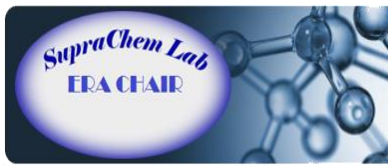
This manuscript was published as formal in:
Journal of Macromolecular Science, Part B: Physics 54 (**2015**) 1105-1121
https://doi.org/10.1080/00222348.2015.1057444
https://www.tandfonline.com/doi/full/10.1080/00222348.2015.1057444



Full paper

Poly(N-isopropylacrylamide-co-N-isopropylmethacrylamide) Thermo-responsive Microgels as Self-regulated Drug Delivery System

Gheorghe Fundueanu*, Marieta Constantin, Sanda Bucatariu, Paolo Ascenzi

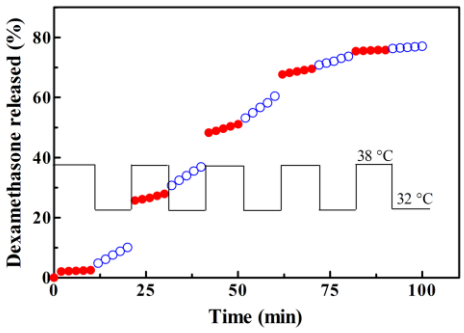
---

G. Fundueanu, M. Constantin, S. Bucatariu
Department of Natural Polymers, Bioactive and Biocompatible Materials, "Petru Poni" Institute of Macromolecular Chemistry, 700487 Iassy, Romania
E-mail: ghefun@icmpp.ro
P. Ascenzi
Interdepartmental Laboratory for Electron Microscopy, University Roma Tre, I-00146 Roma, Italy

---

Abstract

Poly(*N*-isopropylacrylamide-co-*N*-isopropylmethacrylamide) (poly(NIPAAm-co-NIPMAAm)) was synthesized as an attractive thermo-responsive copolymer by an original procedure. Due to the similar structure of the two co-monomers, the poly(NIPAAm-co-NIPMAAm) copolymer displays a very sharp phase transition, under physiological conditions (phosphate buffer solution (PBS) at pH = 7.4). The copolymer, showing the 51/49 co-monomer NIPAAm/NIPMAAm molar ratio, displays a lower critical solution temperature (LCST) close to that of the human body temperature (36.8 °C). The poly(NIPAAm-co-NIPMAAm) microgels obtained at the 51:49 co-monomer ratio displays a volume phase transition temperature (VPTT) slightly smaller than LCST. The deswelling rate of the microgels is very high (k = 0.019 s^{-1}), the shrinkage occurring almost instantaneously, whereas the swelling rate is slightly lower (k = 0.0077 s^{-1}). The microgels were loaded with the model drug dexamethasone and the drug release was investigated at different temperatures, below and above the VPTT. Under thermal cycling operation between 32 and 38 °C, the pulsatile release of dexamethasone was observed.



1. Introduction

The administration of bioactive molecules (e.g., drugs) using controlled drug delivery devices has been deeply investigated over the last thirty years.[1-4] Although these studies contributed to improve drug formulation,[5] patient comfort and treatment efficiency, they are not appropriated for the therapy of some diseases including diabetes, heart rhythm disorders, and angina pectoris. In fact, in patients affected from these diseases, the drugs must be released only under conditions where the physiological parameters are altered. The formulations that achieve these requirements are called self-regulated drug delivery systems.[6,7] Most of them are based on intelligent or stimuli-sensitive polymers,[8,9] which undergo a phase transition only when small changes in physico-chemical parameters/properties, such as temperature,[10] pH,[11] light,[12] and ionic strength[13], occur. Polymers sensitive to temperature are widely used being based on changes of the human body temperature to trigger the solubilization (swelling) or precipitation (collapsing) of the polymeric chains (hydrogel) and, in turn, the drug release. The most commonly used thermosensitive polymer in biomedical applications is poly(*N*-isopropylacrylamide) (poly(NIPAAm)), which displays a phase transition temperature close to that of the human body (32 °C) in aqueous solutions.[14] Below the phase transition temperature, called also lower critical solution temperature (LCST), the polymer is highly hydrated and therefore it is soluble in water, while above the LCST the polymer hydration decreases and it precipitates. Accordingly, the corresponding hydrogel swells under the LCST and collapses above the LCST; then, changes of the polymer volume are at the root of the drug release in a pulsed manner.[15,16]

In comparison with pure water, physiological fluids contain salts and therefore the LCST of the poly(NIPAAm) decreases to a lower temperature.[17] Under these conditions, the increase of the LCST, approaching that of the human body, and a sharp phase transition are imperative to optimize drug release. Generally, the increase of the LCST is obtained by copolymerization of NIPAAm with hydrophilic monomers.[18,19] However, the occurrence of hydrophilic

monomers along the main chain alters the amide and isopropyl sequences of poly(NIPAAm), therefore a temperature higher than LCST is necessary for hydrophobic interactions. Moreover, hydrophilic co-monomers decrease substantially the thermosensitivity of the copolymer. As a whole, the co-monomer should be chosen carefully to preserve the thermosensitivity of the copolymer.

The structure of the co-monomer *N*-isopropylmethacrylamide (NIPMAAm) is similar to that of NIPAAm, preserving the amide and isopropyl sequences. Although poly(NIPMAAm) is more hydrophobic than poly(NIPAAm), the LCST of poly(NIPMAAm) in aqueous solution is higher (~ 46 °C),[20] reflecting the conformation of the monomeric unit in poly(NIPMAAm). The presence of methyl groups along the main chain impairs hydrophobic interactions, therefore a higher temperature is necessary for poly(NIPMAAm) precipitation. Even if the poly(NIPMAAm) LCST is high, the phase transition is very sharp and it appears to be an appropriate partner for copolymerization.

Here, the synthesis of poly(NIPAAm-co-NIPMAAm) with the 51:49 co-monomer ratio is reported. This thermosensitive copolymer displays, under physiological conditions, a sharp phase transition at a temperature close to that of the human body. The copolymer was transformed in microgels by cross-linking with *N,N'*-methylenebisacrylamide (MBAAm) in an appropriate solvent. Due to the similar structures of the co-monomers, the microgels display very fast swelling and deswelling rates. The microgels were loaded with the model drug dexamethasone, and the drug release was investigated below and above the VPTT. Cyclical temperature changes induce the drug release from the microgels in a pulsatile way.

2. Experimental Section

2.1. Materials

N-isopropylacrylamide (NIPAAm) and *N*-isopropylmethacrylamide (NIPMAAm) were supplied from Sigma-Aldrich Co. (St. Louis, USA) and recrystallized from hexane. Ammonium persulfate (APS), *N,N'*-methylenebisacrylamide (MBAAm), *N,N,N',N'*-tetramethylethylenediamine (TEMED) were obtained from Fluka AG (Buchs, Switzerland). Dexamethasone, free base, (see the chemical structures in Figure 1) was kindly provided from Rompharm Company SRL (Otopeni, Romania). All chemicals were of analytical or reagent grade and were used without purification unless stated.

2.2. Synthesis of Linear Poly(NIPAAm-co-NIPMAAm)

The synthesis of linear poly(NIPAAm-co-NIPMAAm) was achieved by free radical co-polymerization of monomers in a water/methanol solution. Typically, 1.13 g of NIPAAm (10 mmol) and 0.846 g NIPMAAm (6.66 mmol) were dissolved in 8 mL of a 1:1 water/methanol mixture. Dry nitrogen was purged through the solution for 30 min prior to polymerization. Then, the initiator (0.030 g of APS) and the accelerator (30 µL of TEMED) were added to the solution and the copolymerization was continued for 8 h at room temperature (22 ± 2 ºC). Afterwards, the polymer solution was diluted with distilled water and dialyzed for 7 days at 22 °C (molecular weight cut off 10,000-12,000 Da; from Medicell International, London, United Kingdom), and recovered by freeze-drying.

2.3. Copolymer Composition

The copolymer structure and composition were determined by ^{1}H-NMR analysis. ^{1}H-NMR spectra of poly(NIPAAm-co-NIPMAAm) were recorded in D_2O on a Varian Mercury Plus

400/Varian VXR 200 spectrometer operating at 400 MHz frequency. The molar fraction of co-monomers was calculated according to Equations (1) and (2):

$$\mathbf{x + y = 1} \quad \textbf{(1)}$$

$$\mathbf{9x + 11y = 10.02} \quad \textbf{(2)}$$

where *x* and *y* are the molar fractions of NIPAAm and NIPMAAm, respectively. Equation (1) indicates the area of methynic protons at 3.86 ppm of both NIPAAm and NIPMAAm. Equation (2) indicates the total area of the peaks between 0.5 and 2.5 ppm corresponding to the main backbone protons (3 NIPAAm + 2 NIPMAAm) and methyl (6 NIPAAm + 9 NIPMAAm) protons.

2.4. Determination of the Molecular Weight of Poly(NIPAAm-co-NIPMAAm)

Values of the the number-average (M_n) and of the weight-average (M_w) molecular weight of poly(NIPAAm-co-NIPMAAm) were determined by GPC using the GPC-PL-EMD 950 instrument (Polymer Laboratories, Shropshire, UK) in dimethylformamide at 120 °C and at the flow-rate of 0.7 mL min^{-1}. Calibration was performed with monodisperse polystyrene standards.

2.5. Determination of the Lower Critical Solution Temperature

Values of the LCST were determined by plotting the temperature-dependence of the absorbance change of the polymer solution (at 450 nm). The absorbance was measured with the UV-Vis Specord 200 spectrophotometer (Analytic Jena, Jena, Germany) equipped with a temperature controller. The copolymer solution (1 %, w/v) was prepared in distilled water, in the standard acidic solution (pH = 1.2; 64 mM HCl + 50 mM KCl), and in the standard phosphate buffer solution (PBS) (pH = 7.4; 50 mM Na_2HPO_4 + NaOH). The heating rate was 0.2 °C every 10 min. The cloud point (CP) was determined as the inflection point of the curve

of the temperature-dependence of the absorbance change by Boltzmann fitting of the experimental data.[21]

2.6. Synthesis of the Poly(NIPAAm-co-NIPMAAm) microgels

Poly(NIPAAm-co-NIPMAAm) hydrogel was synthesized as follows: 1.13 g of NIPAAm (10 mmol), 0.846 g NIPMAAm (6.66 mmol), and 0.025 g of MBAAm (0.16 mmol) were dissolved in 8 mL of a 1:1 water:methanol mixture. Dry nitrogen was purged through the solution for 30 min prior polymerization. Then, the initiator (0.030 g of APS) and the accelerator (30 µL of TEMED) were added to the solution that was immediately transferred into a syringe. The polymerization was conducted for 24 hours at room temperature (22 ± 2 ºC).

After polymerization, the hydrogel was kept in a large volume of water that was periodically refreshed. After 7 days, the un-reacted species were removed. One aliquot of the hydrogel was washed with a water/acetone mixture on increasing the acetone level. Then, the hydrogel was washed with diethylether and dried under vacuum. Finally, the hydrogel was fragmented in small particles with a diameter ranging between 5 and 250 µm. Another aliquot of the hydrogel was dried by lyophilization; thus, the hydrogel swollen in distilled water at room temperature was frozen in liquid nitrogen and then freeze-dried (−57 °C, 5.5×10^{-4} mbar) to remove all the solvent.

2.7. Morphological Analysis

The hydrogel dried with a mixture of water/acetone and the freeze-dried hydrogel were fractured carefully and the interior morphology was examined with an Environmental Scanning Electron Microscope (ESEM, type Quanta 200, Netherlands).

2.8. Determination of the Swelling Degree

The volume of the microparticles at different temperatures was determined at equilibrium in PBS by placing the microparticles in a graduated glass cylinder (i.d. = 12 mm). The swelling degree (q) was calculated according to Equation (3):

$$\mathbf{q} = \frac{\boldsymbol{V}_\mathbf{s}}{\boldsymbol{V}_\mathbf{d}} \quad \textbf{(3)}$$

where V_s is the volume of the microparticles in the swollen state and V_d is the volume in the dried state.

2.9. Determination of the Volume Phase Transition Temperature

The value of the volume phase transition temperature (VPTT) of microgels was determined as the inflection point of the curve representing the temperature dependence of the swelling degree by Boltzmann fitting of the experimental data.[21]

2.10. Determination of the Swelling and Collapsing Kinetics

Swelling kinetics of poly(NIPAAm-co-NIPMAAm) microgels were determined under simulated physiological conditions (PBS at pH = 7.4). The microgels were placed in a graduated glass cylinder (i.d. = 12 mm) and added at equilibrium at the desired temperature (45°C). Then, the cylinder was transferred into a water bath at 4 °C. The volume increase was measured periodically. The dynamic swelling factor (q_{ds}) was calculated according to Equation (4):[22]

$$\mathbf{q_{ds}} = \left(\boldsymbol{V}_\mathbf{t} - \boldsymbol{V}_\mathbf{d}\right) / \left(\boldsymbol{V}_{\mathbf{0(45°C)}} - \boldsymbol{V}_\mathbf{d}\right) \quad \textbf{(4)}$$

where $V_{0\ (45\ ^\circ C)}$ is the microgel volume at 45 °C, V_t is the microgel volume at a given time, and V_d is the volume of microgels in the dried state.

The collapsing kinetics were determined by placing the microgels (in PBS at pH = 7.4) in a graduated glass cylinder (i.d. = 12 mm) and added at equilibrium at 4 °C. Then, the cylinder was transferred into a water bath at 45 °C. The dynamic collapsing factor (q_{dc}) was calculated according to Equation (5):[22]

$$q_{dc} = (V_t - V_d) / (V_{0(4\,^\circ C)} - V_d) \quad \textbf{(5)}$$

where $V_{0\ (4\ ^\circ C)}$ is the microgel volume at 4 °C, V_t is the microgel volume at a particular time, and V_d is the volume of microgels in dried state.

2.11. Drug Loading

Drug loading was performed by soaking in a vial (i.d. = 20 mm, h = 45 mm) 0.150 g of microparticles in 7 mL of ethanol containing 15 mg of dexamethasone. The suspension was kept under stirring for 24 hours at room temperature (22 ± 2 °C), then the cap of the vial was removed and the vial was placed in a vacuum oven at room temperature for 48 hours. The encapsulation efficiency was calculated as the ratio between the actual and theoretical amount of drug trapped in microparticles. The actual amount of dexamethasone was determined by placing 10 mg of loaded microparticles in 100 mL of ethanol. The solubilized drug was determined by a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with an injection valve (sample loop, 20 μL) and a C-18 column connected to the UV-Vis detector (observation wavelength = 241 nm). The mobile phase was acetonitrile-PBS (35:65 v/v) and the flow rate was 0.6 mL min^{-1}.

2.12. Differential Scanning Calorimetry (DSC)

DSC measurements were recorded on a DSC 200 F3 Maia device (Netzsch, Germany). A mass of 5.5 mg of each sample was placed in sealed non-hermetic aluminum pans and heated from 20 to 300 °C at a heating rate of 10 °C min^{-1}. Nitrogen was used as inert gas at a flow rate of 150 mL min^{-1}. Previously, for glass transition temperature (Tg) determination, samples were heated from 20 to 180 ºC at 10 ºC min^{-1} to remove all the residual moisture and erase the effect of previous thermal history. A cooling run was made between the two heatings at a cooling rate of –10 °C min^{-1}. Cooling was made with liquid nitrogen. Tg was considered as the mid-point temperature between the onset and end temperatures.

2.13. Kinetics of Dexamethasone Release

Kinetics of dexamethasone release was performed by sinking 0.150 g of loaded microparticles in 100 mL of PBS at pH = 7.4, under gentle stirring. At given time intervals, 1 mL of the release fluid was collected and the drug content was determined by HPLC as described above. The same volume of the solvent was added to replace the volume of the removed sample.

2.14. Statistical Analysis

All values are the mean ± standard deviation of at least three independent experiments unless stated. The parameter difference was statistically tested for significance with the one-way procedure ANOVA followed by Turkey-Kramer post test using GraphPad Instant 3 (GraphPad software, Inc., La Jolla, CA) software for Windows; $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preparation and Characterization of the Copolymer

In biomedical applications, thermosensitive polymers should display a rapid phase transition in physiological fluids at a temperature close to that of the human body. As largely recognized, poly(NIPAAm) is one of the most studied thermosensitive polymer since it shows a sharp phase transition at about 32 °C, in aqueous solution.[14] However, the transition temperature in physiological fluids, which are complex systems with high ionic strength, may be lower than that determined in pure water.[17] With the purpose of increasing the transition temperature to a value close to that of the human body, NIPAAm is usually copolymerized with hydrophilic monomers. The LCST of the resulting copolymer is similar to that of the human body. Although the alteration of the acrylamide sequences of NIPAAm induces low thermosensitive properties of the copolymer, the copolymerization of NIPAAm with monomers with similar structures, such as NIPMAAm, seems to be an ideal alternative to obtain a copolymer with a sharp phase transition at a temperature close to that of the human body.

As shown in Table 1 and Figure 2, the percentage of co-monomers in copolymers does not follow exactly the composition of the feed. The NIPAAm/NIPMAAm ratio in the copolymer is always lower than that in the feed, suggesting that NIPMAAm is more reactive than NIPAAm.

Under simulated physiological conditions (*i.e.*, in PBS), the LCST of poly(NIPAAm-co-NIPMAAm) increases with the NIPMAAm content in the copolymer. In fact, the phase transition of the copolymer is between the LCST of pure poly(NIPAAm) and that of pure poly(NIPMAAm). Pure poly(NIPAAm) displays a LCST of 29.9 °C in PBS at pH = 7.4 (see Table 1), which is lower than that determined in aqueous solution. Despite the fact that poly(NIPMAAm) is more hydrophobic than poly(NIPAAm), the LCST of poly(NIPMAAm) in PBS (44.8 °C) is higher than that of poly(NIPAAm). In fact, the presence of the methyl

groups in poly(NIPMAAm) induces steric hindrance and the association of the hydrophobic groups requires a higher temperature.

As shown in Figure 3, the LCST increases linearly with the content of NIPMAAm in the copolymer. Similar results were obtained by Djokpé and Vogt.[20] Moreover, the intercept of the line with the *y*-axis gives a LCST value of the poly(NIPAAm) of 28.8 °C in PBS at pH =7.4, this value being consistent with literature data.[23]

However, the most important characteristics of these copolymers are their sharp phase transition at any molar ratio of monomers in the copolymers (Figure 4). In fact, values of the phase transition of the copolymers fall between the LCST values of pure poly(NIPAAm) and of pure poly(NIPMAAm).

The copolymer showing the NIPAAm/NIPMAAm molar ratio of 51/49 shows a LCST value close to that of the human body temperature (36.8 °C), making it suitable for biomedical applications.

3.3. Preparation and Characterization of Microgels

The poly(NIPAAm-co-NIPMAAm) hydrogel was synthesized using a water:methanol mixture of 1:1 (v/v) by free radical copolymerization of NIPAAm and NIPMAAm, in the presence of a low amount of MBAAm. The copolymerization process was initiated by a redox system based on APS and TEMED. The amount of MBAAm was adjusted to obtain a hydrogel with good mechanical properties and a high degree of swelling. The NIPAAm:NIPMAAm molar ratio corresponded to that used for the synthesis of linear copolymer with LCST = 36.8 °C was used for the preparation of hydrogel (sample S_2). The resulting hydrogel was crushed in small particles for an easier handling in the following experiments.

As shown in Figure 5, the microparticles obtained by progressively removal of the solvent display a compact structure with almost no pores. On the contrary, the microparticles obtained

by lyophilization preserve the structure of the microgel in the swollen state which is highly porous.

An important requirement of the microgel is to preserve the thermosensitive characteristics of the linear copolymer. Therefore, the value of VPTT of the microgels was determined under simulated physiological conditions (PBS, pH = 7.4). The method is based on the determination of the swelling degree of microgels at equilibrium under and above the LCST.[24] In general, the swelling degree is an indirect index of the water uptake by hydrogels at different temperatures and can be applied to microparticles. As shown in Figure 6, the swelling degree decreases slowly to the critical temperature and then undergoes a dramatic shrinkage, on increasing temperature. The value of VPTT, calculated by Boltzman fitting of the experimental data was 33.7 °C, being smaller than that of the LCST of the linear copolymer (36.8 °C). In fact, the VPTT is influenced by two opposite effects. On the one hand, cross-linking limits the flexibility of the polymer chains and therefore a high energy (i.e., temperature) is necessary for the formation of hydrophobic interactions. On the other hand, under conditions where the cross-linking degree is low, the hydrogel should be considered as a concentrated polymer solution, the value of LCST being smaller than that of a dilute solution.[23] Of course, hydrophobic interactions occur more rapidly in a highly concentrated solution.

In biomedical applications, microgels must display fast swelling and collapsing rates upon small temperature body changes. Of note, the values of rates of the swelling and deswelling processes of thermosensitive hydrogels depend on the diffusion rate of water into and out of the hydrogel. The diffusion rate is controlled mainly by the size and the porosity of the hydrogel.[25,26] Of course, hydrogels with micrometric dimensions and a high degree of porosity are characterized by fast swelling and deswelling rates. Moreover, since poly(NIPAAm-co-NIPMAAm) microgels are built by two similar co-monomers that individually hold very sharp phase transitions (see Figure 4), the phase transition of the

copolymer seems to be even sharper than that of the homopolymers. Of note, microgels with these characteristics swell and collapse very rapidly (Figure 7), the collapsing process being almost instantaneous. The collapsing rate is faster than the swelling rate (k = 0.019 s^{-1} and k = 0.0077 s^{-1} for collapsing and swelling processes, respectively) (Figure 7B) because, during collapsing, the water is pushed out mechanically, a process faster than that of the water diffusion into microgels. The delay-time of about 30 seconds (see Figures 7A and B) reflects the time necessary for heating transfer from the bath to the samples to equilibrate the temperature of microgels at 45 °C or 4 °C upon changing temperature from 4 °C to 45 °C and vice-versa.

3.4. Drug Loading

The loading of purified microgels was performed by immersion in a drug/ethanol solution, followed by a progressive evaporation of the solvent. Ethanol was chosen for the following reasons. (i) Ethanol is a good solvent for dexamethasone. (ii) Microparticles swell extensively in ethanol (q = Vs/Vd = 21.5 ± 2.2). (iii) The evaporation rate of ethanol is relatively low, allowing the progressive diffusion and the uniform spread of the drug within microgels. As a result, the entrapment efficiency is very high (= 94.6 ± 3.4 %).

The DSC diagram of pure dexamethasone shows a melting peak at around 270 °C (Figure 8A). This peak is also present in the diagram of the physical mixture between poly(NIPAAm-co-NIPMAAm) microgels and drug (Figure 8B (a)). However, the lack of the melting peak of dexamethasone in the loaded microgels indicates the molecular dispersion of the drug within the polymeric network (Figure 8B (b)).

To determine the affinity of dexamethasone for the polymer, values of the glass transition temperature (Tg) were determined (Figure 8B). Since the water acts as a plasticizer and influences the Tg,[27] the samples were previously heated at 180 °C for the removal of any residual solvent. In the physical mixture, the Tg of poly(NIPAAm-co-NIPMAAm) was found

to be 154 °C, being intermediate between those of pure poly(NIPAAm) (Tg = 138 °C) and that of pure poly(NIPMAAm) (Tg = 175 °C).[28] Notably, the loaded microgels show a lower Tg at 151 °C, suggesting an intimate interaction between the drug molecules and the polymeric chains (plasticizing effect of the drug).

3.5. Dexamethasone Release Studies

Kinetics of dexamethasone release from the microgels were obtained under simulated physiological conditions (PBS at pH = 7.4) at temperatures below and above the VPTT of the microgels. Above the VPTT, the microgels are in the shrunken state and therefore the diffusion of the drug from microspheres towards the simulated physiological fluid is hindered by steric interactions. Moreover, the polymeric network is more hydrophobic at this temperature, therefore hydrophobic interactions between microgels and dexamethasone may occur. As follows, the release rate is low (Figure 9 A). Below the VPTT (i.e., at 32 °C), the microgels are in the swollen state and are hydrophilic, therefore no interactions with the drug take place. As a result, the release rate is fast, even if the diffusion coefficient is low at this temperature (Figure 9A). Moreover, the time-course of drug release is biphasic suggesting the occurrence of at least two different binding modes of dexamethasone to the microgels. On the other hand, the time-course of drug release from the microgels at 38 °C is monophasic and the rate constant corresponds to that of the slow phase of the time-course obtained at 32 °C.

The different rate of drug release at 32 °C and 38 °C is at the root of a pulsatile-type release mechanism if the temperature is cyclically changed below and above the VPTT (Figure 9 B). Most of the drug is released when the temperature increases from 32 °C to 38 °C. In fact, during this temperature change, a rapid collapsing process occurs and a large amount of the drug is expelled mechanically. The amplitude of the drug pulse decreases gradually with increasing the number of cycles until almost the whole drug is released (Figure 9 B).

4. Conclusions

Here, a new method for the synthesis of the poly(NIPAAm-co-NIPMAAm)) copolymer is reported, it is based on the free radical polymerization of co-monomers in a mixture of water/methanol. Under simulated physiological conditions, the LCST was tuned by modification of the NIPAAm:NIPMAAm ratio; all samples display a very sharp phase transition due to the similar chemical structure of the two co-monomers. Poly(NIPAAm-co-NIPMAAm) with the co-monomer molar ratio of 51/49 shows a LCST value near to that of the human body temperature (36.8 °C). The microgels obtained at this co-monomer ratio exhibit high swelling and deswelling rates when the temperature changes below to above the VPTT and vice-versa. The microgels were loaded with dexamethasone by the solvent evaporation method and the release experiments were conducted at temperatures below and above the VPTT. These particles have shown the ability to release the drug by a pulsatile mechanism when the temperature is cyclically changed below to above the VPTT and vice-versa.

Abbreviations: AIBN, *N,N'*-azobisisobutyronitrile; APS, ammonium persulfate; CP, cloud point; ESEM, environmental scanning electron microscope; LCST, lower critical solution temperature; MBAAm, *N,N'*-methylenebisacrylamide; NIPAAm, *N*-isopropylacrylamide; NIPMAAm, *N*-isopropylmethacrylamide; PBS, phosphate buffer solution; TEMED, *N,N,N',N'*-tetramethylethylenediamine; Tg, glass transition temperature; VPTT, volume phase transition temperature.

Acknowledgements: This work was partially supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-PTPCCA-

2013-4-1570. The authors are also grateful for the financial support from the H2020 ERA Chairs Project no 667387: SupraChem Lab Laboratory of Supramolecular Chemistry for Adaptive Delivery Systems ERA Chair initiative.

Keywords: *N*-isopropylacrylamide; *N*-isopropylmethacrylamide; lower critical solution temperature; volume phase transition temperature; smart polymers

[1] A. González, L. I. Tártara, S. D. Palma, C. I. Alvarez Igarzabal, *Mater. Sci. Eng. C* **2015**, *51*, 73.

[2] M. Cui, W. Wu, L. Hovgaard, Y. Lu, D. Chen, J. Qi, *Int. J. Pharm*. **2015**, *489***,** 277.

[3] W. T. Liu, Y. Yang, P. H. Shen, X. J. Gao, S. Q. He, H. Liu, C. S. Zhu, *Express Polym. Lett.* **2015**, *9*, 1068.

[4] J. Trygg, E. Yildir, R. Kolakovic, N. Sandler, P. Fardim, *Macromol. Mater. Eng.* **2015**, *300*, 210.

[5] Y. Zhang, H. F. Chan, K. W. Leong, *Adv. Drug Deliver. Rev*. **2013**, *65*, 104.

[6] W. Wu, S. Zhou, *Macromol. Biosci*. **2013**, *13***,** 1464.

[7] P. Mi, X. J. Ju, R. Xie, H. G. Wu, J. Ma, L. Y. Chu, *Polymer* **2010**, *51***,** 1648.

[8] J. Y. Fang, Y. K. Lin, S. W. Wang, Y. C. Li, R. S. Lee, *React. Funct. Polym*. **2015**, *95*, 46.

[9] V. Stoyneva, D. Momekova, B. Kostova, P. Petrov, *Carbohyd. Polym*. **2014**, *99*, 825.

[10] Y. B. Schuetz, R. Gurny, O. Jordan, *Eur. J. Pharm. Biopharm*. **2008**, *68***,** 19.

[11] E. Krisch, L. Messager, B. Gyarmati, V. Ravaine, A. Szilágyi, *Macromol. Mater. Eng.* **2016**, *301*, 260.

[12] H. M. Lin, W. K. Wang, P. A. Hsiung, S. G. Shyu, *Acta Biomater*. **2010**, *6***,** 3256.

[13] B. Zhang, B. Sun, X. Li, Y. Yu, Y. Tian, X. Xu, Z. Jin, *Int. J. Biol. Macromol.* **2015**, *79*, 392.

[14] M. Heskins, J. E. Guillet, *J. Macromol. Sci. Chem.* **1968**, *2*, 1441.

[15] T. G. Park, *Biomaterials* **1999**, *20*, 517.

[16] A. D'Emanuele, R. Dinarvand, *Int. J. Pharm*. **1995**, *118*, 237.

[17] G. Fundueanu, M. Constantin, P. Ascenzi, *Acta Biomater*. **2009**, *5*, 363.

[18] X. Z. Zhang, Y. Y. Yang, S. T. Chung, K. X. Ma, *Langmuir* **2001**, *17*, 6094.

[19] B. Wang, X. D. Xu, Z. C. Wang, S. X. Cheng, X. Z. Zhang, R. X. Zhuo, *Colloid. Surface. B* **2008**, *64*, 34.

[20] E. Djokpe, W. Vogt, *Macromol. Chem. Phys*. **2001**, *202*, 750.

[21] S. Sun, J. Hu, H. Tang, P. Wu, *J. Phys. Chem. B* **2010**, *114*, 9761.

[22] B. Yıldız, B. Işık, M. Kış, *Eur. Polym. J*. **2002**, *38*, 1343.

[23] M. Constantin, M. Cristea, P. Ascenzi, G. Fundueanu, *Express Polym. Lett*. **2011**, *5*, 839.

[24] M. Constantin, S. Bucatariu, P. Ascenzi, B. C. Simionescu, G. Fundueanu, *React. Funct. Polym*. **2014**, *84*, 1.

[25] T. Tanaka, D. J. Fillmore, *J. Chem. Phys*. **1979**, *70*, 1214.

[26] X. S. Wu, A. S. Hoffman, P. Yager, *J. Polym. Sci. Pol. Chem*. **1992**, *30*, 2121.

[27] M. Pouplin, A. Redl, N. Gontard, *J. Agr. Food Chem*. **1999**, *47*, 538.

[28] L. Ruiz-Rubio, J. L. Vilas, M. Rodríguez, L. M. León, *Polym. Degrad. Stabil*. **2014**, *109*, 147.

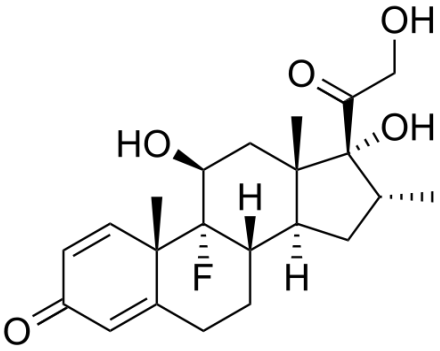


Figure 1. Chemical structure of dexamethasone.

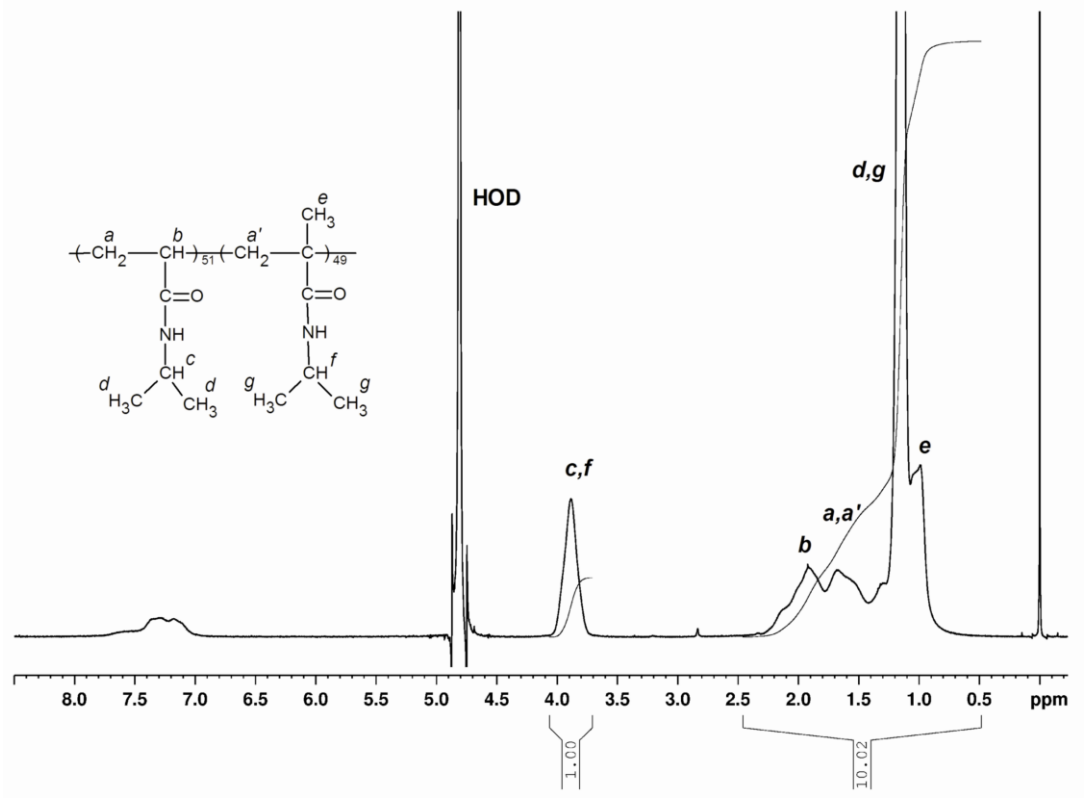


Figure 2. ^{1}H-NMR spectrum of poly(NIPAAm-co-NIPMAAm) (sample S_2 in Table 1).

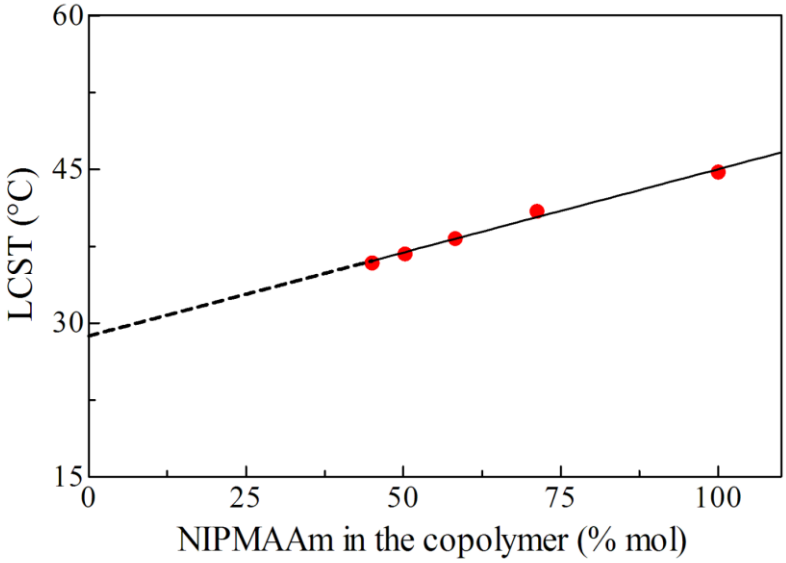


Figure 3. Dependence of the LCST on the amount of NIPMAAm in the copolymer. The experiments were performed in simulated intestinal fluid (PBS at pH = 7.4). The concentration of the copolymer solution was 1 % (w/v). The intercept with the *y* axis of the straight line is 28.8 °C. Data are the results of three independent experiments.

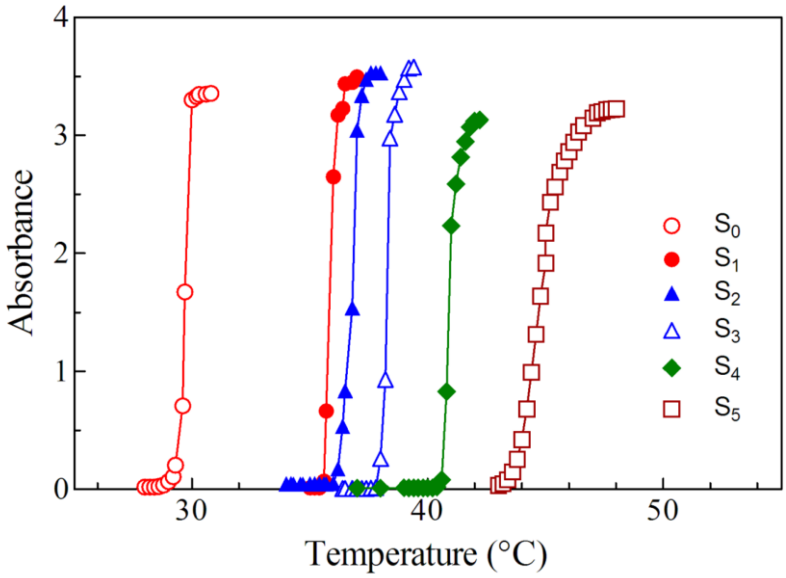


Figure 4. LCST curves of poly(NIPAAm-co-NIPMAAm) in simulated intestinal fluid (PBS at pH = 7.4). The concentration of the copolymer solution was 1 % (w/v). Data are the results of three independent experiments.

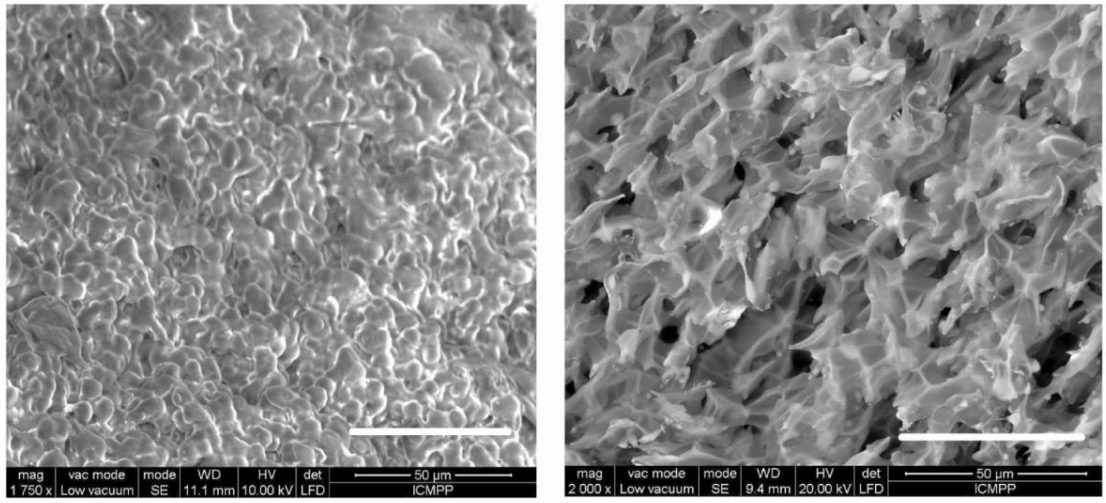


Figure 5. Scanning electron micrographs (cross-section) of poly(NIPAAm-co-NIPMAAm) microgels dried by progressive removal of water (left panel) and by lyophilization (right panel). The bars correspond to 50 μm in both panels.

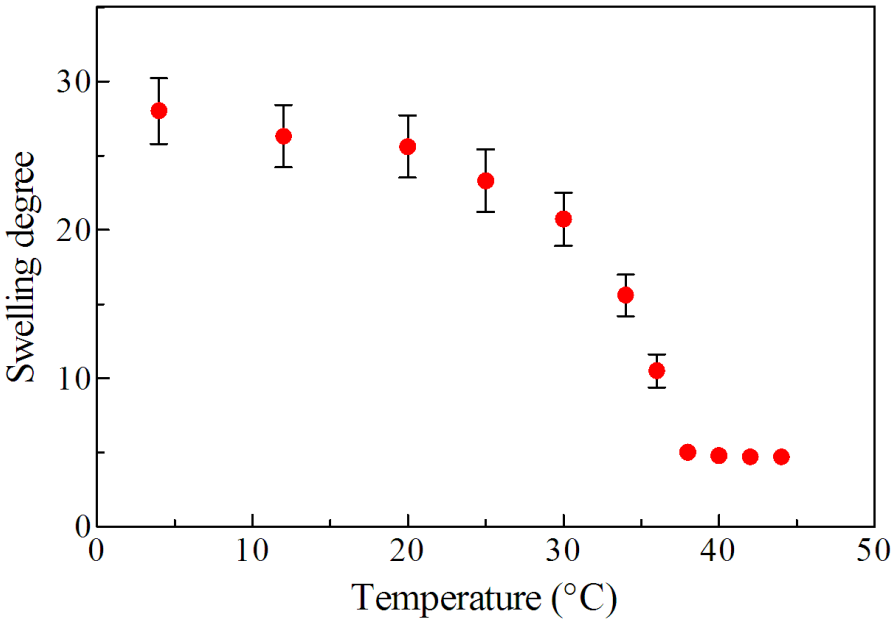


Figure 6. Effect of temperature on the swelling degree of the poly(NIPAAm-co-NIPMAAm) microgels. Data were obtained in simulated physiological conditions (PBS at pH = 7.4). Data are the results of three independent experiments.

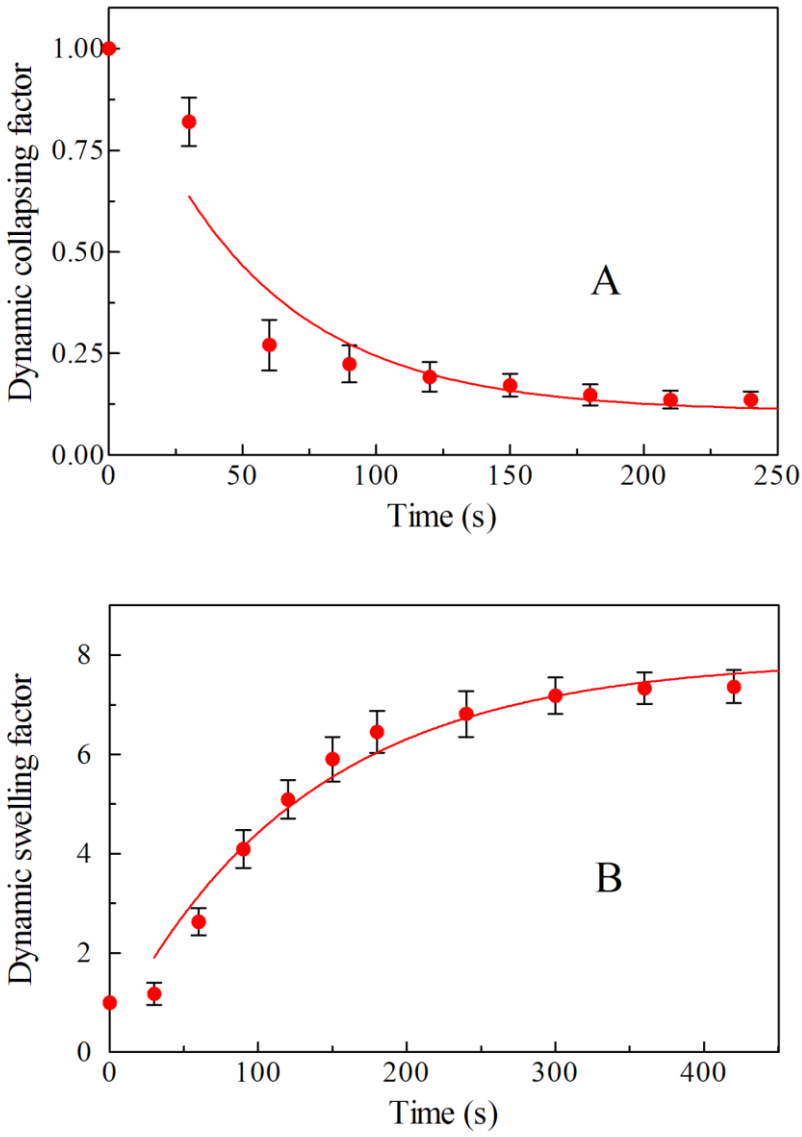


Figure 7. Dynamic collapsing factor (panel A) and dynamic swelling factor (panel B) of poly(NIPAAm-co-NIPMAAm) microgels in simulated physiological conditions (PBS at pH = 7.4). The continuous lines in panels A and B were calculated according to the one-phase dissociation and association equations (GraphPad Prism version 5.0), respectively, with the following sets of parameters: panel A, plateau = 0.11 and k = 0.019 s^{-1}; and panel B, plateau = 7.9 and k = 0.0077 s^{-1}. The lag time of about 30 s reflects the time that is necessary to reach the desired temperature (for details see text). Data are the results of three independent experiments.

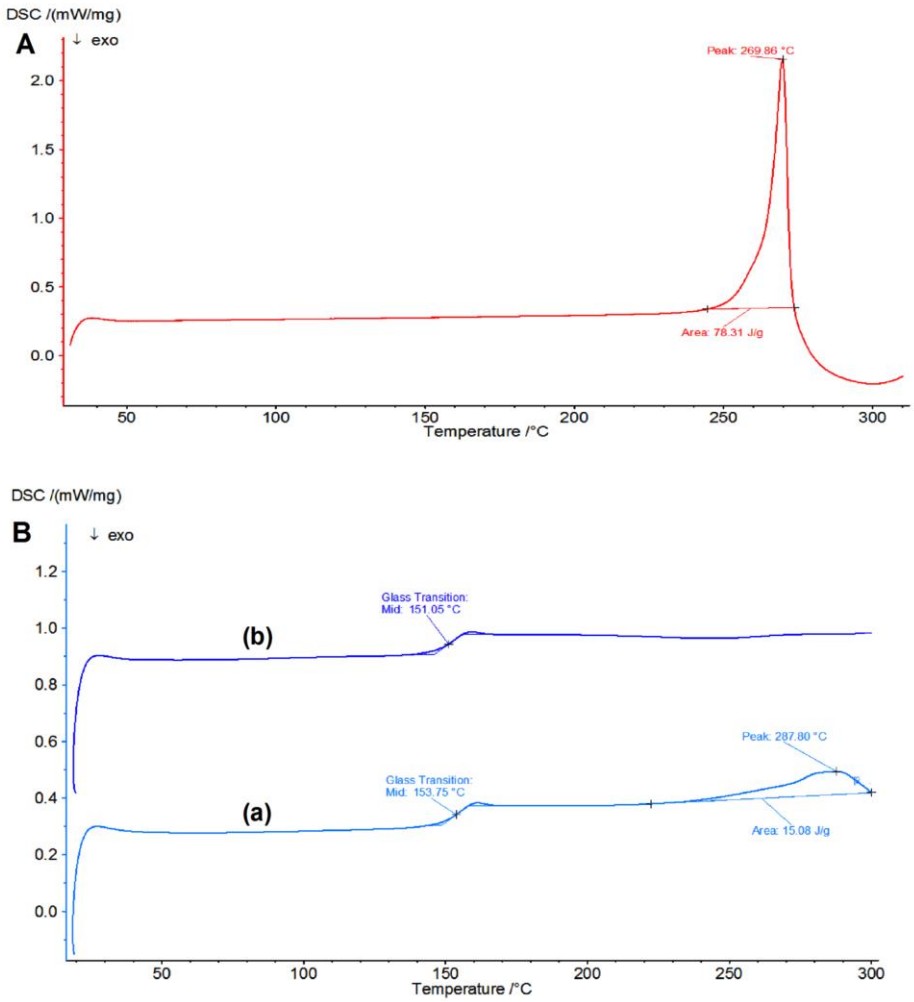


Figure 8. DSC thermograms of dexamethasone (panel A), of the poly(NIPAAm-co-NIPMAAm)/dexamethasone physical mixture (panel B (a)), and of dexamethasone loaded by poly(NIPAAm-co-NIPMAAm) microparticles (panel B (b)).

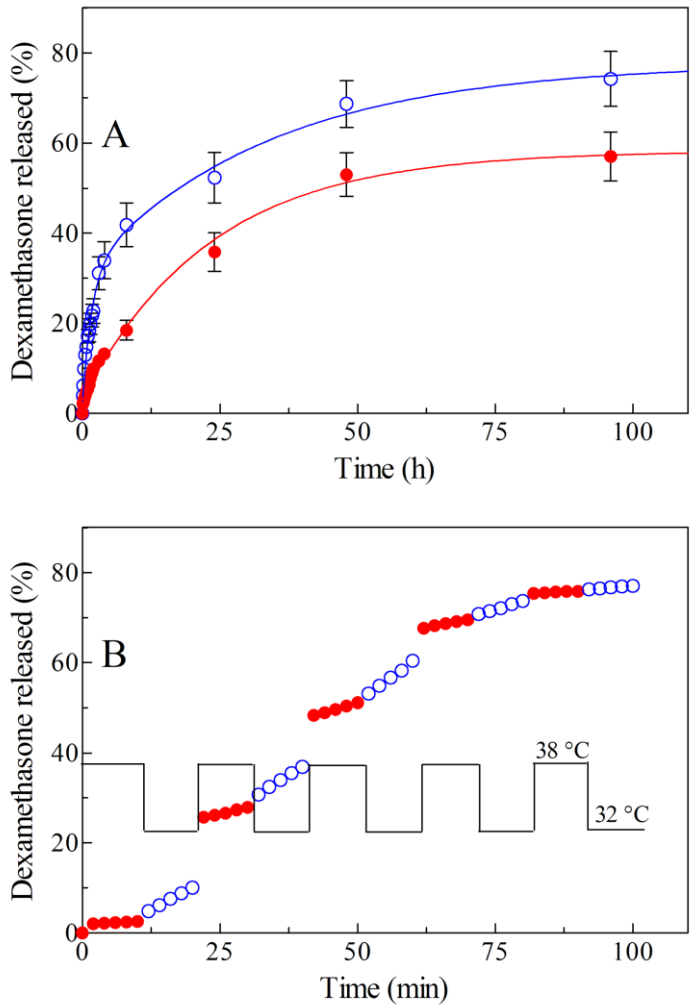


Figure 9. Influence of temperature (panel A) and of cyclically temperature change (panel B) on dexamethasone release from the poly(NIPAAm-co-NIPMAAm) microgels. Data were obtained in simulated physiological conditions (PBS at pH = 7.4) at 32 °C (○) and 38 °C (●). The continuous lines in panel A were calculated according to the one-phase and the two-phase association equations (GraphPad Prism version 5.0), respectively, with the following sets of parameters: (○), plateau = 77.7, fast phase = 37.8 %, slow phase = 62.2 %, k_{fast} = 0.57 h^{-1}, and k_{slow} = 0.030 h^{-1}; and (●), plateau = 58.2 and k = 0.043 h^{-1}. Data are the results of three independent experiments.

Table 1. Dependence of LCST on the co-monomer ratio in the feed and in the copolymer. The concentration of the copolymer solution was 1 % (w/v).

Sample	**Co-monomer composition**				**LCST (°C)**		
	In the feed (% mol ratio)		**In copolymer (% mol ratio)**		**pH=7.4**	**pH=1.2**	**H_2O**
	NIPAAm	**NIPMAAm**	**NIPAAm**	**NIPMAAm**			
S_0	100	0	100	0	29.9±0.2	31.6±0.2	32.6±0.2
S_1	66.67	33.33	55.00	45.00	35.9 ± 0.3	-[a)]	-[a)]
S_2[b)]	60	40	51.00	49.00	36.8±0.3	38.2±0.3	38.8±0.2
S_3	50	50	41.75	58.25	38.3±0.3	-[a)]	-[a)]
S_4	33.33	66.67	28.75	71.25	40.9±0.2	-[a)]	-[a)]
S_5	0	100	0	100	44.7±0.3	45.0±0.2	45.5±0.3

Data are the results of two independent experiments

[a)]not done; [b)]M_n = 89,543 g/mol, M_w = 108,483 g/mol, IP = 1.212; (IP, index of polydispersity).

Porous microgels with excellent swelling/collapsing characteristics around the human body temperature (36.8 °C) have been obtained from **a copolymer with the appropriate molar ratio of the two co-monomers *N*-isopropylacrylamide and *N*-isopropylmethacrylamide** characterized by similar properties. The microgels release dexamethasone by a pulsatile mechanism when the temperature is cyclically modified from below to above the critical temperature.

G. Fundueanu*, M. Constantin, S. Bucatariu, P. Ascenzi

Poly(N-isopropylacrylamide-co-N-isopropylmethacrylamide) Thermo-responsive Microgels as Self-regulated Drug Delivery System