

Regulating the thermal response of PNIPAM hydrogels by controlling the adsorption of magnetite nanoparticles

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Abstract Thermoswitchable magnetic hydrogels are being extensively investigated because of their great potential for medical applications. Indeed, they can behave as smart carriers able to transport drugs to a chosen part of the body and release them via magneto-thermal activation by an external alternating magnetic field. We report on the magnetization of the thermosensitive poly(N-isopropylacrylamide) hydrogel through the adsorption of controlled amounts of magnetite nanoparticles. We show that the temperature at which the hydrogel contraction occurs (i.e. the lower critical solution temperature) can be controlled from 32 °C to 52 °C by varying the concentration of adsorbed nanoparticles. This is clearly shown by photon correlation spectroscopy. The results are an advance in the use of the magnetized poly(N-isopropylacrylamide) hydrogel as a flexible and adjustable nanomaterial and are of great interest in numerous applications which require drug release on demand.

1 Introduction

Sub-micrometric drug carriers, that are able ‘to move’ and ‘to eject’ a drug on demand in a specific part of the human body with a minimum risk for health, represent a highly desirable goal in several biomedical applications. In the last 5 years the research in this direction focused on magnetic composite materials, and in particular on the one formed by

the polymer poly(N-isopropylacrylamide) (PNIPAM) and magnetic nanoparticles [1–7].

PNIPAM is able to generate hydrogels and belongs to the class of the so-called smart polymers. In fact, when heated in water above 32 °C (lower critical solution temperature, LCST), it shrinks via a reversible phase transition from a swollen hydrated state to a shrunken dehydrated state. During this process PNIPAM contracts and behaves like a hydrostatic pump, ejecting outside the inner water with its solute [7–10]. In these hydrogels the presence of magnetic nanoparticles can provide a remote temperature control mechanism via inductive heating, by which the energy is transferred from an external alternating magnetic field to the magnetic nanoparticles [11–16].

Since the LCST of the pure PNIPAM (32 °C) is lower than the normal temperature of the human body (37 °C), tuning the LCST of PNIPAM is a crucial goal for biomedical applications of this smart material [17]. Therefore, the development of a method for the control of the LCST and the achievement of a LCST higher than 37 °C is of great significance for the control of drug release after the injection of the hydrogel in the body. Indeed, no control of the delivery could be exerted if the expulsion occurred at body temperature, that is, immediately after injection. It is important to remark that the use of magnetic nanoparticles not only provides a means to switch on the drug delivery by inductive heating (remote control), but it also provides a versatile method to target the material to a chosen part of the body by using an external static magnetic field [18–22].

Although very little literature exists on the effect that the inclusion of nanoparticles has over the LCST of thermosensitive hydrogels, recently it has been shown that the inclusion of magnetite nanoparticles (MNPs) can indeed increase the LCST of PNIPAM [6]. Based on this finding, in this paper we demonstrate the possibility to tune the LCST of

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Table 1 Properties of the produced PNIPAM/MNP hydrogels

Sample	Added MNP ^a (mg)	Added MNP ^a (mL)	% Fe ₃ O ₄ in PNIPAM (w/w ^b)	M_s (emu/g)	LCST (°C)
<i>O</i>	0	0	0	0	32
<i>A</i>	8.4	0.4	40	9.44	32
<i>B</i>	10.5	0.5	30		30
<i>C</i>	12.6	0.6	27		35
<i>D</i>	14.7	0.7	49.4	29.77	44
<i>E</i>	16.8	0.8	65.14	35.28	50
<i>F</i>	21.3	1.0	67.5		49
<i>G</i>	31.5	1.5	70.76	45.80	52

^aAqueous MNP suspension 21 mg/mL, pH = 10;

^bConcentration measured by TGA

PNIPAM by controlling the concentration of the MNPs. We also define the maximum LCST variation achievable by this method.

In the literature, the synthesis of magnetic PNIPAM has followed mainly three approaches: the first approach consists in the fabrication of core-shell particles where the core is magnetic and the shell is made of PNIPAM. In the second approach the PNIPAM hydrogel is used as a template for the deposition of MNPs [23]. The third consists in the synthesis of magnetic microcapsules with a supra-colloidal structure obtained by suspension polymerization based on Pickering emulsion droplets [24]. In our article we followed the second approach. In all cases the magnetic moment of the resulting magnetic hydrogels shows predominantly the superparamagnetic behavior of the nanoparticles used in their fabrication. The magnetic PNIPAM we report on in this article is tailored for a wide range of temperatures in order to be potentially effective for medical applications in the different parts of the body to be cured [14, 25].

2 Experimental section

2.1 Materials

Bare MNPs of about 10 nm diameter [26] were obtained by chemical co-precipitation of a stoichiometric mixture of Fe²⁺ and Fe³⁺ salts (FeSO₄ and FeCl₃, respectively) with ammonium hydroxide [27]. All the reagents were purchased from Aldrich and used without further purification.

The PNIPAM hydrogels were prepared by surfactant-free radical polymerization [6, 24] under nitrogen atmosphere in 50 mL of boiled Milli-Q water using an aqueous solution of N-isopropylacrylamide 0.1 M (550 mg) and the cross linker N,N-methylenebis-acrylamide, 0.007 M (50 mg). First the temperature was raised to 80 °C and 250 mg of ammonium persulfate were added to start the polymerization. After 10 min the temperature was decreased to 70 °C. After

15 min from the start of the polymerization, sodium acrylate (40 mg) was added to generate a shell enriched with carboxylic groups.

The mixture was refluxed for 4 h in N₂ atmosphere and, subsequently, the warm hydrogels were purified by centrifugation at a relative centrifugal force of 5591 g for 3 min. After the centrifugation, the tube used for centrifugation was heated back to 70 °C to make the PNIPAM deposit visible. The deposit was separated and re-dispersed at room temperature in 50 mL of Milli-Q water.

PNIPAM/MNP hydrogels were obtained by adsorbing MNPs on the shrunken dehydrated state of PNIPAM. The MNP suspension is characterized by a pH value of about 10. In order to shrink PNIPAM, the hydrogel was heated to 50 ± 1 °C in N₂ atmosphere and different amounts of MNPs in water were added to form the composite. The amount of added MNPs, ranging from 0.4 mL to 1.5 mL (samples A and G respectively in Table 1), was diluted in 50 mL of freshly synthesized PNIPAM solution. Thus, the final pH of PNIPAM/MNP suspensions varied between 5 and 6 depending on the added volume of the MNP suspension. All the other parameters were kept constant in all syntheses. The magnetic hydrogels in the shrunken state at 50 ± 1 °C allowed the magnetic separation of PNIPAM/MNP by leaving it overnight (≅12 h) at 50 ± 1 °C in close proximity of a NdFeB magnet with a surface magnetic field of 0.48 T. The removal of the supernatant liquid left the PNIPAM/MNP deposit ready to be tested.

2.2 Characterization methods

Thermogravimetric analyses (TGA) were performed on magnetic hydrogels dried in air at 70 °C. A STA1500 system (simultaneous thermal analyzer) was used in air. A temperature ramp of 5 °C/min was used.

Photon correlation spectroscopy (PCS) was carried out using a Malvern zeta sizer 3000 HS system (Malvern Instruments Ltd., Malvern, UK) equipped with a 10 mW He-Ne 633 nm wavelength laser with a beam diameter of 200 μm

working at a fixed scattering angle of 90° at temperatures from 25°C to 55°C with 5°C increments. We assumed the optical absorption of the laser radiation by the MNPs to be negligible, as the absorption coefficient by magnetite for 633 nm radiation is 10^6 m^{-1} [28]. This means that no local heating of the magnetic nanoparticles caused by the absorption of the laser light should be expected.

A delay time of 10 min was used at each temperature to ensure that the sample viscosity reached equilibrium before the measurements were taken. The suspension of microgels was diluted to a concentration of 0.02 % (w/w) with a NaCl solution of concentration 10 mM to prevent coagulation and to enhance heat dissipation.

Scanning electron microscopy (SEM) observations on dried samples were performed using a Zeiss FEG-SEM LEO 1530 electron microscope at 5.00 kV equipped with an energy dispersive X-ray spectrometer (EDX). To collect good quality images, SEM was performed on samples coated with gold films about 15 nm thick and deposited by Ar sputtering.

Magnetic characterizations were performed by using a vibrating sample magnetometer (VSM). Magnetic hysteresis loops were obtained by cycling the field between -1 T and 1 T at room temperature.

3 Results and discussion

In this article PNIPAM doped with acrylate was used as a template to adsorb MNPs [6]. The acrylate strongly interacts with Fe atoms at the MNP surface providing a binding mechanism for the MNP–PNIPAM composite formation [23]. Thermogravimetric analysis (TGA) was used on dried hydrogels to measure the amount of magnetite adsorbed on PNIPAM. Photon correlation spectroscopy (PCS) was used to estimate the mean size of the hydrodynamic radius of the hydrogel aggregates [29, 30]. All the experimental details and characterization results are reported in Table 1.

First, we tested the magnetic properties of PNIPAM/MNP samples. The magnetization was measured on a set of samples {A, E, D, G} in order to test a wide range of compositions. Results have been normalized to the total sample mass in order to obtain comparable figures.

In Fig. 1 magnetization curves are shown as a function of the applied magnetic field. PNIPAM/MNP samples are mostly superparamagnetic, but showed a slight hysteresis in the magnetization cycles, as can be observed in the inset of Fig. 1. We have observed a similar hysteresis also in the pristine MNPs used in the present work. For this reason, we attributed the hysteresis of PNIPAM/MNP samples to the pre-synthesized MNPs used for the PNIPAM magnetization. The samples reached different values of saturation magnetization. Sample A showed the lowest saturation magnetiza-

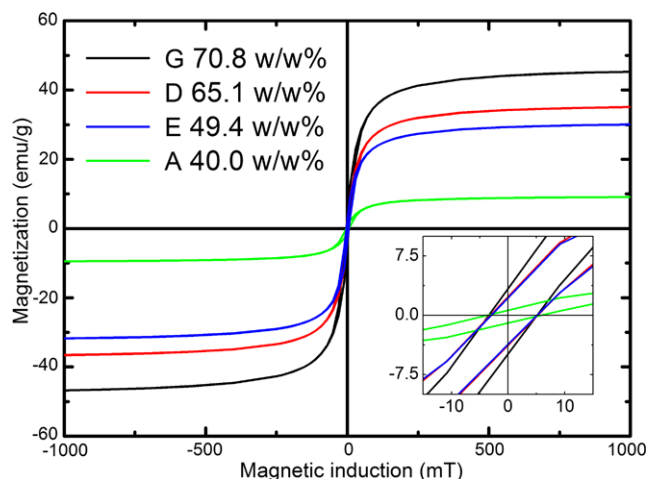


Fig. 1 Magnetization cycles of PNIPAM/MNP hydrogels at different % weight concentrations of MNPs in PNIPAM

tion, followed by samples E and D which had similar saturation magnetizations and then by G, which had a slightly higher magnetization. The large difference of $\sim 25\text{ emu/g}$ between the magnetizations of sample A and all the rest confirmed that it contained less magnetite than the others.

After having magnetically characterized the developed materials, we moved to the investigation of the fine control of the thermal response achieved in these PNIPAM/MNP hydrogels. The compositional results and the corresponding LCSTs of the obtained magnetic hydrogels are shown in Fig. 2a. The LCST was obtained by measuring the hydrodynamic radius of the hydrogel microparticles by PCS as a function of temperature.

The MNP concentration in the hydrogels (open red circles) and the LCST of the magnetic PNIPAM hydrogels (blue dots) are both shown as functions of the MNP content. The samples A, E, D, G are highlighted in the figure. MNP concentrations varying from 15 % to 65 % (w/w) were obtained. Sample A has the lowest added amount of Fe_3O_4 but not the lowest w/w concentration. This apparently contradictory result can be explained by the fact that magnetic separation, which is one of the steps in the fabrication process, is less effective at lower concentrations. Sigmoidal trends were obtained for both concentration and LCST. Adsorption of MNPs on PNIPAM reached a plateau at 65 % w/w, due to saturation of the adsorption loci. The adsorption of MNP on PNIPAM is mainly controlled by the presence of carboxylic groups on the microgel's surface [23]. The saturation occurs when all the carboxylic groups have been anchored to the magnetite surface. At this concentration the LCST of the samples showed an abrupt increase due to the steric hindrances of the nanoparticles on the polymer that prevented the polymer chains from aggregating above the LCST [23]. It is important to note that a LCST lower than 40°C was achieved at MNP concentrations smaller than 50 % w/w. On

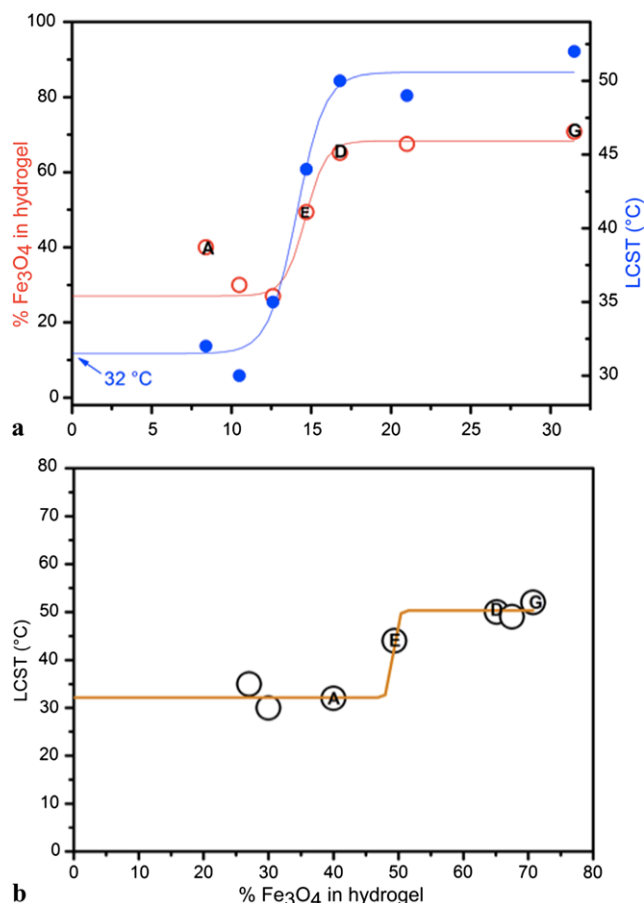


Fig. 2 (a) Red open circles: magnetite concentration (% w/w) in the magnetic PNIPAM hydrogels as measured by TGA. Samples A, E, D, G are highlighted in the figure. The blue disks represent the LCST of each magnetic hydrogel. The red and blue sigmoidal curves are guides for the eye; (b) LCST of PNIPAM/magnetite at different magnetite concentrations in the hydrogels. Samples A, E, D, G are evidenced by the open circles. The orange line is a guide for the eye

the other hand, a LCST greater than 45 °C was achieved when the MNP content was 65 % w/w.

In Fig. 2b we emphasize the effect of adsorbed MNP on the LCST and we show for the first time the sigmoidal trend of the LCST as a function of MNP concentration. The sigmoidal curve in the figure is a ‘guide for the eye’ that best represents the trend of the experimental data. These results allowed us to put in relation MNP concentration and LCST.

Samples A, E, D, G were also tested by energy dispersive X-ray spectrometry (EDX). It is worth noting that the resulting elemental analyses were in good agreement with the results from TGA (Fig. 3).

The morphology of PNIPAM/MNP was investigated by SEM on lyophilized samples. As an example, Fig. 4 shows a SEM micrograph of sample F after lyophilization. Separate PNIPAM/MNP microparticles are visible, as highlighted by the white circle. At the bottom of the figure, the microparticles are fused together and the lyophilized sample appears as a smooth combination of ‘sheets’ and ‘branches’. The

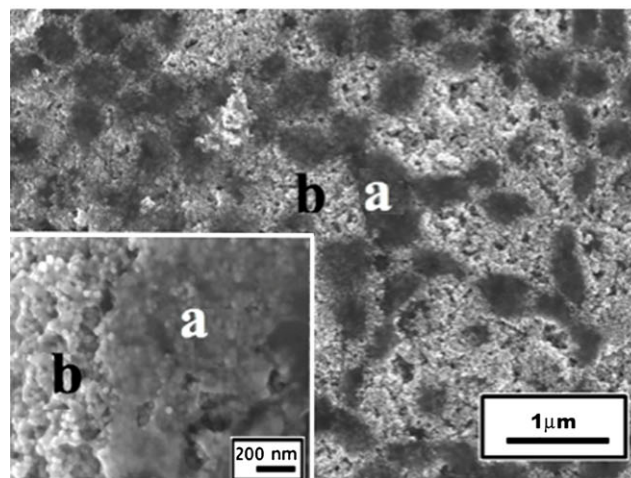
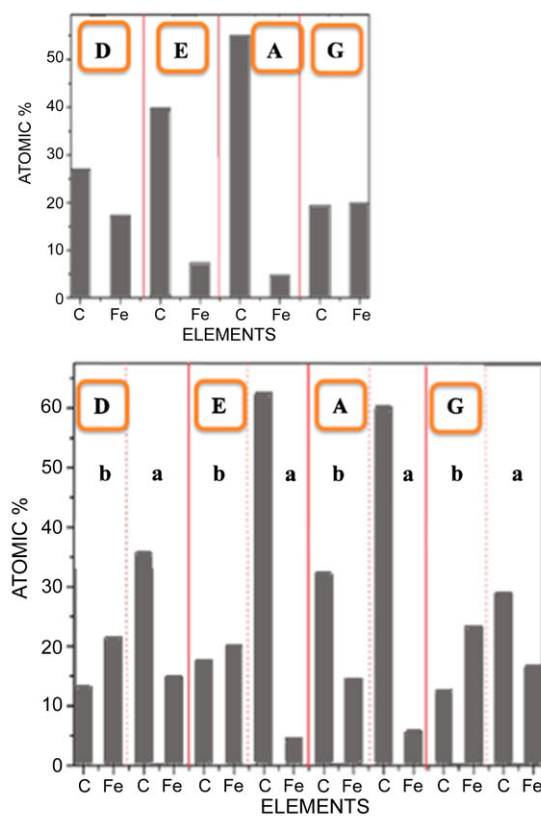


Fig. 3 Elemental analysis of dried magnetic PNIPAM hydrogels: elemental compositions measured by energy dispersive X-ray microanalysis (EDX) of the dried samples of the magnetic hydrogel (samples D, E, A, G) are shown in the graph. The magnetic hydrogels generate two distinct regions after drying, named *a* and *b* and evidenced in the SEM image on a drop-cast sample on silicon (sample D is taken as an example). SEM/EDX microanalyses were performed on 10 μm × 10 μm wide windows that comprised both *a* and *b* regions to extract the average compositions (top graph) and on 0.5 μm × 0.5 μm wide windows to select only one of the two regions (bottom graph). In the dried samples the area marked by *a* appears to be made of submicrometric dark circles in which magnetic nanoparticles are embedded, while the area *b* appears as an aggregate of magnetite particles

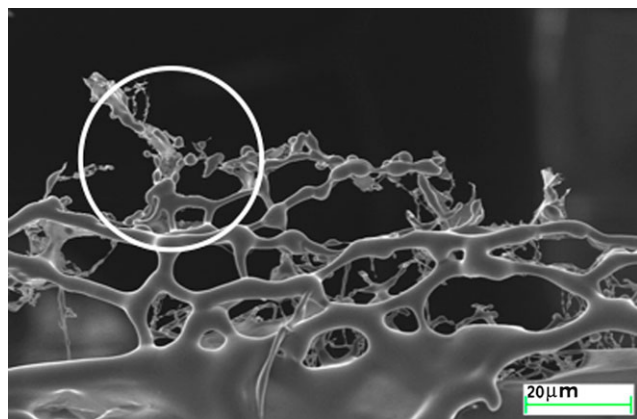


Fig. 4 SEM micrograph (EHT = 5.00 kV, WD = 4 mm, Signal A = InLens) of lyophilized PNIPAM/MNP hydrogel (sample F). The circle highlights a zone where PNIPAM/MNP composite microparticles are still visible as separate entities. In the bottom part of the figure the microparticles are fused into smooth 'sheets' and 'branches' made of the composite material

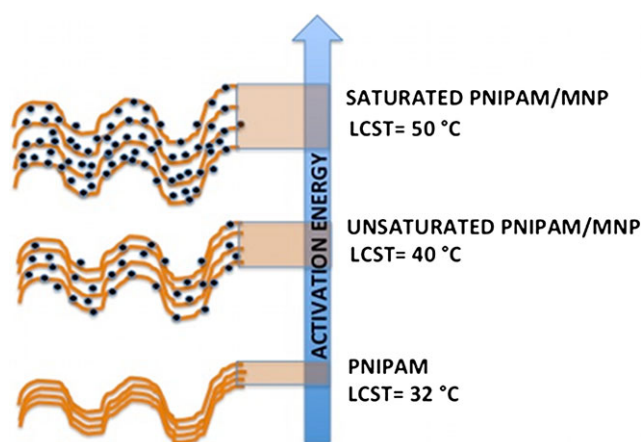


Fig. 5 Schematic diagram of the model for the hindrance effect of MNPs on PNIPAM chain aggregation. The diagram shows graphically how MNPs hinder the collapse of the PNIPAM chains; the steric hindrance caused by the presence of MNPs is an obstacle to their packing. We chose to visualize this effect with the width of the packed PNIPAM chains as a function of the amount of adsorbed MNPs. The higher the hindrance, the more difficult is the polymer aggregation at the phase transition into the dehydrated phase and the higher the LCST required for the packing of the chains

presence of Fe in the sample was verified by EDX, although MNPs were not directly imaged in the SEM micrographs. We believe that this was due to the fact that MNPs are embedded in the PNIPAM matrix and therefore cannot be directly imaged.

The phase transition in PNIPAM is a thermally activated process: an increase in the LCST corresponds to an increase of the activation energy of the process. In Fig. 5 we present a pictorial model for both PNIPAM and PNIPAM/MNP hydrogels. The model shows graphically how MNPs hinder the collapse of the PNIPAM chains; the steric hindrance caused

by the presence of MNPs is an obstacle to their packing. We chose to visualize this effect with the width of the packed PNIPAM chains as a function of the amount of adsorbed MNPs. The more difficult the polymer aggregation at the phase transition into the dehydrated phase, the higher the LCST required for the packing of the chains [23]. We suppose that the difference between the LCST of pure PNIPAM (32 °C) and the LCST at the plateau could be attributed to the hindrance effect of the MNPs in the packing of the PNIPAM/MNP hydrogels and supports the model visualized in Fig. 5.

Nevertheless, the specific effect of the MNP interactions cannot be excluded as reported for a thermosensitive copolymer like acetoacetoxyethyl methacrylate (AAEM) and N-vinylcaprolactam (VCL) by Pich et al. [6].

It is important to note that the LCST of pure PNIPAM is conserved for all the concentrations below 48 % w/w. Hence, the LCST trend demonstrates that there is a threshold MNP concentration below which steric hindrance of the adsorbed MNPs does not modify the LCST of PNIPAM. In our model this limit corresponds to the maximum quantity of adsorbed MNPs that still allows the aggregation of PNIPAM in the dehydrated phase without any increment of the activation energy. Above a concentration of 48 % w/w, MNPs start to hinder chain aggregation and an abrupt increase of LCST is observed.

4 Conclusions

We presented for the first time a technique that allows the fine control of the LCST of the PNIPAM hydrogel. A wide range of LCST values, ranging between 32 °C and 52 °C, demonstrates that PNIPAM is a highly tunable thermosensitive polymer. The sigmoidal trend in the adsorption of MNPs on the PNIPAM hydrogels supports the proposed model by which the PNIPAM hydrogel is a template for the deposition of MNPs. In particular, this is confirmed by the existence of a maximum in the quantity of adsorbed MNPs which would not exist if the process consisted in a simple mixing of two materials.

We showed that the PNIPAM/MNP w/w ratio is fully responsible for the thermal response and magnetic properties of these hydrogels. The results support the aim of this article to propose custom-made hydrogels for targeted medical applications.

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