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MICROSTRUCTURED HYDROGELS WITH MODULATED TRANSITION TEMPERATURE FOR POSITIVE CONTROL RELEASE

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In this work we prepared microgels of N-isopropylacrylamide (NIPAAm) with the swollen-unswollen temperature transition modulated above the body temperature (37 °C), by copolymerization with acrylamide or N,N-dimethylacrylamide. The microgels were used to produce microstructured hydrogels of NIPAAm, also with the temperature modulated with the comonomers. We found that the microstructured hydrogels present fast temperature response when temperature is changed from 37 °C to 42 °C. The shrinking-swelling process was reversible when the temperature was cycled between the two temperatures. However, since the temperature induced transition of the hydrogels was continuous, the change in degree of swelling between the two temperatures was smaller than the change between 25°C and 42°C. Solute release studies shows fast release of vitamin B_{12} without temperature control, but temperature controlled release of cytochrome C.

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Keywords: Hydrogels, microgels, NIPAAm, Transition temperature, Drug release

1. Introduction

Hydrogels have become very important for a range of biotechnology applications including drug delivery [1,2], tissue engineering [3,4], artificial muscles [5], wound dressings and diagnostics; due to their unique properties such as high water content, softness, flexibility and biocompatibility [6–8]. These materials can be prepared with different sizes, presenting differences on the speed of response to different environmental stimuli such as temperature, pH, ionic strength, and chemical species [9,10]. Nanogels and microgels are of particular interest given their fast response due to their reduced dimensions [11].

Thermosensitive polymeric materials present changes in the material structure below and above a transition temperature, Ttr, related to the lower critical solution temperature (LCST) [12,13]. Among temperature sensitive hydrogels, poly(N-isopropylacrylamide) (PNIPAAm) is the most commonly studied material. It has a swollen-shrunk Ttr around 32 °C, which is near the human body temperature, and thus has applications in biomedicine [14–16]. Additionally, since the Ttr of PNIPAAm can be adjusted to be higher than the body temperature and, it could be used for positive temperature controlled release; this is, for the release of the loaded drug when the temperature rises above the Ttr, and the hydrogels shrink. This tuning can be obtained by copolymerizing with hydrophilic or ionizable monomers [17–20], such as acrylic acid [21], methacrylic acid [22], poly(N-vinylcaprolactam) [23], N,N-(dimethyl amino)ethyl methacrylate, polyethylene glycol [24], etc. Positive release control is relevant since increase in body temperature above normal occurs in certain pathologies, or can be induced either locally or systemically [25,26].

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The main problems suffered by hydrogels are poor mechanical properties [27], and slow response rates [28]. Additionally, NIPAAm hydrogels present a skin like formation in the surface that limits shrinking and drug delivery, when heated above their Ttr [29]. The solution to such problems have been approached in different opportunities by incorporating nanoparticles in the hydrogels which generates fast shrinking and improved mechanical properties [30,31].

We previously studied NIPAAm hydrogels containing NIPAAm microgels that present fast temperature response and improved mechanical properties. We proposed that when the swollen microstructured hydrogels are exposed to temperatures above their Ttr, fast shrinking of the included microgels generates pores that allow the expulsion of water during the shrinking of the (macro)hydrogels. Those materials presented reproducible swelling-shrinking cycles when temperature was cycled below and above its Ttr (32 °C), producing a pulsed drug release in response to temperature changes between 25 °C and 42 °C [32].

Pulsed drug release is very important for improving the therapeutic effect of many bioactive molecules, particularly hormones [33–35]. However, we require temperature sensitive materials with Ttr above 37 °C so pulsed drug release occurs when temperature rises above this temperature to a maximum of 42 °C, a temperature below the threshold for body heat sensors [36].

In this work we tuned the transition temperature of NIPAAm microgels and microstructured hydrogels by copolymerization with N,N-dimethylacrylamide or acrylamide. Modulated temperature transition materials were used for the temperature controlled release of model substances vitamin B_{12} and cytochrome C.

2. Experimental

N-Isopropylacrylamide NIPAAm (TCI, 98%) was recrystallized from n-hexane. N,N-dimethylacrylamide (DMA) (Sigma-Aldrich) and ethyleneglycoldimethylacrylate (EGDMA) (Aldrich, 98%) were purified by passing for an inhibitor removing resin (Aldrich). Acrylamide (AAm) (Sigma-Aldrich), N,N-methylenebisacrylamide (BIS) (Aldrich, 98%), ammonium persulfate (APS) (Aldrich, 98%), N, N, N, N-tetramethylethylenediamine (TEMED) (Aldrich, 99.5%), Vitamin B_{12} (Sigma Aldrich) and cytochrome C (Sigma Aldrich) were used as received. Milli-Q water was filtered through a filter with a 0.22μm pore size.

Statistical analysis was performed by two way ANOVA using PRISM (GraphPad Software Inc, San Diego CA).

2.1 Synthesis of microgels (MG)

Microgels were prepared through dispersion polymerization. NIPAAm was copolymerized with AAm (10, 15 and 20% mol%) or DMA (10, 20, and 30 mol%), crosslinked by EGDMA 3 mol% with respect to the monomers. The monomers were added in Milli-Q water. The solution was bubbled with nitrogen to remove oxygen, then heated to 85 °C, before adding initiator APS 2% w/w with 200 rpm stirring for 45 min. After that, the reaction mixture was placed in an ice bath to stop the reaction. The resulting dispersion was dialyzed against water for 5 days using a 14 kDa membrane (Spectrum Laboratories). Microgels were finally lyophilized.

2.2 Characterization of microgels

The microgels were characterized by FTIR-ATR with a Thermo Scientific model Nicolet Is5 spectrometer in ATR mode using 16 scans, taken in the range of 4000-600 cm^{-1}

Microgels were reconstituted in MilliQ water with a concentration of 1 mg/mL, the hydrodynamic diameter (Dh) was obtained by dynamic light scattering (DLS) using the Zetasizer (DTS1060; Malvem Instruments, Miami FL), with a green laser 532 nm with a reading angle of 173°.

The size distribution is reported in volume and the effect of temperature on the microgels was studied between 20 to 50 °C.

STEM images of the microgels were acquired with a Field Emission Scanning Electron Microscope, Model JSM-7800F Prime. For this, a droplet of nano/microparticulate dispersion (0.4 w%) was spread onto the surface of a 400- mesh copper carbon grid. Two droplets of uranyl

acetate (2 w%) were added to the copper carbon grid. The samples were vacuum-oven dried at 22 °C for 24 h. The dried specimens were clamped onto a STEM specimen rod, inserted into the sample chamber, and observed at 25 kV.

2.3 Synthesis of copolymeric hydrogels

Hydrogels were prepared with NIPAAm and AAm or DMA at 0, 10, 20 mol% and crosslinker N-methylenebisacrylamide 3% w/w. Monomers were dissolved in Milli Q water, and stirred for 15 min, then APS 0.15 M and TEMED 0.15 M were added and shaken for 1 min. The solution was placed between two 10 x 10 cm glass plates for 24 h at 4 °C. The obtained hydrogels were cut into 1 cm (diameter) discs and were washed with water for 5 days.

Microstructured hydrogels were prepared in the same fashion but a certain weight of microgels was introduced in the feed recipe to obtain the desired composition.

2.4 Scanning electron microscopy of hydrogels

The structure and morphology of lyophilized samples of the hydrogels were studied by scanning electron microscopy (Field Emission Scanning Electron Microscope, Model JSM-7800F Prime).

2.5 Determination of equilibrium swelling ratio

The swelling equilibrium was determined for hydrogels in water at temperatures ranging from 20 to 50 °C. After immersion in distilled water at a predetermined temperature, the hydrogels were removed, the excess water on the hydrogel surface was eliminated, and then the hydrogel was weighed. Degree of swelling, Q, was determined by:

$$Q = \frac{(Ws - Wd)}{Wd}$$

Where Ws is the weight of a hydrogel swollen to equilibrium at a given temperature, and Wd is the weight of the dry gel. The Ttr is defined as the temperature at which a maximum weight change occurs. Measurements were made by triplicate.

2.6 Determination of kinetic swelling of hydrogels

The swelling kinetics of hydrogels was determined gravimetrically. The dried hydrogels were placed in water at 25 °C, and subsequently were weighed at regular times. Finally, the swelling degree at different sampling times (Q_t) was determined by the equation,

$$Q_t = \frac{(Wt - Wd)}{Wd}$$

Where Wt is the weight of hydrogel at the sampling time and the Wd is the dry weight hydrogel. Measurements were made by triplicate.

2.7 Determination of shrinking kinetics

Dried hydrogels were weighed and placed in water at 25 °C. After 24 h, the hydrogels were weighed and immersed in water at 42 °C, during which the weights of the hydrogels were recorded at 10 min intervals. Prior to being weighed, the hydrogels surface water was absorbed with a Kimwipe. Water retentions were calculated with equation:

$$Water\ Retention = 100\ \times \frac{(Wt - Wd)}{Ws}$$

Where Wt is the weight of hydrogel at the sampling time, Wd is dry weight hydrogel and Ws is the weight of a hydrogel swollen to equilibrium at a given temperature. Measurements were made by triplicate.

2.8 Determination of oscillating deswelling-swelling kinetics

Swollen hydrogels were cycled between two sets of temperatures: 42 and 25 °C, and also 42 and 37 °C with one hour at each temperature, weighing the hydrogels every 15 min and evaluating the water retention in each sampling point. Measurements were made by triplicate.

2.9 Loading and release of model substances into hydrogels

Model substances for this study were vitamin B_{12} (1335 Da) and cytochrome C (12000 Da). The loading was done by swelling the hydrogels in a phosphate buffer pH 7.4, containing 10 mg/mL of the solutes, at 4 °C for 7 days. Releases were performed using two different temperature cycles these being 25 to 42 °C and 37 to 42 °C. Before the release study, the hydrogels in loaded media were equilibrated at the release temperature (25 or 37°C) for 2 h. Then, loaded hydrogels were placed in 3 mL of buffer pH 7.4 with stirring at 25 °C, taking a 1 mL sample every 15 min in replacing media with fresh buffer equilibrated at 25 °C. After one hour the hydrogels were placed in the medium at 42 °C. Maintaining the sampling protocol by cycling the temperature every hour, until complete release of the model substances was observed. The same procedure was performed with temperature cycles between 37 °C and 42 °C. Concentration of model substances was determined using a UV–Vis spectrophotometer at a wavelength of 361 nm for vitamin B_{12} and 528 nm for cytochrome C. The assays were performed in triplicate.

3. Results and discussion

3.1 Synthesis and characterizations of microgels

Copolymeric NIPAAm microgels (MG) were synthesized by dispersion polymerization. EGDMA was chosen as the crosslinker since good swelling/deswelling properties of microgels synthetized with this crosslinker have been observed [37,38].

Fig. 1 presents the FT-IR spectra for selected microgels. A broad band appeared between 3600 cm^{-1} and 3100 cm^{-1} (N-H stretching). Peaks appear at 1635 cm^{-1} and 1538 cm^{-1}, which are due to the presence of the amide groups (amide band I and II, respectively), and at 1385 cm^{-1} corresponding to the C-H bending vibrations of $CH(CH_3)_2$. For the microgels NIPAAm/DMA (90:10) a peak is observed at 1725 cm^{-1} corresponding to the stretching of the carbonyl group of the crosslinker EGDMA. The small intensity of the peak is due to the small proportion of the crosslinker (3 mol%). For the NIPAAm/AAm (90:10) microgels this peak overlaps with the signal at 1635 cm^{-1}, so only a shoulder is observed. Similar signals are observed for the rest of the microgels, which correspond to the signals for the chemical bonds expected in the materials.

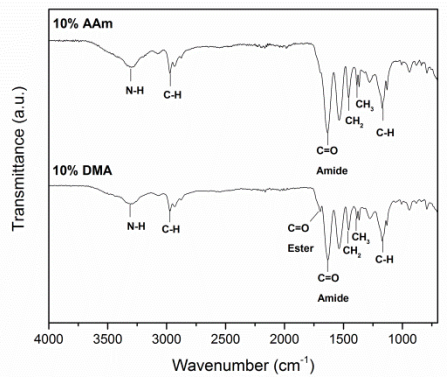


Fig. 1. FTIR of copolymeric NIPAAm microgels

Table 1 shows the average hydrodynamic diameter (Dh) at 25 °C, polydispersity index (PDI), yield, and Ttr of the microgels synthetized. Interestingly, the microgels containing DMA are smaller and decrease in size as the proportion of DMA increases. Apparently DMA has emulsifying properties which decreases the size of the microgels [39]. Figure 2 presents STEM images of microgels NIPAAm/AAm (90:10) and NIPAAm/DMA (90:10), which corroborate the micro/nanometric dimensions obtained by DLS measurements of the materials synthesized.

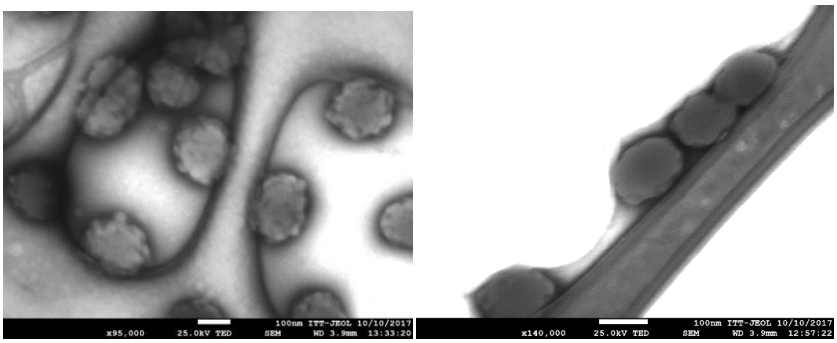


Fig. 2. STEM images of microgels: left NIPAAm/AAm (90:10), right NIPAAm/DMA (90:10)

Fig. 3 shows the effect of temperature on the size of the synthetized microgels. The Ttr was calculated as the point of maximum change of the curves (Table 1). NIPAAm MG´s shows similar size to the reported [40,41] keeping their Ttr at 32 °C. MG's containing different percentages of DMA show a minimal increase in Ttr, with Ttr of 36 °C for the microgels with 30% DMA in the feed. However, Ttr of microgels NIPAAm/AAm in different percentages is higher than body temperature, showing 38 °C at 10% and 15% of AAm and 40 °C for 20% of AAm. These results agree with the reported in the literature where the Ttr of NIPAAm was increased by these copolymers [42,43].

Table 1. Properties of microgels synthetized

	Dh (nm) @ 25 °C	PDI	Yield (%weight)	Ttr (°C)
NIPAAm	579	0.078	71	32
NIPAAm/DMA (90:10)	497	0.111	80	34
NIPAAm/DMA (80:20)	363	0.085	86	34
NIPAAm/DMA (70:30)	360	0.013	86	36
NIPAAm/AAm (90:10)	610	0.172	70	38
NIPAAm/AAm (85:15)	765	0.196	73	38
NIPAAm/AAm (80:20)	804	0.443	68	40

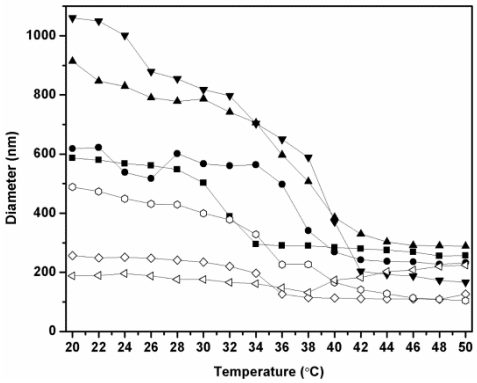


Fig. 3. Size as a function of temperature from 20 at 50 °C of different MG´s: NIPAAm (■), NIPAAm/AAm (90:10) (●), NIPAAm/AAm (85:15) (▲), NIPAAm/AAm (80:20) (▼), NIPAAm/DMA (90:10) (◇), NIPAAm/DMA (80:20) (⬡) and NIPAAm/DMA (70:30) (◁).

3.2 Equilibrium swelling of hydrogels

Fig. 4 shows equilibrium swelling degree for the hydrogels in the temperature range from 20 to 50 °C. NIPAAm hydrogels show the Ttr at 32 °C (point of maximum change) as found in the literature. Meanwhile for the copolymeric hydrogels NIPAAm/DMA (90:10) have a transition at 36 °C while the hydrogel NIPAAm/DMA (80:20) shows a continuous decrease of swelling with temperature, presenting a drop in size at 42 °C. On the other hand NIPAAm/AAm (90:10) and (80:20) show a continuous swelling transition without a clear inflection point, with the curves shifting to higher temperature as the proportion of AAm increases. The continuous transition, lacking a clear critical value, is a phenomenon attributed to inhomogeneities in the composition of the hydrogels [44]. In such case, Ttr is defined as the temperature at which the hydrogels collapsed to half of their maximum shrinkage. These results are similar to those found in the literature [42]. Table 2 presents the Ttr for the hydrogels.

Table 2. Ttr for the synthetized hydrogels

Hydrogels	Ttr (°C)
NIPAAm	32
NIPAAm/DMA (90:10)	36
NIPAAm/DMA (80:20)	42
NIPAAm/AAm (90:10)	36
NIPAAm/AAm (80:20)	36

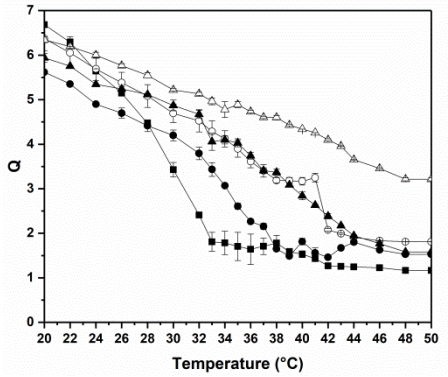


Fig. 4. Temperature dependence of equilibrium swelling degree of hydrogels NIPAAm (■), NIPAAm/DMA (90:10) (●), NIPAAm/DMA (80:20) (○), NIPAAm/AAm (90:10) (▲), NIPAAm/AAm (80:20) (△).

3.3 Swelling properties of microstructured hydrogels

According to the swelling response to temperature found in the previous sections we decide to use NIPAAm/DMA (80:20) as the copolymeric hydrogel composition of study and prepare hydrogels containing 15 w% of NIPAAm microgels, 15 and 30 w% of MG of two compositions NIPAAm/AAm (85:15) and NIPAAm/AAm (80:20), for the further studies.

Conventional hydrogels are transparent whilst microstructured hydrogels are opaque, indicating the microgels introduce inhomogenities during hydrogel synthesis. Figure 5 present SEM micrographs of conventional hydrogels NIPAAm/DMA (80:20) and microstructured copolymeric hydrogel containing 30% MG NIPAAm/AAm (80:20). Conventional hydrogel is observed as a non-porous material while the microstructured hydrogel is observed as porous materials.

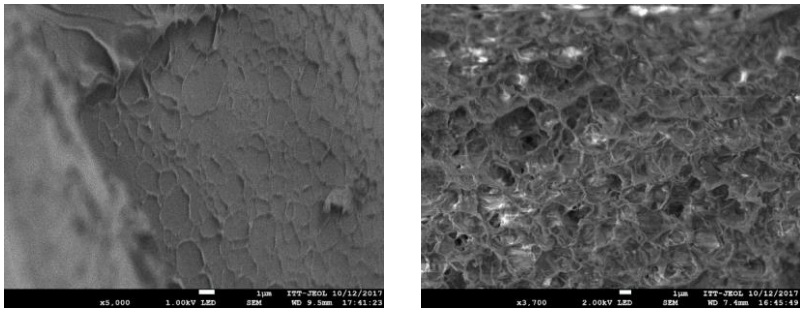


Fig. 5. SEM of hydrogels, left: conventional hydrogel, right: microstructured hydrogel.

Fig. 6 presents the effect of temperature on the degree of swelling for the microstructured gels. All of them present a continuous transition with about the same degree of swelling (P=0.20), except for the microstructured hydrogels with NIPAAm microgels which present less swelling (P<0.05).

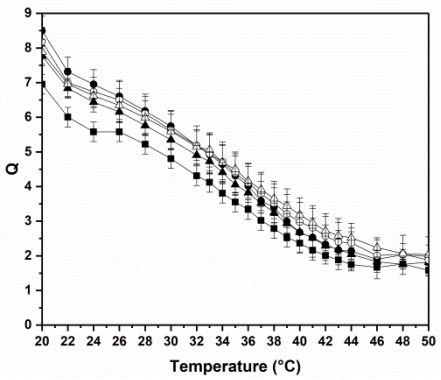


Fig. 6. Degree of swelling as a function of temperature for microstructured hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○), 30% MG NIPAAm/AAm (80:20) (△)

Fig. 7 presents swelling kinetics from dry hydrogels for the different materials studied. Equilibrium is reached in about 200 min. In general the microstructured hydrogels present faster swelling (P<0.05), and higher equilibrium swelling (P<0.05), attributed to the inhomogeneities introduce by the microgels. The highest swelling is for the hydrogels with higher proportion of microgels (30%), with the highest proportion of AAm (20%).

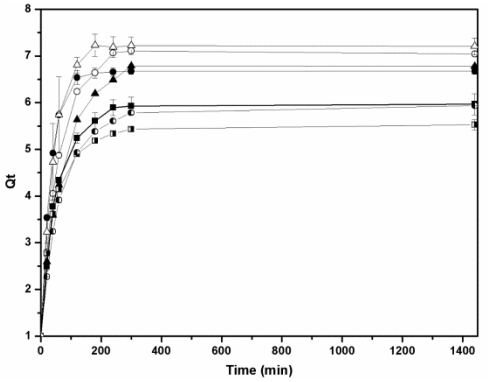


Fig. 7. Degree of swelling as a function of time at 25 °C for microstructured hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○), 30% MG NIPAAm/AAm (80:20) (△) and, for conventional hydrogels: NIPAAm/DMA (90:10) (◨), and NIPAAm/DMA (80:20) (◐).

Shrinking kinetics was determined in two different experiments. In the first the hydrogels were swollen at equilibrium at 25 °C and then placed at 42 °C. In Fig. 8 we observe that conventional hydrogels (without microgels) shrink slowly due to the formation of the skin that precludes water expulsion [29,45]. On the other hand microstructured hydrogels have fast response reaching minimum water retention at the first sampling period of 10 min (Figure 9) ($P<0.01$). The lowest water retention is observed for the hydrogel that contains NIPAAm microgels ($P<0.05$). The tendency is lower water retention for the hydrogels containing microgels with less proportion of AAm. As mentioned earlier, the fast shrinking response is produced by the formation of pores in the hydrogels due to the fast shrinking of the included microgels.

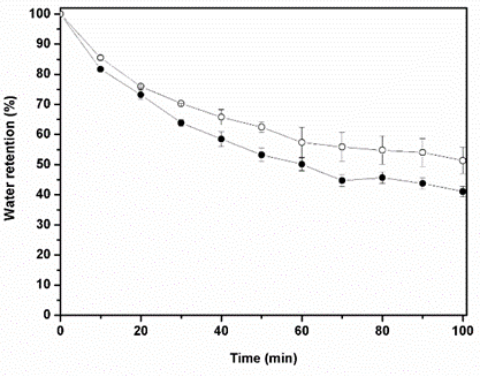


Fig. 8. Shrinking kinetics from 25 to 42 °C, for conventional hydrogels NIPAAm/DMA (90:10) (●), NIPAAm/DMA (80:20) (○).

The second study was to measure water retention after equilibration at 37 °C and heating at 42 °C. Fast response of the microstructured hydrogels is also observed in Figure 9, but with less water lost ($P<0.01$). This occurs because, given the continuity of the transition, a considerable shrinking of the microstructured hydrogels has occurred at 37 °C, as a consequence the difference in water content in the hydrogels at both temperatures is decreased.

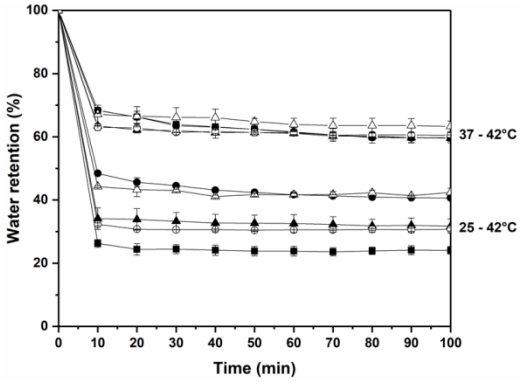


Fig. 9. Shrinking kinetics for hydrogels as a function of time at different equilibrium temperature for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (100:0) (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (⊿).

The swelling-deswelling kinetics was determined by cycling the temperature from 25 °C to 42 °C (Figure 10a) or from 37 °C to 42 °C (Figure 10b) for the microstructured hydrogels. Temperature was maintained at each temperature for 1 h. Due to the fast shrinking the process was reversible in both cases. There is about 30% difference in water retention between the swollen and shrunken states for the cycles from 25 °C to 42 °C and about 15% for the change from 37 °C to 42 °C. Again, the difference in water retention is due to the continuity of the Q vs temperature curves, since at 37 °C significant shrinking of the hydrogels had occurred.

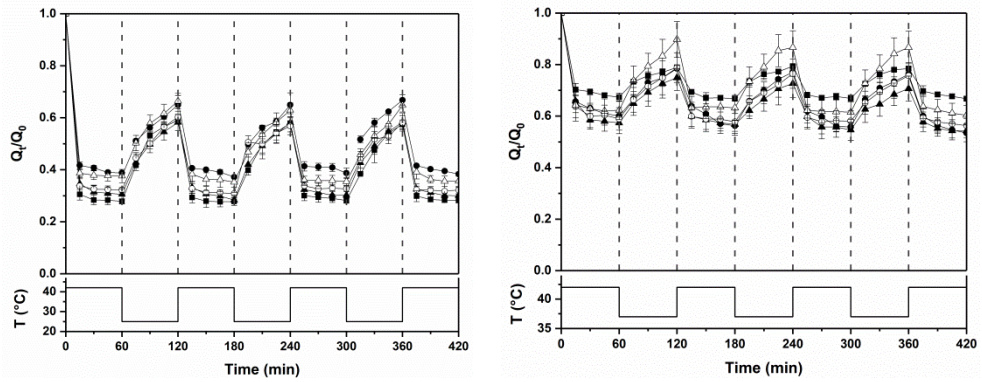


*Fig. 10. Relative swelling degree (Q_t/Q_0) with cycles of temperature **a)** 25 and 42 °C, **b)** 37 and 42 °C for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (100:0) (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (⊿).*

3.4 Release for model substances

Table 3 presents the loading of vitamin B_{12} and cytochrome C in the microstructured hydrogels. Higher loadings of cytochrome C than of vitamin B12 are observed. Loading occurs at 4 °C and the samples are equilibrated at higher temperature before the release study resulting in trapping of the higher molecular weight solute (cytochrome C).

Table 3. Loading of the solutes in the studied hydrogels

Hydrogels NIPAAm/DMA(80:20) with	Temperature before release (°C)	Loading vitamin B_{12} (mg/g)	Loading cytochrome C (mg/g)
15% NIPAAm		0.69	1.33
15% NIPAAm/AAm (85:15)		0.59	1.15
15% NIPAAm/AAm (80:20)	25	0.54	1.52
30% NIPAAm/AAm (85:15)		0.69	1.69
30% NIPAAm/AAm (80:20)		0.67	1.40
15% NIPAAm (100:0)		0.34	0.78
15% NIPAAm/AAm (85:15)		0.65	1.00
15% NIPAAm/AAm (80:20)	37	0.47	2.16
30% NIPAAm/AAm (85:15)		0.69	2.75
30% NIPAAm/AAm (80:20)		0.55	2.51

Fig. 11 presents a microstructured hydrogel loaded with vitamin B_{12} at 25 ^{o}C, during the release process.

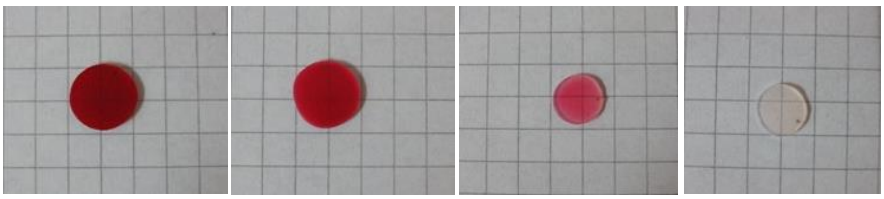
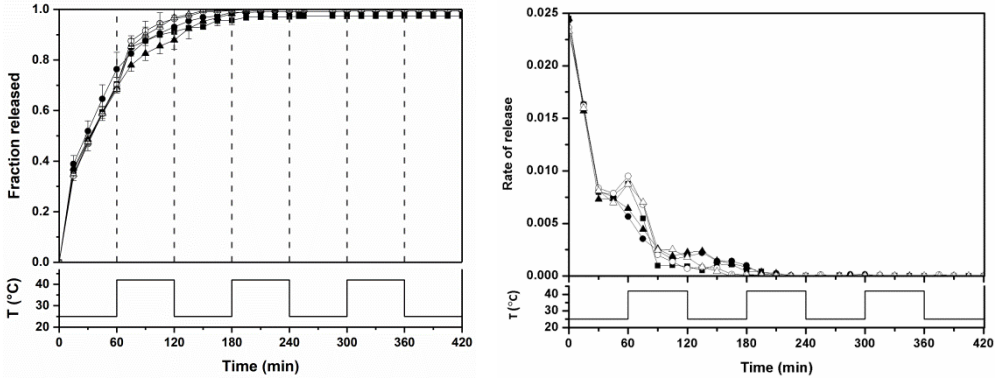


Fig. 11. Hydrogel NIPAAm/DMA (80:20) microstructured with 15 % MG NIPAAm/AAm (85:15), loaded with vitamine B_{12}, a different sampling times during the release process, a) a time 0 at 25 ℃ , b) 1 hour at 25 ℃, c) at the end of the first cycle at 42 ℃, d) at the end of the release.

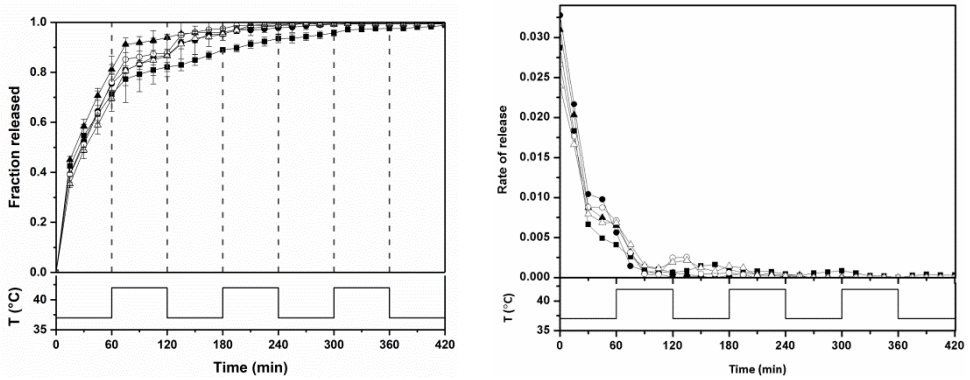
The release of Vitamin B_{12} (1335 Da) was performed by oscillating the temperature between 25 °C and 42 °C. Fig. 12a shows about 70% release in the first hour at 25 °C for all microstructured hydrogels with only slight peak on rate at 42 °C (Figure 12b) for the microstructured hydrogels with 30% MG´s. Almost complete release is observed in the first temperature cycle.



a) *b)*

Fig. 12. a) Releasing vitamin B_{12} in cycles of temperature 25 and 42 °C, and b) rate of release (mg/min) normalized by drug loading, for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (Δ).

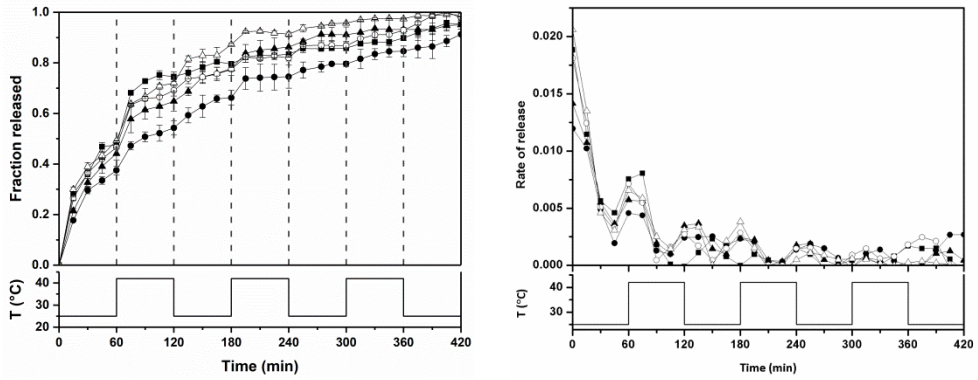
On the other release study temperature was cycled from 37 to 42 °C (Figure 13a) with similar results to the previous one, with a high proportion of solute released at 37 °C, but without change on rate at 42 °C (Fig. 13b); by contrast, there is a small increase of rate when temperature is decreased back at 37 °C and the hydrogels partially reswells. The slower release occurs for the hydrogel containing 15% MG NIPAAm taking three complete cycles to reach 100% release. This is the microstructured hydrogel with the lowest swelling according to the previous figures.



a) b)

Fig. 13 ***a)*** *Releasing vitamin B_{12} in cycles of temperature 37 and 42 °C, and* ***b)*** *rate of release (mg/min) normalized by drug loading for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (△).*

Fig. 14a shows the release of cytochrome C (12,000Da) in temperature cycles of 25 °C to 42 °C. The microstructured hydrogels show a release of around 40% in the first hour. However, in this case, rate of release increases with each change of temperature, either increase to 42 °C or decrease to 25 °C (Figure 14b). This is, the rate of release increases when the hydrogels shrinks and expels water, but also when the hydrogel re-swells and allows diffusion of the solute. The hydrogel with the better temperature controlled release is the one containing 15% MG NIPAAm/AAm (85:15).

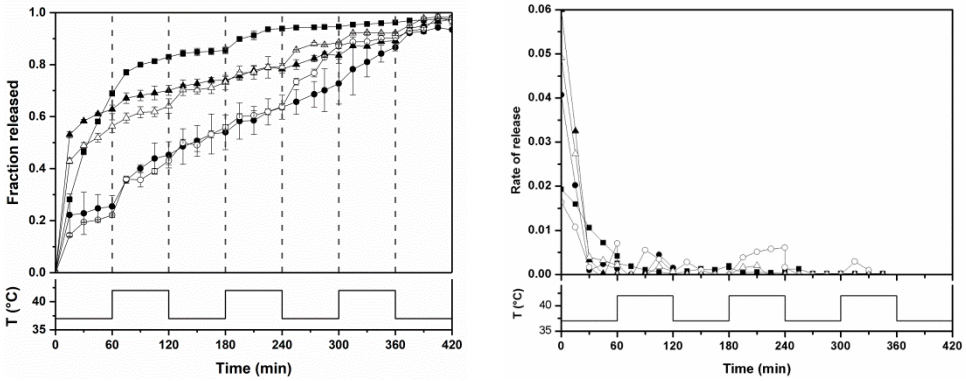


a) b)

Fig. 14 ***a)*** *Releasing cytochrome C in cycles of temperature 25 °C and 42 °C and* ***b)*** *rate of release (mg/min) normalized by drug loading for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (△).*

In Fig. 15a shows the release in cycles of temperature from 37 °C to 42 °C, the hydrogel with 15% NIPAAm shows a rapid release of 70% in the first hour (Figure 15b). In this case the

microparticles are in the shrunk state, while the hydrogels are partially swollen, which explains the fast release of the solute through the nanopores created by the shrinking of the MG´s. The hydrogels with 15% and 30% MG NIPAAm/AAm (80:20) also present a high release (60%) in the first hour and a continuous release after that, independently of temperature. The hydrogels of 15% and 30% MG NIPAAm/AAm (85:15) show a release of only 20% in the first hour at 37 °C. At this temperature the MG´s are swollen (Figure 3) and block the diffusion of cytochrome C. The hydrogel containing 30% MG presented an increase in the rate of release of each cycle at 42 °C, indicating positive control release for at least two temperature cycles. The rest of the hydrogels presented a continuous release. All hydrogels need the 3 cycles to achieve complete release.



a) b)

Fig. 15 ***a)*** *Releasing cytochrome C in cycles of temperature 37 and 42 °C and b) rate of release (mg/min) normalized by drug loading for hydrogels NIPAAm/DMA (80:20) with: 15% MG NIPAAm (■), 15% MG NIPAAm/AAm (85:15) (●), 15% MG NIPAAm/AAm (80:20) (▲), 30% MG NIPAAm/AAm (85:15) (○) and 30% MG NIPAAm/AAm (80:20) (Δ).*

4. Conclusions

Nano and microstructured NIPAAm hydrogels have demonstrated interesting properties such as fast temperature response and improved mechanical properties. Nevertheless, to our knowledge, there are not studies tuning the Ttr of both the nano/microgels and hydrogels, to achieve positive temperature control with these materials. Most studies reported on nanostructured hydrogels use NIPAAm nanoparticles prepared by inverse emulsion methods which requires organic solvents as the continuous media. In this case we use a simple and fast dispersion polymerization method using water as dispersion medium. In this study we demonstrated that it possible to modulate the Ttr of microstructured NIPAAm based hydrogels, above the body temperature, by copolymerizing with DMA or AAm. The materials present fast response to temperature from 37 °C to 42 °C. However, there is considerable shrinking of the copolymeric hydrogels at 37 °C, due to the continuity of the swelling transition, and for instance the magnitude of the swelling change between 37 °C and 42 °C is smaller than the swelling change between 25 °C and 42 °C. The swelling-deswelling cycles with changes of temperature from below and above the Ttr are reproducible to due to the fast response of the materials.

Release studies show fast release of low molecular weight solutes (vitamin B_{12}), with practically no temperature control for the microstructures hydrogels studied. On the other hand there is some temperature control for the release of higher molecular weight molecules (cytochrome C) with the hydrogels containing MG´s with Ttr above 37 °C.

The results indicate that, even when some control of the release can be obtained, there is still the need to develop materials with a discontinuous swollen-unswollen transition above the normal body temperature for applications on the temperature sensitive, positive control release of macromolecules.

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