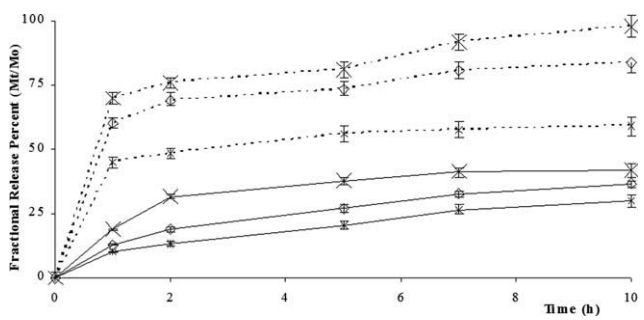

Figure 5 Drug release expressed as percent of DDA delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads I (*), II (◇), and III (×) at 25°C (solid lines) and 40°C (dashed lines) and at pH 7.0 (PBS solution 10^{-3} mol L^{-1}).

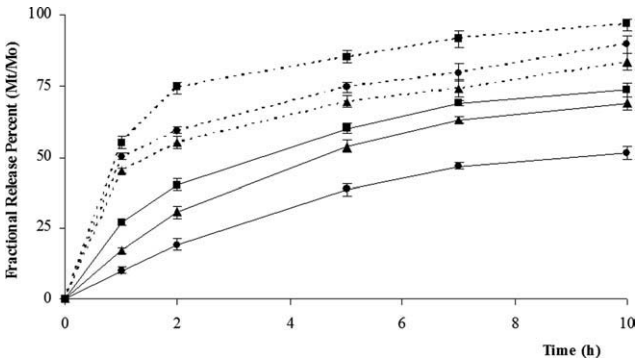

Figure 6 Drug release expressed as percent of DDA delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads IV (▲), V (●) and VI (■) at 25°C (solid lines) and 40°C (dashed lines) and at pH 7.0.

The determination of the drug dispersion state in all the hydrogels was performed by X-ray analysis. Figure 4 reports the X-ray diffraction patterns of pure drugs (Curves A), unloaded (Curves B) and drugs-loaded V hydrogels (Curves C). It is evident that pure drug is in the crystalline state; on the contrary, both the drug unloaded and loaded microparticles are in the amorphous state. This result demonstrates 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 the other spherical microparticles.

In Figures 5 and 6 the release profiles are reported for I–III and IV–VI respectively, and a different release profiles were recorded depending of the temperature of the surrounding medium. The amount of drug molecules moving from the polymeric beads to the medium was higher at 40°C than 25°C for all microspheres at each experimental time; furthermore, a shape rise at the first hour was noted at 40°C, comparing with a slow increase at 25°C. This jump in the release profile was concerned as a result of rapid collapse of the hydrogel from a swelled to a shrinked state.

Since the microparticles have a well-defined geometry and a narrow dimensional distribution, we determined the mechanism of drug release (Fickian or non-Fickian). In particular, the kinetics of DDA release at 25°C, under the LCST value, were analyzed by the semiempirical eq. (4) for $M_t/M_0 \leq 0.6$.[19]

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

TABLE III
Release Kinetics Parameters of Different Formulations

	$M_t/M_0 = Kt^n$					
	K 10^3 (min^{-n})		N		r	
Sample	25°C	40°C	25°C	40°C	25°C	40°C
I	9.67 ± 0.62	45.10 ± 0.76	0.43 ± 0.03	0.12 ± 0.01	0.98	0.97
II	13.62 ± 0.52	60.92 ± 1.49	0.49 ± 0.02	0.14 ± 0.01	0.99	0.96
III	22.88 ± 2.72	68.40 ± 2.75	0.29 ± 0.06	0.14 ± 0.02	0.85	0.91
IV	29.80 ± 2.22	45.41 ± 0.76	0.41 ± 0.04	0.26 ± 0.01	0.97	0.99
V	20.97 ± 2.72	49.74 ± 0.73	0.53 ± 0.07	0.25 ± 0.01	0.97	0.99
VI	13.12 ± 2.24	59.47 ± 3.09	0.62 ± 0.08	0.22 ± 0.03	0.96	0.94
	$M_t/M_0 = K_1 \cdot t^{1/2} + K_2 \cdot t$					
	K_1 10^3 ($min^{-1/2}$)		K_2 10^3 (min^{-1})		r	
	25°C	40°C	25°C	40°C	25°C	40°C
I	29.67 ± 0.72	48.86 ± 1.58	−0.02 ± 0.01	−9.86 ± 0.34	0.99	0.97
II	24.24 ± 0.48	65.41 ± 2.64	−0.82 ± 0.07	−12.83 ± 1.01	0.99	0.96
III	15.89 ± 1.96	72.04 ± 2.94	−3.96 ± 0.10	−13.64 ± 1.90	0.98	0.94
IV	32.19 ± 1.76	48.77 ± 1.84	−0.25 ± 0.05	−7.39 ± 1.06	0.99	0.99
V	21.27 ± 1.36	53.24 ± 1.48	−0.89 ± 0.05	−8.23 ± 1.30	0.98	0.98
VI	12.28 ± 1.07	65.49 ± 1.18	−1.55 ± 0.14	−11.34 ± 1.19	0.97	0.99

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. If $n = 0.85$, a completely non-Fickian or Case II release kinetics is operative. The intermediary values ranging between 0.43 and 0.85 are attributed to anomalous type diffusive transport. The least-squares estimations of the fractional release data along with the estimated correlation coefficient values, r, are presented in Table III. As the results shown, the exponents n was in the range between 0.43 and 0.50 in the experiments at 25°C for the hydrogels I, II, IV, and V, which were meant the polymers at this temperature mainly followed a Fickian diffusion way. When the amount of the functionalized BSA was increased in the polymerization feed (Copolymers III and VI), the release profile of the drug appears to be away from the Fickian behavior. A more informative analysis can be obtained by fitting the data with the model proposed by Peppas and Sahlin.[18] 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). The term $K_1 t^{1/2}$ is greater than the term $K_2 t$, for Samples I, II, IV, and VI indicating that the predominant mechanism of these microparticles, at 25°C, for DDA release is the Fickian diffusion through the swollen microparticles. On the contrary, the values of K_1 and K_2 for Copolymers III and VI are comparable as consequence of the decrease of Fickian contribution on the release mechanism. 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 to the medium. The collected experimental data show that, in the swollen state, the cross-liking degree and the hydrophilicity of the network greatly influence the release profile and the release rate of the drug. Introducing HEMA in the polymerization feed, an increase in the amount of drug released was observed as a result of the increased water affinity of the microparticles due to the presence of the pendant hydrophilic groups in the polymeric backbone.

When the polymers were at 40°C, above their LCST, the release behaviors were complex and not only diffusional effects of drug through the polymer network has to be considered, but also the squeezing effect of swelled polymer contributed to the apparent release rate. The n values for all formulations

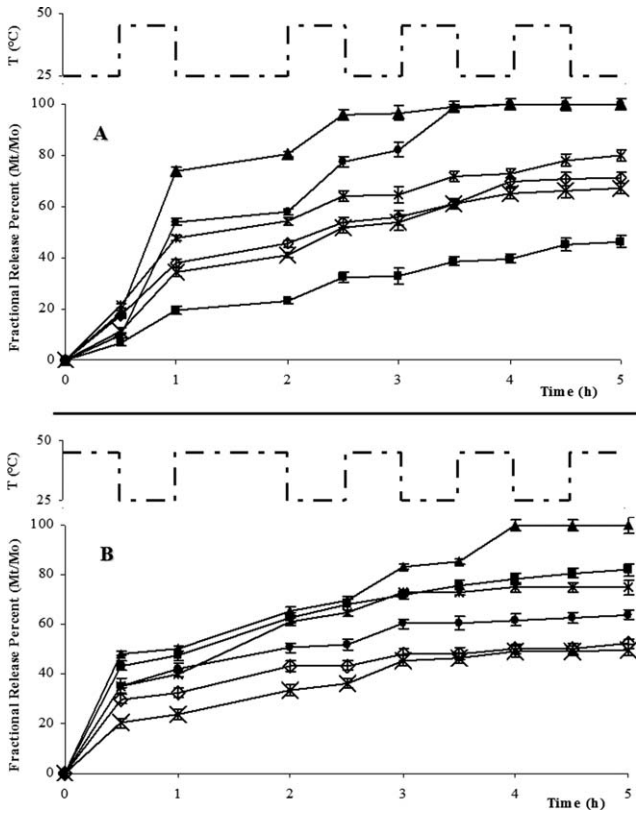

Figure 7 Temperature-dependent stepwise DDA release profile expressed as percent of drug delivered (M_t) related to the effectively entrapped total dose (M_0), as a function of time for beads I (*), II (◇), III (×), IV (▲), V (●), VI (■) hydrogels first exposed to a temperature of 25°C (A) and 40°C (B), with alternating temperature between 25°C and 40°C at pH 7.0 (PBS solution 10^{-3} mol L^{-1}).

are lower than 0.43, indicating that a non-Fickian diffusion transport was recorded. Fitting the experimental data using the Peppas-Sahlin equation, more information can be obtained; in particular, at 40°C, it is possible to observe a greater contribution due to K_2 respect to the fitting at 25°C as a consequence of the reduced role of the diffusional component in the release mechanism.

In vitro release profiles of pulsatile device during 5 h studies were found to have a sustaining efficacy and the cycling temperature may affect the response to the environmental modifications and thus the drug delivery profile. Figure 7(A,B) shows the DDA release profile and release rate from the thermoresponsive hydrogels as a function of the temperature cycling at fixed pH value (pH 7.0). The experiments were initiated placing the samples both in a swelling (25°C) and in a collapsed state (40°C) and recording the amount of DDA released, expressed as M_t/M_0 percent, in the surrounding environment after each temperature change. As reported in Figure 7(A) DDA-loaded samples were first exposed to a temperature of 25°C followed by 40°C and after each temperature cycling a higher release amount and release rate were generally observed at 40°C versus 25°C. As the temperature reached the LCST, strong hydrophobic interactions between the NIPAAm moieties occurred, so that a significant amount of aqueous drug solution was dispelled from the collapsed hydrogels. Exposing the DDA-loaded samples to an initial temperature of 40°C [Fig. 7(B)], a significant burst effect was observed for all copolymers, with M_t/M_0 percent values ranged from 20.3 to 48.3 after 30 min and the highest amount of drug was released in the collapsed state at the same time interval. In the oscillatory experiments, the drug release profiles approximately in the first 3 h greatly depend on the temperature of the surrounding environment. After this time, no significant differences in the release profiles were recorded changing the medium temperature. These data can be explained with the hypothesis that the drug concentration in the medium after 3 h is close to the highest attainable concentration. Pulsatile drug release of the hydrogels meant that an effective modulation of the drug release rate was achieved. This conclusion is vital for future practical applications.

CONCLUSIONS

In this work a novel class of stimuli-responsive hydrogels was designed and synthesized by reverse phase suspension radical polymerization of BSA-derivative with an hydrophilic (HEMA) and/or a temperature-sensitive (NIPAAm) monomers.. Thermal analyses showed negative thermoresponsive behavior for all samples and the LCST value was modified by modulating the ratio of hydrophilic and hydrophobic segment in the macromolecular system. In particular, increasing the content of hydrophilic moieties in the network, higher LCST values, close to the body temperature, were recorded. To test the preformed materials as drug carriers, DDA was chosen as drug and the entrapment percent was determined. Hydrogels crosslinking degree and drug-bead interactions were found to greatly influence the drug release profiles. Depending on temperature of the surrounding environment, drug release across the polymeric matrices takes place by abrupt modification of volume hydrogels and by diffusion of the therapeutic agent through the network. The diffusional contribute on the drug delivery were estimated by introducing semiempirical equations.

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