Hybrid drug carriers with temperature-controlled on-off release: A simple and reliable synthesis of PNIPAM-functionalized mesoporous silica nanoparticles

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Abstract

MCM-41-like mesoporous silica nanoparticles (MSNs) grafted with a thermoresponsive copolymer of N-isopropylacrylamide were synthesized, fully characterized and tested to assess their efficiency as drug delivery systems. The hybrid nanoparticles were prepared by carrying out the optimized copolymer synthesis within the mesopores of MSNs after infiltration of monomers and initiator. Polymerization and grafting of the thermoresponsive copolymer occurred simultaneously by exploiting the reactive sites of the 3-methacryloxypropyltrimethoxysilane comonomer which carries a polymerizable group and alkoxysilane groups prone to condensation with surface silanols on silica. The grafted copolymer through its coil-to-globule transition acts as a gatekeeper for the temperature-controlled release of ibuprofen molecules loaded inside the pores. Significant difference in the quantitative release of ibuprofen was observed at 25 and 40 °C, which are below and above the lower critical solution temperature of the thermoresponsive copolymer. Importantly, the ordered mesoporous structure of the MSNs remained intact in all synthetic steps, loading of drug and during the *in vitro* release tests.

Keywords: Thermoresponsive polymer; Poly(N-isopropylacrylamide); Mesoporous silica nanoparticle; Drug delivery; Controlled release

1. Introduction

Mediated transport of drugs has emerged as a powerful methodology for the treatment of various diseases. The possibility to address therapeutic substances to target tissues or cells, to control their release kinetics and to protect drugs from the attack of biological, chemical and physical agents has been receiving growing attention, and many attempts have been made in the last decades to design drug delivery systems exhibiting one of these properties or a combination of them. In this context mesoporous silica nanoparticles (MSNs) are growing in importance due to their biocompatibility, nontoxicity, controllable mesopore structure and ease of surface modification. In recent years, the use of mesoporous materials for hosting and further delivering of a variety of pharmaceutical compounds has been described [1-6]. It has been shown that both small and large molecular drugs can be entrapped within the mesopores by an impregnation process and released via a diffusion-controlled mechanism. MCM-41 type silica nanoparticles having high surface area, relatively large pore volume, tunable pore size and good chemical and thermal stability are potentially suitable for various drug delivery applications and have been proposed as drug delivery systems since 2001 [7]. Many attempts have been made to test the efficiency of these types of nanoparticles in delivery of drugs either as such or with chemical modifications [8-10]. A variety of functional groups and polymers have been attached on the nanoparticle surface in order to increase drug loading and to modulate rate and duration of drug release. Much more interesting is the possibility to precisely control where and when the drug will be released and eventually to dose it by application of an external stimulus. To this aim mesoporous silica nanoparticles need to be functionalized with appropriate release triggers, specifically reacting to perturbations from chemicals [11-13], temperature [14, 15], pH [16-19], light [20], magnetic field [21, 22], ultrasounds [23] etc.

Among thermoresponsive polymers, poly(N-isopropylacrylamide) (PNIPAM) has been widely investigated [24, 25]. The thermosensitivity of NIPAM-based polymers is due to a coil-to-globule transition of the polymer chains in aqueous solution, which takes place at the lower critical solution temperature (LCST). Above the LCST the extended and hydrated polymer chains in solution shrink into a very compact mass. The range of temperature at

which the coil-to-globule transition of PNIPAM occurs (i.e. 30-35 °C) is interesting for drug delivery applications.

Recently, different strategies have been proposed to synthesize silica/PNIPAM mesoporous particles with thermoresponsive properties with the final aim of governing the opening and closing of the pores through thermal cycles, thus controlling the release of loaded molecules [26-28]. These strategies generally include surface activation, the use of grafting agents, initiators, co-initiators, chain transfer agents and other chemicals required in post-synthesis functionalization of MSNs. Although these methods allow an efficient grafting of PNIPAM chains on the silica surface and a fine control of polymer chains at a compositional and molecular level, most of these strategies appear too complex and imply the use of several substances whose fate in *in vivo* trials has not been fully disclosed. Moreover, most of the reported thermoresponsive MSNs are based on materials with larger mesopores than MCM-41. This is intended to allow an efficient encapsulation of drugs with higher molecular weight, but at the same time larger mesopores increase the risk of significant drug leakage at room temperature.

Here, we present a simple, efficient and convenient method to obtain hybrid silica/PNIPAM thermoresponsive nanoparticles with ordered, highly dense nanopores which can be opened and closed by changing the temperature. Grafting of the thermoresponsive copolymer on MSNs is achieved by carrying out the free radical copolymerization of NIPAM and 3-methacryloxypropyltrimethoxysilane (MPS) inside the mesopores of MCM-41-like nanoparticles, after infiltration of monomers and initiator into the mesopores. The hybrid mesoporous nanoparticles obtained following this one-step synthesis exhibit a temperature dependent on-off drug release that can facilitate the in-situ dosage of drugs and the reduction of dose frequency.

2. Experimental Section

2.1. Materials and methods

All reagents and solvents were purchased from Sigma-Aldrich and used as received.

MCM-41-like nanoparticles with an average diameter of approximately 100 ± 23 nm were prepared according to literature procedures using cetyltrimethylammoniumbromide (CTAB) as a structure directing agent [29, 30].

Both untethered and tethered Poly(NIPAM-*co*-MPS) chains were prepared by AIBN-initiated radical polymerization. Untethered copolymers with different compositions were synthesized by changing the monomer to initiator molar ratio and the relative amount of the comonomer pair in the feed. The main molecular characteristics and properties of synthesized copolymers are reported in Table 1.

The same synthesis conditions used for the untethered polymers were also applied to the synthesis of Poly(NIPAM-*co*-MPS)-modified MSNs. Before initiation of the polymerization reactants were infiltrated into the MSNs in order to assure the presence of the thermoresponsive polymer not only on the nanoparticle surface but also inside the pores. Once infiltrated in the mesopores reactants were assumed to polymerise as in homogeneous solution and to give Poly(NIPAM-*co*-MPS) chains with similar composition and properties of the untethered polymer. As detailed below in section 3, this assumption is supported by both FTIR spectra and critical coil-to-globule transition temperatures (T_c) of tethered and untethered copolymers, which are basically the same. Reactivity of trimethoxysilane groups of MPS with hydroxyl groups of the silica surface was exploited to anchor Poly(NIPAM-*co*-MPS) chains to the MSNs.

2.2. Synthesis of untethered Poly(NIPAM-*co*-MPS)

Poly(NIPAM-*co*-MPS) copolymers were synthesized by free radical polymerization of NIPAM and MPS initiated by AIBN. Copolymerization conditions are listed in Table 1. Polymerization was carried out in absolute ethanol at 70 °C for 16 hours under nitrogen atmosphere. Then products were purified by three dissolution/precipitation cycles from acetone into hexane and were dried overnight at room temperature (RT).

Molecular weight, solubility and coil-to-globule transition temperature were modulated by changing the monomer to initiator molar ratio and relative amount of the comonomer pair in the feed, thus obtaining the series of copolymers whose main molecular characteristics and properties are reported in Table 1.

NMR and ATR-FTIR data of representative sample 5 are shown below.

^{1}H NMR (D_2O) δ=1.0–1.2 ($CH(C\underline{H}_3)_2$, $C(C\underline{H}_3)$), 1.5–1.8 (main chain CH_2, $O–CH_2–C\underline{H}_2–CH_2$), 1.9–2.1 (main chain CH), 3.6 ($Si–O–C\underline{H}_3$), 3.8–4.0 ($N–C\underline{H}(CH_3)_2$, $O–C\underline{H}_2–CH_2$)

ATR-FTIR: 3290 (νN–H), 2970 ($\nu_{as}CH_3$), 2933 ($\nu_{as}CH_2$), 2874 (ν_sCH_3), 1720 (νC=O, MPS), 1638 (νC=O amide I), 1537 (νC–N + δNH amide II), 1458 ($\delta_{as}CH_3$), 1386-1367 ($\delta_sCH(CH_3)_2$), 1172 (νC–C), 1130 ($\rho CH(CH_3)_2$), 1086 cm^{-1} (νC–O).

2.3. Synthesis of hybrid Poly(NIPAM-*co*-MPS)/MSNs

Hybrid mesoporous nanoparticles were obtained by carrying out the synthesis of copolymer 5 in an ethanol dispersion of MSNs. In a typical reaction 280 mg of nanoparticles was used and dispersed in 10 mL of ethanol solution of NIPAM and AIBN by sonication at room temperature. The NIPAM/MSN weight ratio was 1:4. After 30 minutes MPS was added into the suspension and dispersed evenly by sonication for another 30 minutes. The dispersion was then injected in a three neck round bottom flask equipped with condenser and nitrogen inlet. The injected suspension was then heated at 70 °C under nitrogen for 16 hours. The product was dried by nitrogen flow, washed three times with deionized water and recovered by centrifugation.

2.4. Characterization of untethered Poly(NIPAM-*co*-MPS)

NMR spectra were recorded with a JEOL EX 400 spectrometer (1H operating frequency 400 MHz) at 298 K; data were treated by Jeol Delta Software. 1H chemical shifts are relative to TMS ($\delta = 0$ ppm) and referenced against solvent residual peaks (D_2O at 4.79 ppm).

Fourier Transform Infrared Spectroscopy (FTIR) spectra were collected with a Thermo-Nicolet FTIR Nexus instrument equipped with an attenuated total reflectance (ATR) device (Thermo Nicolet Smart Endurance) and with a DTGS detector. Spectra were collected in the range of 4000–400 cm^{-1} and with a resolution of 4 cm^{-1}.

A Q200 (TA instruments) differential scanning calorimeter was used to determine glass transition temperatures (T_g) and critical coil-to-globule transition temperatures (T_c). For T_g measurements analyses were performed in the temperature range from 20 to 200 °C, with a scanning rate of 10 °C/min and under a nitrogen flow of 50 ml/min. Sealed aluminium pans containing ~5 mg of samples were used. For T_c measurements 25 mg of polymer were dissolved in 0.5 mL of water, a couple of drops were poured in an aluminium pan and analyzed by raising temperature from 20 to 60 °C with a heating ramp of 5 °C/min.

Size exclusion chromatography (SEC) was performed with a Viscotek modular instrument equipped with a VE 1122 pump, a VE 7510 degasser, manual injection valve, VE 3580 refractive index detector, column oven and two PLgel 10µm MIXED-B columns (Polymer Laboratories). N,N-Dimethylformamide (DMF) (1.0 mL/min) was used as eluent and analyses were performed setting the column oven at 70 °C. DMF solutions of the samples (3

mg/mL) were filtered through 0.45 μm PTFE membrane filters. Calibration was obtained with poly(methyl metacrylate) (PMMA) molecular weight standards.

2.5. Characterization of hybrid Poly(NIPAM-*co*-MPS)/MSNs

Powder X-ray diffraction (XRD) patterns were collected by a PW3040/60 X'Pert Pro diffractometer (PANalytical B.V.) using Cu Kα radiation (40 mA and 45 kV), with a scan speed of 0.01°/min.

Gas-volumetric analysis, specific surface area (SSA), pore volume and size were measured by N_2 adsorption–desorption isotherms at 77 K using an ASAP 2020 (Micromeritics) gas-volumetric analyzer. SSA was calculated using the Brunauer–Emmett–Teller (BET) method; average pore size and volume were calculated on the adsorption branch of the isotherms according to the Barrett–Joyner–Halenda (BJH) method, by employing the Kruk–Jaroniec–Sayari equations which are optimized for MCM-41-type materials. Prior to analyses, samples were outgassed at room temperature overnight.

Thermogravimetric analyses (TGA) were carried out by a TA Q500 model from TA Instruments by heating samples contained in alumina pans at a rate of 10 °C/min from 25 to 700 °C in air.

High resolution Transmission Electron Microscopy (HRTEM) images were obtained with a JEOL 2010 instrument (300 kV) equipped with a LaB6 filament. For specimen preparation powdery samples were supported onto holed carbon coated copper grids by dry deposition. Specimens from water dispersions were prepared by pouring a few drops of dispersion of MSNs onto carbon-coated copper grids and were imaged after complete evaporation of the solvent.

Dynamic Light Scattering (DLS) measurements were carried out by using a Nano-ZS90 Zetasizer (Malvern Instruments). Suspensions of nanoparticles (0.1% w/v) were prepared in deionized water and sonicated for 20 minutes before the analysis. The measurements of hydrodynamic diameter (HD) were performed in triplicate for each sample and the mean value is reported.

2.6. Drug loading and release tests

Ibuprofen was selected as model drug for loading and release tests. Nanoparticles were outgassed at RT overnight to remove adsorbed water and then were dispersed under magnetic stirring in a hexane solution of ibuprofen (0.16 M) at 40 °C for 48 hours. Then MSNs were

separated by centrifugation, washed once with hexane and dried at RT. Ibuprofen loading was determined by TGA and UV-visible spectroscopy by monitoring the absorbance at 272 nm. The loading was calculated from TGA by subtracting from the total weight loss the contribution due to the copolymer, adsorbed water and dehydroxylation. By UV-Vis spectroscopy the loading was estimated from the total amount of ibuprofen released at 40 °C in 24 hours of stirring in physiological saline solution (PSS). UV-Vis analyses were performed using a Lambda 15 UV-Vis spectrophotometer (Perkin Elmer). For ibuprofen quantification a calibration curve (ε = 185.1 M^{-1}, R^2 = 0,9989) was constructed by plotting absorbance vs. ibuprofen concentration between 6.0×10^{-5} and 4.8×10^{-4} M.

In vitro drug release tests were carried out in PSS. Firstly, loaded nanoparticles were washed with PSS at low temperature (approximately 4 °C) to get rid of ibuprofen adsorbed on the outer surface of the nanoparticles. Then release profiles in PSS were obtained by monitoring the characteristic absorbance peak of ibuprofen at 272 nm at different time intervals and at different temperatures: below the LCST (at 25 °C) and above the LCST (at 40 °C).

3. Results and discussion

Coupling agents have been widely used to modify the surface chemistry of mesoporous silica thus providing reactive sites for grafting of various compounds (i.e. functional groups, biomolecules and polymers) which are unstable under the severe experimental conditions used for the synthesis of MSNs [31]. Hence, post-synthesis grafting of such moieties on mesoporous silicas has become a common practice. Alkoxysilanes are often used as coupling agents to modify silica surfaces because siloxane groups easily react with silica silanols by condensation reaction. In addition to alkoxy groups 3-methacryloxypropyltrimethoxysilane (MPS) also carries a polymerizable group that can be used to grow polymer chains by reaction with vinyl monomers.

Recently we have proposed a post-synthesis two-step controlled grafting of PNIPAM on ordered MSNs by first functionalization of the mesopores with polymerizable methacryloxy groups and their subsequent polymerization by distillation precipitation polymerization (DPP) with N-isopropylacrylamide [32].

Here we describe the one-step synthesis of hybrid thermoresponsive MSNs by copolymerization of NIPAM and MPS and the simultaneous grafting of the copolymer chains on the inner and external surface of MSNs.

At first we synthetized a series of untethered Poly(NIPAM-*co*-MPS) with different composition and molecular weight. NMR spectra (Supplementary Information, Figure S1) confirmed the presence in the polymer chains of both structural units coming from comonomers, but peaks assigned to alkoxysilane groups have very low intensity because of post-synthesis hydrolysis and condensation reactions and do not allow for an accurate determination of the copolymer composition. However, both FTIR spectra and T_g values demonstrate that the fraction of MPS units in the polymer chains increases accordingly to the feed composition, that is by increasing the amount of MPS in the reaction mixture the intensity of *ν*C=O of ester groups (MPS) increases with respect to the intensity of νC=O of amide groups (NIPAM) (Supplementary Information, Figure S2). Similarly, a higher content in MPS units shifts glass transition temperature to higher values (Table 1).

Based on the properties of all synthesized copolymers reported in Table 1, sample 5 was selected for the next steps of the research. In addition to be water soluble, sample 5 was the copolymer with the best molecular characteristics, having a molecular weight reasonably high enough to allow for an efficient pore opening-closing mechanism once grafted inside the nanoparticle mesopores, but not so high to fully block the mesopores [33, 34]. Therefore, according to the feed composition used for sample 5, NIPAM and MPS were infiltrated in the MSNs and then polymerized. At the same time stable anchoring of the copolymer chains is provided by condensation of trimethoxysilane groups with silanols on the silica surface. Thus, assuming that under the same polymerization conditions, reactivity of NIPAM and MPS remains unchanged even if they are confined in mesopores, thermoresponsive copolymer chains having similar properties of sample 5 were anchored to MSNs by a one-step reaction as shown in Figure 1.

The final hybrid nanoparticles were characterized by ATR-FTIR spectroscopy to confirm the presence of the polymer on the nanoparticles. Figure 2 shows the overlaid spectra of bare MSNs, untethered Poly(NIPAM-*co*-MPS) and hybrid Poly(NIPAM-*co*-MPS)/MSNs.

The FTIR spectrum of the hybrid nanoparticles shows intense absorptions due to the inter-intra tetrahedral fundamental vibrational modes of the silica framework between 1300 and 800 cm^{-1}.The most interesting spectral range is between 1750 cm^{-1} and 1300 cm^{-1} and contains the characteristic infrared absorptions of poly(NIPAM-*co*-MPS) at 1713, 1638, 1536, 1458, 1386 and 1367 cm^{-1}.

Infiltration of monomers and initiator inside the nanoparticles prior to polymerization allows Poly(NIPAM-*co*-MPS) to initiate and grow inside the mesopores and to anchor silica not

only on the outer nanoparticle surface but also on the pore walls, thus improving the polymer efficiency as gatekeeper. The presence of polymer inside the silica pores is confirmed by nitrogen adsorption-desorption analyses. Both bare and hybrid MSNs show type IV adsorption-desorption isotherms typical for mesoporous materials, with a step around a p/p_0 value of 0.3 (typical of MCM-41-like materials) and an hysteresis loop at high values of p/p_0 related to interparticle porosity (Supplementary Information, Figure S3). The specific surface area and more importantly the pore volume decreased after grafting of Poly(NIPAM-*co*-MPS) (Table 2). This is a strong evidence of the presence of the polymer inside the mesopores. In addition, the changes in the values of specific surface area and pore size demonstrate that the porosity of the hybrid MSNs is still accessible and pores are coated, but not plugged by the polymer chains [14, 35].

Poly(NIPAM-co-MPS) chains can also extend out of the mesopores as demonstrated by the change in the hydrodynamic diameter (HD) of the hybrid MSNs observed by DLS measurements. Figure 3a shows the temperature dependence of size of the hybrid MSNs. The average HD that was approximately 170 nm at 20 °C decreases to 150 nm at 40 °C. At 20 °C the polymer chains are present in their full extended hydrated conformation thus increasing the overall HD of the particles. The extension of the polymer layer decreases as temperature increases. By raising temperature the hydrogen bonding between NIPAM units and water molecules is disrupted, the attraction between the hydrophobic polymer segments is increased and the extended chains of the polymer shrink in the globular form and fall on the particles. Hence, the effective diameter of the particles decreases at this temperature. These results confirm the thermoresponsive behaviour of the grafted polymer and also prove the good dispersibility of the nanoparticles [36-38]. It is worth noting that below and also above the LCST no significant aggregation of the hybrid MSNs was observed due to the fact that polymer chains which are grafted on the surface of the particles apply steric stability to the suspension. TEM analyses of hybrid nanoparticles stirred at 40 °C for 24 hours confirm the good dispersibility of the nanoparticles (Figure 3b and Figure S4) and the average HD determined by DLS at 40 °C is in well agreement with the diameter of single nanoparticles determined by TEM. The zeta potential value for the bare, as synthesized nanoparticles at pH 7.5 was -35.2 mV, which upon polymer grafting was reduced to -16 mV. This change of zeta potential to lower value can be attributed to the fact that upon grafting some of the free hydroxyl groups are consumed for condensation of the polymer and some of them are involved in hydrogen bonding with the groups on polymer chains.

The amount of thermoresponsive copolymer grafted to MSNs was quantified by TGA. The TGA curve of bare nanoparticles (Figure 4) shows a first weight loss at around 100 °C, due to adsorbed water molecules, followed by a smooth weight loss related to surface dehydroxylation accounting for approximately 2% of total mass. In hybrid MSNs this second weight loss increases to about 10% because of the thermal decomposition of the polymeric component. Thus, considering the contribution to weight loss due to surface dehydroxylation, the total amount of grafted copolymer results to be approximately 8% wt. This value is probably an underestimation, due to the fact that a fraction of the surface silanol groups was consumed in the grafting reaction.

To test the efficiency of thermoresponsive hybrid MSNs as temperature-controlled drug delivery systems ibuprofen was used as model drug molecule. Ibuprofen was loaded under experimental conditions assuring that Poly(NIPAM-*co*-MPS) chains are in their globular conformation, leaving the pores open to enter the drug molecules. Drug loading was quantified by TGA (Supplementary Information, Figure S5) and by UV-visible spectroscopy as described in the experimental section. The resulting values, listed in Table 3, are in good agreement with each other.

In vitro drug release tests were carried out at 25 and 40 °C, which are below and above the coil-to-globule transition temperature of the thermoresponsive copolymer. Figure 5 shows the quantitative release profiles of ibuprofen. At both temperatures ibuprofen is released from bare MSNs in short time, while hybrid nanoparticles have release profiles that are temperature dependent. At 25 °C the thermoresponsive copolymer present on the external and internal surface of the nanoparticles is in its full extended form covering the pores and hindering the release of ibuprofen. Instead at 40 °C, which is above the LCST, the copolymer undergoes a coil-to-globule transition and shrinks in a compact mass allowing pore opening and resulting in the complete release of the loaded ibuprofen.

The structural and hydrothermal stability of mesoporous drug carriers is a fundamental requirement in a variety of biomedical and clinical applications, likewise colloidal stability is important for drug carriers based on nanoparticles. Especially bare mesoporous silica nanoparticles in biological environments may show poor hydrothermal stability [39] and high tendency to aggregate into larger agglomerates. TEM micrographs in Figure 6 show that both the particle size and the characteristic ordered porous structure of MCM-41-like nanoparticles (Figure 6A) remained intact after functionalization with polymer chains (Figure 6B), after ibuprofen loading (Figure 6A) and after release tests (Figure 6D). It is important to mention

here that all these subsequent steps were carried out in different solvents (i.e. water, ethanol, hexane) and at different temperatures (40 °C ÷ 70 °C). Moreover, the colloidal stability of the nanoparticles in aqueous solutions was increased after grafting of Poly(NIPAM-*co*-MPS). Bare particles tend to aggregate fast (this was observed by TEM and DLS), but this aggregation rate is considerably reduced upon grafting of thermoresponsive copolymer. Altogether these evidences prove that the thermoresponsive copolymer can be grown and grafted on the MSNs without affecting the integrity of the ordered porous system, once grafted the copolymer acts as a gatekeeper for the molecules loaded inside and it also improves the colloidal stability of the mesoporous nanoparticles by minimising the particle-particle interactions.

4. Conclusions

MSNs were functionalized with a thermoresponsive copolymer of NIPAM and were tested *in vitro* for their efficiency in temperature-controlled drug delivery. Synthesis and grafting of the thermoresponsive Poly(NIPAM-*co*-MPS) were carried out simultaneously according to a single-step reaction. Both polymer characteristics and grafting procedure were optimized and tested for their reproducibility. Results demonstrated that these hybrid nanoparticles are stable in different environments and the thermoresponsive copolymer efficiently acts as a gatekeeper for the controlled release of ibuprofen molecules loaded inside the mesopores. *In vitro* ibuprofen release tests carried out at two different temperatures, below and above the LCST of the thermoresponsive polymer, have clearly shown significant difference in the profile of drug released. The single-step polymer grafting strategy reported here can be potentially applied to graft such copolymers on different particles bearing surface hydroxyl groups.

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Figure captions

Figure 1. Scheme of the grafting of Poly(NIPAM-*co*-MPS) on MSNs.

Figure 2. FTIR spectra of Poly(NIPAM-*co*-MPS) (solid line), Poly(NIPAM-*co*-MPS)/MSNs (dashed) and bare MSNs (dotted).

Figure 3. Hydrodynamic diameter of hybrid MSNs vs. temperature (a); TEM image of a dispersion of hybrid MSNs stirred at 40 °C for 24 hours (b).

Figure 4. Thermogravimetric analysis of bare (dashed) and hybrid MSNs (solid line).

Figure 5. Ibuprofen release profiles at 25 °C (solid line/square) and 40 °C (dashed line/circles).

Figure 6. TEM images of as synthesized MSNs (A), Poly(NIPAM-*co*-MPS)/MSNs (B), Poly(NIPAM-*co*-MPS)/MSNs loaded with ibuprofen (C) and Poly(NIPAM-*co*-MPS)/MSNs after ibuprofen release at 40 °C (D).

Table 1. Synthesis conditions and properties of poly(NIPAM-*co*-MPS) samples.

Sample	NIPAM/MPS	NIPAM/AIBN	$\bar{M}_n$	$\bar{M}_w$	PDI	T_g (°C)	T_c (°C)	Solubility DMF	Solubility Water
1	10/1	20/1				126		-	-
2	20/1	20/1				115		+/-	-
3	40/1	20/1	2500	7200	2.9	93		+	+/-
4	10/1	30/1				119		-	-
5	20/1	30/1	8500	19000	2.2	116	36	+	+
6	40/1	30/1	4800	12000	2.5	107	36	+	+

+: soluble; -: non soluble; +/-: partially soluble

Table 2. Specific surface area (SSA), pore volume, size of bare and hybrid MSNs measured by N_2 adsorption–desorption isotherms.

Sample	Specific surface area (m^2/g)	Cumulative pore volume (cm^3/g)	Mean pore diameter (Å)
MSNs	1228	1.45	35
Hybrid MSNs	805	0.40	31

Table 3. Ibuprofen loading on bare and hybrid MSNs as determined by TGA and UV-Vis spectroscopy.

Sample	Ibuprofen loading (TGA)	Ibuprofen loading (UV-Vis)
MSNs	28.9	30.9
Hybrid MSNs	17.2	17.8

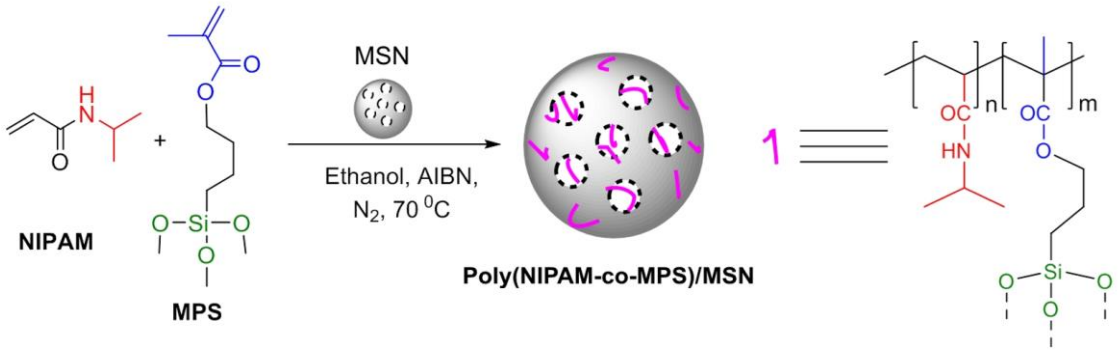

Figure 1

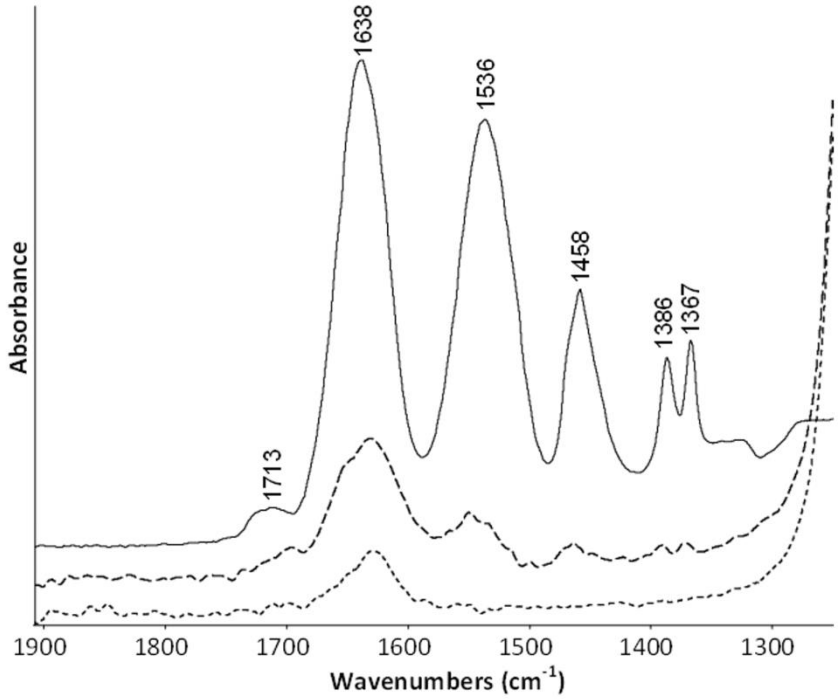
1638
1536
1458
1386
1367
1713
Absorbance
1900
1800
1700
1600
1500
1400
1300
Wavenumbers (cm-1)

Figure 2

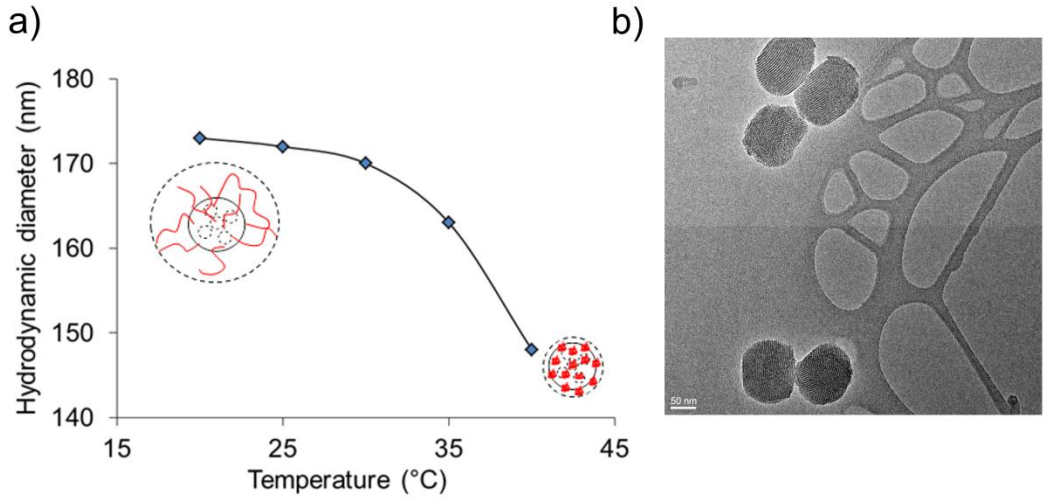
a)
b)
Hydrodynamic diameter (nm)
180
170
160
150
140
15
25
35
45
Temperature (°C)
50 nm

Figure 3

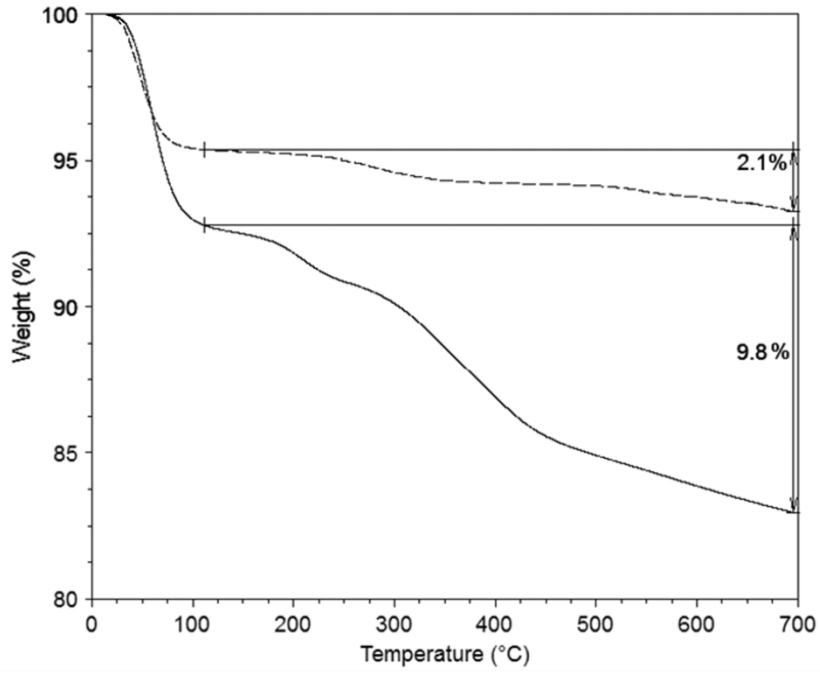
100
95
90
85
80
Weight (%)
0
100
200
300
400
500
600
700
Temperature (°C)
2.1%
9.8%

Figure 4

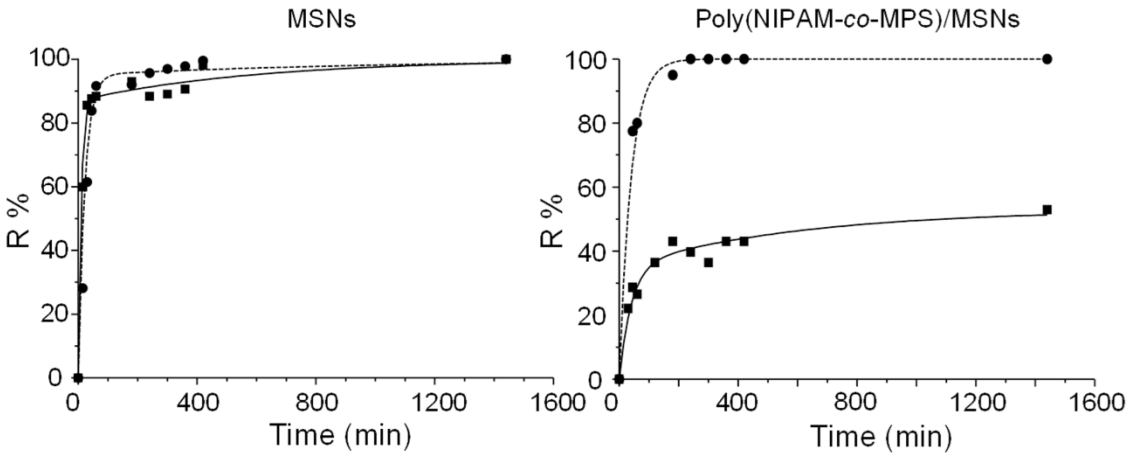
MSNs
Poly(NIPAM-co-MPS)/MSNs
R %
Time (min)
0
20
40
60
80
100
400
800
1200
1600

Figure 5

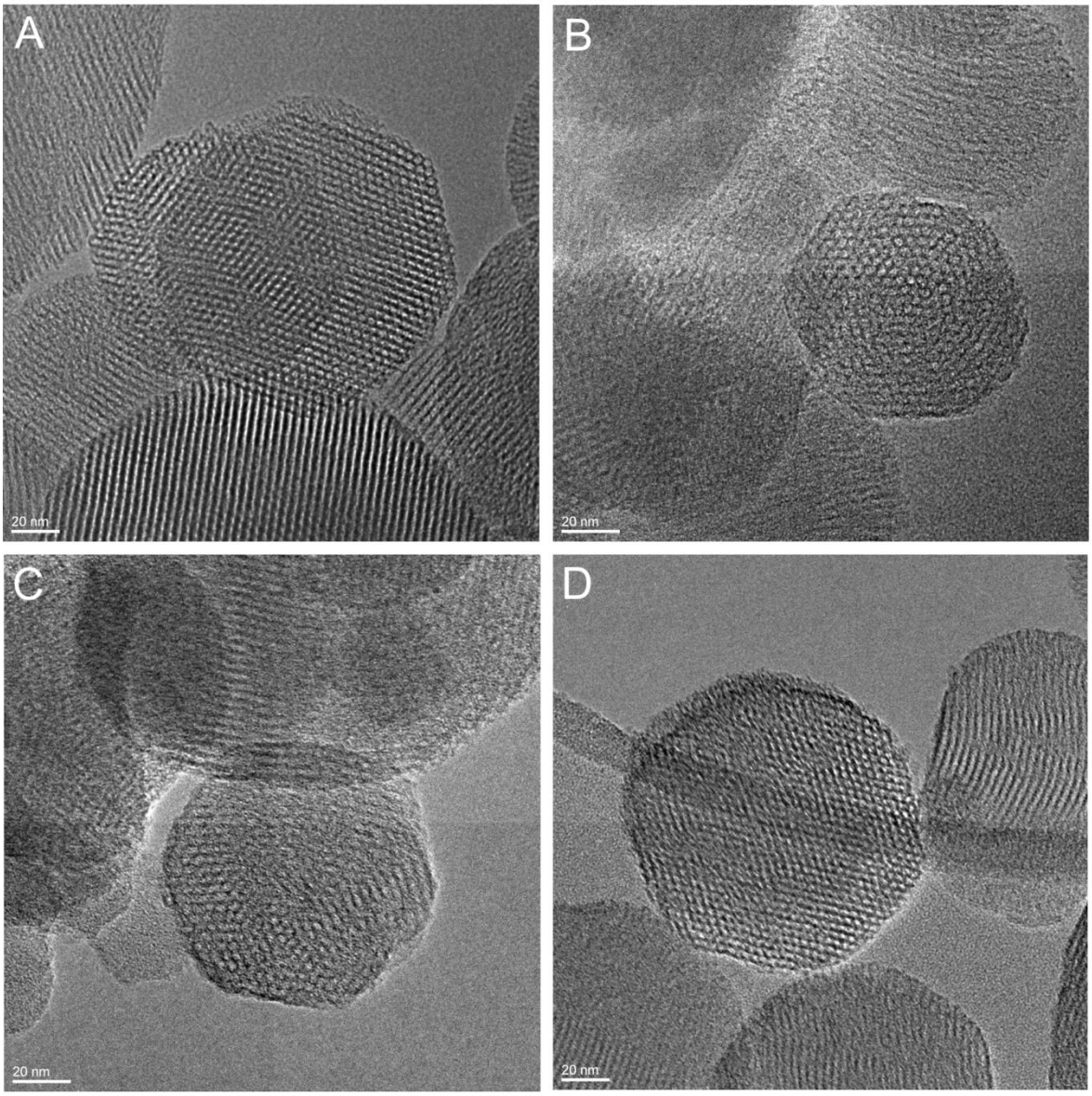

Figure 6

Supplementary Information

Hybrid drug carriers with temperature-controlled on-off release: A simple and reliable synthesis of PNIPAM-functionalized mesoporous silica nanoparticles

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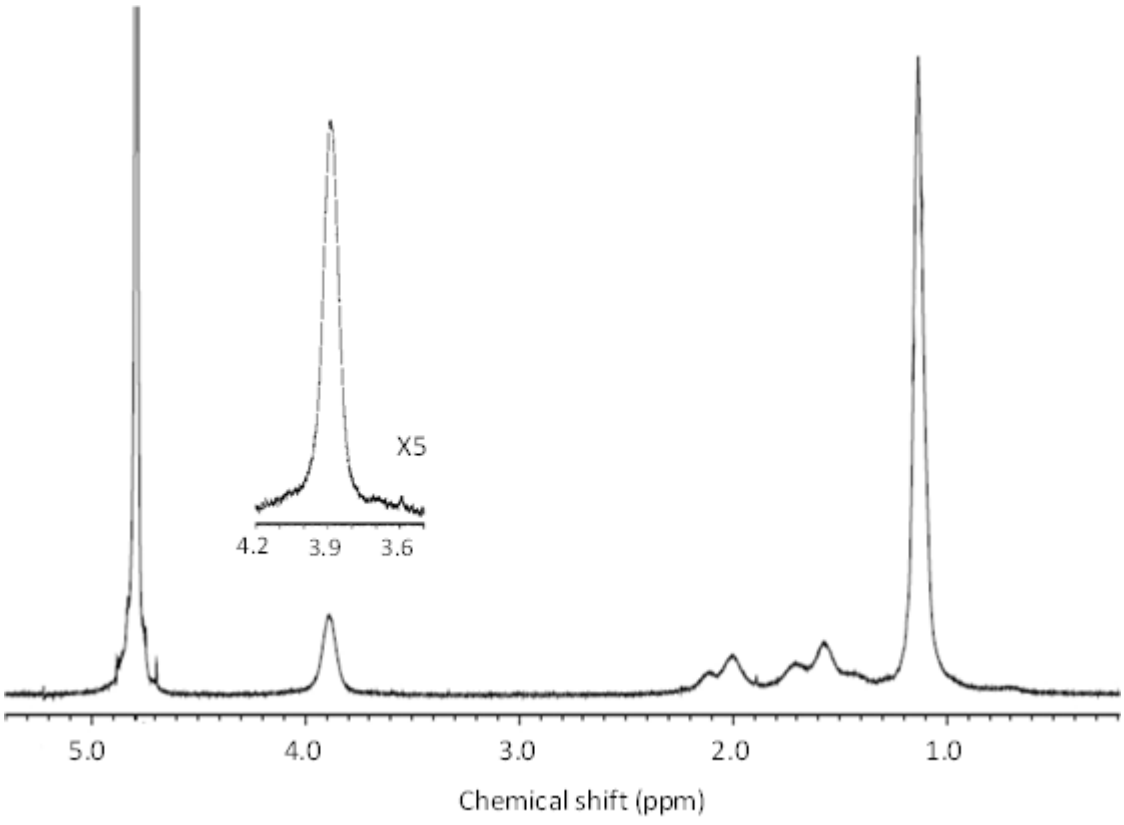

Fig. S1 ^{1}H NMR spectrum of Poly(NIPAM-*co*-MPS) (sample 5 in Table 1).

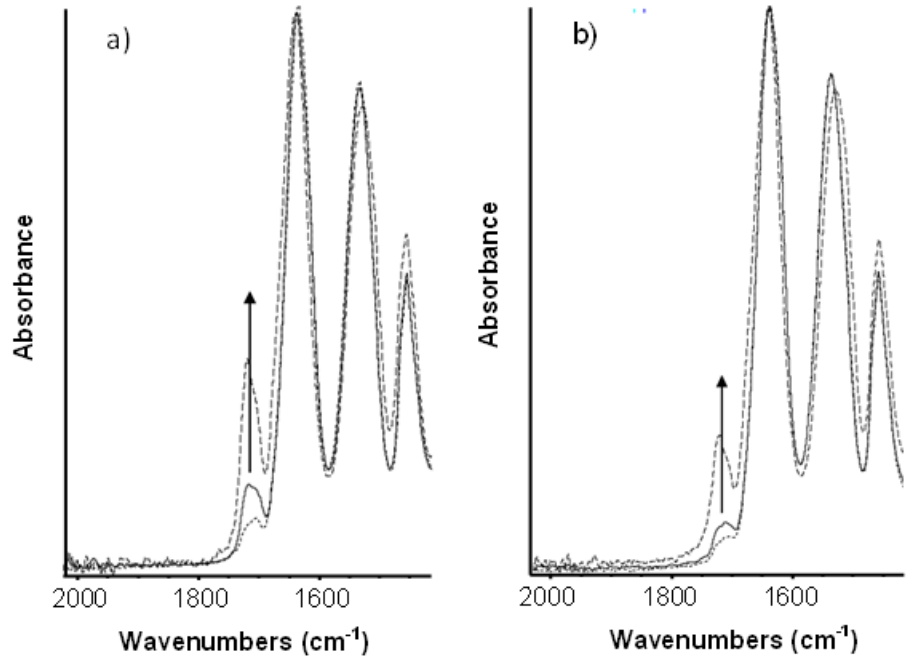

Fig. S2 ATR-FTIR spectra of Poly(NIPAM-*co*-MPS) copolymers (see Table 1): a) sample 1 (dashed), sample 2 (solid line) and sample 3 (dotted); b) sample 4 (solid line), sample 5 (dashed) and sample 6 (dotted). The intensity of νC=O of ester groups (MPS) at 1720 cm^{-1} increases with respect to the intensity of νC=O of amide groups (NIPAM) at 1638 cm^{-1} accordingly to the amount of MPS in the reaction mixture.

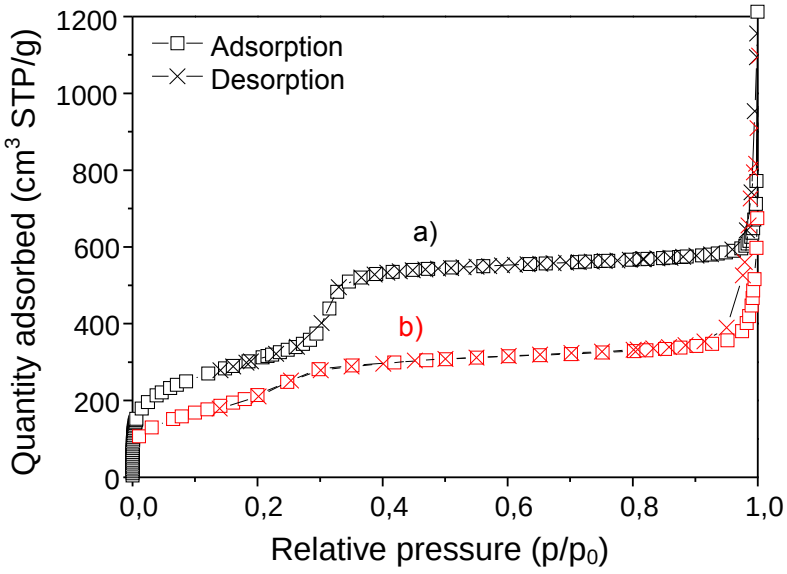

Fig. S3 Nitrogen adsorption isotherm (□) and desorption (×) on: (a) bare MSNs and (b) Poly(NIPAM-*co*-MPS)/MSNs.

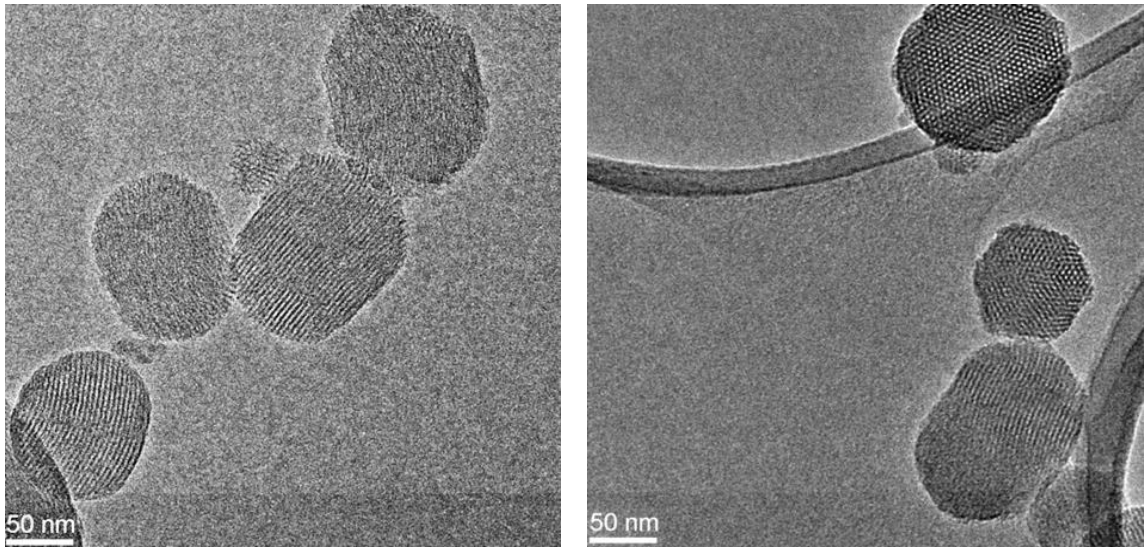

Fig. S4 TEM analyses of specimens prepared by pouring a few drops of dispersion of hybrid nanoparticles after release tests at 40 °C. TEM images show well dispersed nanoparticles and confirm DLS data reported in Figure 3.

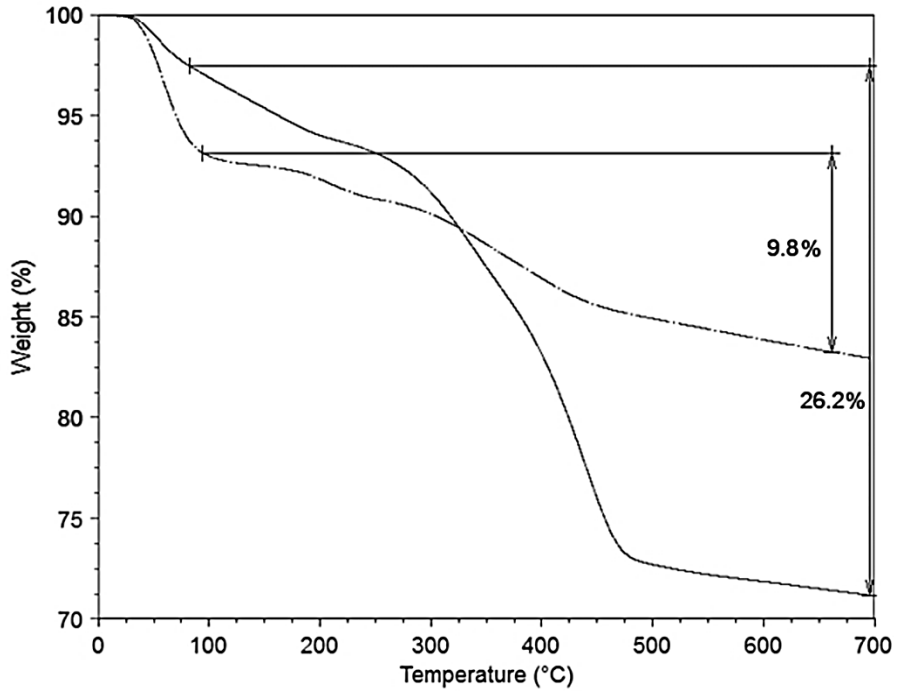

Fig. S5 Thermogravimetric analysis of hybrid thermoresponsive nanoparticles before (dash-dot line) and after ibuprofen loading (solid line).

Determination of ibuprofen loading by thermogravimetric analysis was done by measuring the total weight loss from the TGA curve of the hybrid thermoresponsive nanoparticles loaded with ibuprofen (Fig. S5, solid line) and subtracting the contribution due to the copolymer (Fig. S5, dash-dot line). The final value is corrected to take into account the weight contribution due to adsorbed water (weight loss at temperature less than 100 °C).