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(Article begins on next page)

18 December 2024

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Thermoresponsive copolymer-grafted SBA-15 porous silica particles for temperature-triggered topical delivery systems

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Abstract

A series of poly(*N*-isopropylacrylamide-*co*-acrylamide) thermoresponsive random copolymers with different molecular weights and composition were synthesized and characterized by attenuated total reflection Fourier-transform infrared (ATR-FTIR), differential scanning calorimetry (DSC), size exclusion chromatography (SEC) and proton nuclear magnetic resonance (NMR) spectroscopy. The lower critical solution temperatures (LCST) of the copolymers were tuned by changing the mole ratios of monomers. Copolymer with highest molecular weight and LCST (41.2 ^{o}C) was grafted on SBA-15 type mesoporous silica particles by a two-step polymer grafting procedure. Bare SBA-15 and the thermoresponsive copolymer-grafted (hybrid) SBA-15 particles were fully characterized by scanning electron microscope (SEM), ATR-FTIR, thermogravimetric analysis (TGA) and BET analyses. The hybrid particles were tested for their efficiency as temperature-sensitive systems for dermal delivery of the antioxidant rutin (quercetin-3-O-rutinoside). Improved control over rutin release by hybrid particles was obtained which makes them attractive hybrid materials for drug delivery.

Keywords: Smart polymers, lower critical solution temperature, nanomaterial, SBA-15 particles, temperature-triggered delivery to skin

1. Introduction

Thermoresponsive polymers, especially poly(N-isopropylacrylamide) (PNIPAM) and its copolymers are investigated with great interest for their applications in biomedical fields [1–5]. These polymers in aqueous solution show a typical coil-to-globule transition above its lower critical solution temperature (LCST), that is upon heating the water soluble extended chains of the polymer collapse to form compact insoluble globules [6,7]. This property of PNIPAM and its copolymers is used for designing of hybrid thermoresponsive drug delivery systems (DDS). In order to adjust the hydrophobicity of the PNIPAM and thereby its LCST various random and block copolymers of N-isopropylacrylamide (NIPAM) with different composition and microstructure have been synthesized [8,9]. Copolymerization of NIPAM with different monomers thus allows tuning of the properties especially LCST of PNIPAM according to the requirements of the applications under investigation. There is increased interest in hybrid thermoresponsive DDS as several studies reported their synthesis and testing for the delivery of various drugs in different environments and physiological conditions, including dermal drug delivery and also their use in dermocosmetic applications [10]. For the preparation of hybrid thermosensitive DDS, the thermoresponsive polymer needs to be anchored on a solid support. For instance, grafting of thermoresponsive polymers on porous particles leads to the formation of hybrid thermosensitive porous materials useful in temperature-triggered drug delivery [11,12]. The porous particles in such DDS act as reservoirs for various molecules and the thermoresponsive polymer, though its typical coil-to-globule transition at its LCST, applies a pore opening and closing mechanism to the porous particles [13,14]. This mechanism helps in the temperature-triggered release of the loaded molecules by the hybrid DDS.

The main difficulty in the use of PNIPAM homopolymer in the preparation of topical delivery systems is its LCST which is around 31-33 ^{o}C. This transition temperature is very close to the human skin, hence they are not efficient. As the LCST of PNIPAM is low, an uncontrolled release of the loaded drug is observed due to immediate transition of PNIPAM when it comes in contact with the skin. To address this important issue, in the present study first we report synthesis of a series of random copolymers of NIPAM with acrylamide (AM) to tune the LCST to higher values. Later grafting of the copolymer on SBA-15 type mesoporous silica particles was carried out. The higher values of LCST (41-42 ^{o}C) obtained for the copolymers can help to trigger the thermoresponsive transition of the copolymer with an external mild heating device. The hybrid thermoresponsive copolymer-grafted porous particles were tested for their efficiency in the temperature-triggered delivery of rutin. The

flavonoid rutin is believed to exhibit significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic, anti-adipogenic, and in hormone therapy [15,16]. The *in vitro* tests showed improved control over the release of the loaded rutin by the hybrid particles with respect to the bare porous silica particles. Thus the present work is an important step towards transformation of conventional porous materials used for drug delivery into hybrid thermoresponsive DDS with improved properties. It is believed that the same synthetic strategy presented in the work can be applied to various porous micro and nanoparticles to transform them into thermoresponsive delivery systems, which further increases the general scope of the study.

2. Experimental

2.1 Materials

N-isopropylacrylamide (NIPAM), acrylamide (AM), 3-(methacryloxypropyl) trimethoxysilane (MPS), azobisisobutyronitrile (AIBN) and rutin were purchased from Sigma-Aldrich, Italy. Porous silica particles of SBA-15 type used in this study were purchased from Zecasin S.A., Romania. The chemical analysis of particles provided by the producers was SiO_2 (wt. % 99.91) and Na_2O (wt. % 0.005). All the solvents mentioned in the experimental procedure were purchased from Sigma-Aldrich, Italy and were of high purity and used as received.

2.2 Instruments and methods

Fourier-transform infrared (FTIR) spectra were recorded on a Perkin Elmer Spectrum 100 in the attenuated total reflectance (ATR) mode with a diamond crystal, using 32 scans per spectrum, a resolution of 4 cm^{-1} and a spectral range of 4000-600 cm^{-1}.

Proton nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance NMR (200 MHz) spectrometer in duterated dimethyl sulfoxide (DMSO-D_6) with tetramethylsilane (TMS) as reference. Thermogravimetric analyses (TGA) were carried out on a TA Q500 model from TA Instruments by heating samples contained in alumina pans at a rate of 10 °C min^{-1} from 25 to 600 ^oC in a nitrogen flow and from 600 to 800 °C in air. Change of the gas at 600 °C was used to remove completely the carbonaceous residues from pyrolysis reactions and measure the exact amount of organic component.

The cloud points of the polymers were measured using a digital thermometer by heating the aqueous solution of the polymers. A differential scanning calorimeter (DSC Q200, TA Inc.) was used to collect DSC thermograms of the copolymers in water solution. 50 mg of polymer were dissolved in 0.5 mL of water, a couple of drops were poured in an aluminum

pan and DSC measurements were performed under nitrogen atmosphere with heating rate of 10 °C/min, from 20 up to 60 °C.

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 10 μm MIXED-B columns (Polymer Laboratories). *N,N*-Dimethylformamide (1.0 mL min^{-1}) 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 PMMA molecular weight standards.

Scanning electron microscope (SEM) images were obtained with SEM Zeiss Evo-50 instrument equipped with a secondary electron detector. The samples were previously coated with gold by a sputter coater (Bal-tec SCD 050) for 60 s under vacuum at a current intensity of 60 mA after mounting the sample on an aluminum stub with double-sided conductive tape.

Gas-volumetric analysis, specific surface area (SSA), pore volume and size were measured by N_2 adsorption–desorption isotherms at -196 °C using an ASAP 2020 (Micromeritics) gas-volumetric analyser. 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 (Kruk–Jaroniec–Sayari equations). Prior to analyses, samples were outgassed at room temperature (RT) overnight.

2.3 Synthesis

2.3.1 Synthesis of poly(NIPAM-*co*-AM) copolymers

Solution of weighted quantities of monomers in 3 mL degassed anhydrous 1,4-dioxane was prepared in a two-neck round-bottom flask equipped with heating bath and nitrogen balloon. Solution of initiator (AIBN) prepared in 1 mL of degassed 1,4-dioxane was injected into the flask with monomer solution and the polymerization reaction was continued for 5 h at 70 °C under nitrogen atmosphere. The obtained polymers were recovered by pouring the reaction mixture on ice cold ether. Then polymers were reprecipitated from cold ether. All the polymer samples were dried under vacuum at 30 °C for 5-6 h before characterization. Table 1 enlists the prepared samples.

2.3.2 Synthesis of SBA-MPS grafted particles (figure 4A first step)

Suspension of 500 mg of SBA-15 particles in 18 mL toluene was prepared in a round-bottom flask to it 50 μL of MPS was injected. The suspension was then sonicated for 15-20 min to allow the diffusion of MPS molecules inside the pores. The flask was then equipped

with water condenser and nitrogen balloon and heated at reflux with magnetic stirring at 125 °C for 16 h. At the end of the reaction, the suspension was cooled to RT and centrifuged to separate the particles. Particles were washed once with fresh 5 mL toluene and twice with 5 mL ethanol. Each time the particles were separated from the suspension by centrifugation. The obtained final product was then dried in vacuum for several hours to remove solvent traces.

2.3.3 Synthesis of poly(NIPAM-*co*-AM)/SBA hybrid particles (figure 4A second step)

Reaction conditions and mole ratio of monomers were the same used for copolymer 7 from table 1.

In a two-neck round-bottom flask, a solution of 112.5 mg of NIPAM and 9.5 mg of AM was prepared in 3 mL of anhydrous and degassed 1,4-dioxane. To it 250 mg of SBA-MPS particles were added. The resulting suspension was sonicated for 15-20 min. The solution of initiator AIBN (NIPAM/AIBN, 100/1 mole ratio) in 1 mL of anhydrous 1,4-dioxane was prepared in a separate vial. The round-bottom flask was then kept in heating bath at 70 °C equipped with a water condenser, magnetic stirrer and nitrogen balloon. The solution of the initiator was injected into the flask. The polymerization reaction was continued for 5 h at 70 °C under nitrogen. At the end of the reaction, the suspension in the flask was cooled and centrifuged to obtain polymer-grafted particles. Particles were washed twice with acetone to remove solvent traces. Then polymer-grafted particles were then dried in vacuum for several hours before characterization.

2.4 Loading and release tests

Loading of model antioxidant drug rutin inside the pores of SBA-15 and poly(NIPAM-co-AM)/SBA particles was carried out by wet mixing method. In a typical procedure the particles were mixed with rutin in 1/1 weight ratio in 1 mL of ethanol and mixed well. The obtained complex was dried in desiccator to remove the solvent. The complex was washed quickly with ethanol to remove physisorbed rutin. Quantitative loading of rutin was determined by extraction with DMSO, which is its best solvent. Loading conditions and results are reported in table 3.

For release tests a weighted amount of hybrid particles loaded with rutin were dispersed in 25 mL of phosphate buffer (pH 6.5)/DMSO (75/25). These dispersions were placed in incubator at 20 °C (below LCST) and 45 °C (above LCST). At a predetermined time interval, 1.0 mL aliquots from the dispersions were withdrawn and replaced with fresh release media. After centrifugation at 16000 r.p.m. for 10 min, the concentrations of the released

molecules in the supernatant were determined via UV–visible spectrophotometry at $\lambda_{max} =$ 260 nm. The results of the release tests are reported as percentage of released rutin vs time.

3. Results and Discussion

3.1 Synthesis of poly(NIPAM-co-AM) copolymers

Random copolymers of NIPAM and AM were synthesized by standard radical polymerization method using AIBN as the initiator [17,18]. The characterization data of the synthesized polymers are shown in table 2. Molecular weights of the copolymers were in the range of 97300 to 172300 Da. As expected, molecular weights of the copolymers increased with increasing the monomer to initiator ratio. LCSTs of the copolymers were increased from 37 to 41 ^{o}C with increase of AM mole fraction from 6.0 to 11.8%. Representative ATR-FTIR spectra of copolymers 2 and 7 (one from each series of AM fraction) are shown in figure 1. The characteristic peaks of NIPAM and AM units are evident. Peaks at 3445, 3441, 3319, and 3318 cm^{-1} are attributed to the asymmetric stretching of the –NH$_2$ group from AM and –N–H stretching from NIPAM units, respectively [19]. Peaks at 1650 and 1545 cm^{-1} in both spectra are due to the carbonyl (C=O) group stretching and –N–H bending in secondary amines [19]. These data confirmed the formation of the copolymers and the presence of groups from both structural units.

From the characterization data of the copolymers it was observed that copolymer 7 shows desired properties with highest molecular weight and cloud point around 41 °C. High molecular weight of the copolymer 7 can guarantee good surface coverage and helps to apply the pore opening and closing mechanism to the pores on the particles. Furthermore, a cloud point of 41 °C, being significantly higher than the skin temperature, is suitable for cutaneous applications. Due to this, the composition and polymerization conditions used for the synthesis of copolymer 7 were chosen to prepare the copolymer-grafted SBA-15 particles. Copolymer 7 was subjected to additional structural and thermal characterization by NMR spectroscopy and DSC analysis, respectively. The NMR spectrum of copolymer 7 is shown in figure 2. It shows characteristic signals for all the protons present in the copolymer and is well in agreement with the reported data [20,21]. The feed composition calculated by NMR for copolymer 7 was 89.5% NIPAM and 10.5 % AM which is well in agreement with the used feed composition (refer to table 1). The DSC heating cycle curve of copolymer 7 in water solution (50 mg/mL) is shown in figure 3. The minimum point indicates that the LCST of the copolymer at 41.2 ^{o}C is very near to the cloud point observed (refer to table 2).

3.2 Grafting of copolymer on SBA-15 particles

The SEM images of SBA-15 type mesoporous silica particles used in this work (figure 5) show aggregates of several micrometers, consisting of primary microparticles of around 0.8 to 1 μm. The synthesis protocol followed for the grafting of random copolymer on mesoporous silica particles is shown in figure 4A. In the first step of the reaction the reactivity of surface silanols on SBA-15 particles were exploited as anchoring points because they easily react with alkoxysilane groups of MPS. In the procedure afforded grafting of MPS on bare SBA-15 particles with polymerizable acryloxy groups [22]. In the second step in situ copolymerization of acryloxy-grafted particles with NIPAM and AM was carried out. In general silanol groups do not interfere with the chain propagation during the polymer formation. In fact, their reactivity is considerable in aqueous solvents (where they dissociate, with an isoelectric point around 2-3), but is much less in anhydrous organic solvents [23]. Structure of the polymer and its grafting by using the reactivity of surface silanols inside the pores are shown in figure 4B. The coil-to-globule transition of the copolymer upon heating above LCST inside the pores is shown in figure 4C.

3.3 Characterization of poly(NIPAM-co-AM)/SBA particles

The ATR-FTIR spectra of the starting bare SBA-15 porous silica particles, SBA-MPS particles and copolymer-grafted particles are shown in figure 6. The spectrum of SBA-15 particles showed an intense broad band around 990-1000 cm^{-1} due to the stretching vibrations of Si–O–Si groups [24–26]. The spectra of MPS functionalized particles (inset zoomed area) showed two peaks at 1704 and 1630 cm^{-1} due to carbonyl (C=O) and C=C stretching vibrations, respectively [27]. The copolymer-grafted particles (lower spectrum in inset zoomed area) showed peaks at 1702, 1668, 1551, 1460 cm^{-1} due to MPS carbonyl, secondary amide carbonyl, secondary amide –N–H bending and C–H asymmetric bending of the isopropyl group respectively [28,29].

TGA curves of the SBA-15, SBA-MPS and poly(NIPAM-co-AM)/SBA samples are shown in figure 7. SBA-15 particles show good thermal stability and upon programmed heating up to 800 °C only 0.25% weight loss for the particles was observed which is due to the adsorbed humidity. The weight loss for SBA-MPS particles was 10.9% and for the poly(NIPAM-co-AM)/SBA sample was 14%. The copolymer grafting percentage calculated was 3.1%. Results of the ATR-FTIR and thermal analysis confirmed grafting of the copolymer on the particles. The copolymer-grafted SBA particles were further characterized by nitrogen adsorption-desorption analysis. The adsorption-desorption isotherms obtained are

shown in figures 8A and 8B. The specific surface area for the SBA-15 particles calculated by BET analysis was 816 m^2/g, which was reduced to 622 m^2/g for the copolymer-grafted particles. Due the grafting of the copolymer inside the mesopores, the pore volume was decreased from 1.14 to 0.96 cm^3/g and the pore diameter (shown in figure 9) was decreased from 56.3 to 54.9 Å [30]. These results altogether confirmed the effective grafting of the thermoresponsive copolymer insides the pores of the SBA-15 particles.

3.4 Rutin loading and release tests

Rutin (quercetin-3-O-rutinoside) was chosen as the model molecule for loading inside the porous particles. The SBA-15 type mesoporous silica particles were purposely chosen to prepare the delivery vehicle as they have large pores, hence big molecules such as rutin can easily enter and be loaded within the pores [31]. The obtained rutin loading was 35.3% and 27.9% for bare SBA-15 and poly(NIPAM-co-AM)/SBA particles respectively (refer to table 3).Rutin, being an unstable antioxidant is vulnerable to oxidation and undergoes degradation to form various products [32] hence its stability in the release medium was tested by monitoring its concentration by UV-visible spectroscopy (figure 10). It was observed that rutin remains stable in the release medium over a period of 24 hours. This stability study confirms that the quantitative release of rutin calculated at each time interval (figure 10) can be directly related to the concentration of rutin in the release medium.

In the case of rutin-loaded SBA-15 (figure 11A), a burst release was observed both at 20 and 45°C. This can be related to the competition of the release medium (buffer solution) with the silica surface, displacing physically adsorbed rutin molecules [33,34]. In the case of thermoresponsive copolymer-grafted SBA-15 particles a considerable difference of the quantitative release of rutin at two temperatures, below and above the LCST of the polymer, was observed (figure 11B). Below the LCST the soluble extended copolymer chains block the immediate release of the rutin molecules by plugging the SBA pores and when the temperature is raised to 45°C, which is well above the LCST of the copolymer, the polymer chains collapse to open the pores thus allowing the release of rutin. The initial burst release observed for bare SBA-15 particles was significantly reduced by the copolymer-grafted particles. The transition of the thermoresponsive copolymer from its hydrophilic coil conformation to hydrophobic globules applies a slightly hydrophobic character to the surface of the particles inside the mesopores which helps to minimise the interactions of the loaded molecules with the silica surface (silanols) and allows complete release of rutin. Additionally the hydrophobic copolymer layer also helps to minimize the readsorption of the released molecules back on the silica surface. Thus, release tests confirmed improved control of the hybrid thermoresponsive copolymer-grafted particles over rutin release.

4. Conclusions

A series of thermoresponsive random copolymers of NIPAM with acrylamide were synthesized to tune the LCST of the copolymers. *In situ* copolymer grafting on mesoporous silica particles was carried out by replicating the optimized synthesis conditions for the selected copolymer with highest LCST. Effective grafting of the thermoresponsive copolymer on SBA-15 type porous silica particles was carried out to transform the particles into thermoresponsive dermal delivery systems. *In vitro* release tests performed by using rutin as the model molecule showed that the hybrid i.e. thermoresponsive copolymer-grafted porous silica particles are characterized by an improved control over rutin release. Burst release of the drug and readsoption of the drug was minimized. The synthesis of thermoresponsive copolymer-grafted porous material presented in this work proves to be an important step towards the transformation of porous materials into temperature-triggered drug delivery systems. Besides the results presented, the general importance of the work lies in the fact that the synthetic strategies reported can be applied easily to various other porous materials to transform them in to temperature-triggered delivery systems.

References

[1]   Gandhi A., Paul A., Sen S. O., Sen K. K.: Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications. Asian Journal of Pharmaceutical Science, **10,** 99–107 (2015).
DOI: 10.1016/j.ajps.2014.08.010

[2]   Guan Y., Zhang Y.: PNIPAM microgels for biomedical applications: from dispersed particles to 3D assemblies. Soft Matter, **7,** 6375 (2011).
DOI: 10.1039/c0sm01541e

[3]   Tan L., Liu J., Zhou W., Wei J., Peng, Z. A.: Novel thermal and pH responsive drug delivery system based on ZnO@PNIPAM hybrid nanoparticles. Material Science and Engineering C, **45,** 524–529 (2014).
DOI: 10.1016/j.msec.2014.09.031

[4]   Yadavalli T., Ramaswamy S., Chandrashekharan G., Michal I., Therese, H. A., Chennakesavulu R.: Dual responsive PNIPAM-chitosan targeted magnetic nanopolymers for targeted drug delivery. Journal of magnetism and Magnetic Materials, **380,** 315–320 (2015).
DOI: 10.1016/j.jmmm.2014.09.035

[5]   Ward M. A., Georgiou T. K.: Thermoresponsive polymers for biomedical applications. *Polymers*, **3,** 1215–1242 (2011).
DOI: 10.1080/10601326808051910

[6]   Taylor P., Heskins M., Guillet, J. E.: Solution Properties of Poly (N-isopropylacrylamide). Journal of Macromolecular Science : Part A, 37–41 (2006).
DOI:10.1080/10601326808051910

[7]   Schild H. G.: Poly (N-Isopropylacrylamide): Experiment , Theory and Application. Progress in Polymmer Science, **17,** 163–249 (1992).
DOI: 10.1016/0079-6700(92)90023-R

[8]   Jain K., Vedarajan R., Watanabe M. Ishikiriyama M., Matsumi N.: Tunable LCST behavior of poly(N-isopropylacrylamide/ionic liquid) copolymers. Polymer Chemistry, **6,** 6819–6825 (2015).
DOI: 10.1039/C5PY00998G.

[9]   Lee S. C., Chang, J. Y.: Thermosensitive Block Copolymers Consisting of Poly (N-isopropylacrylamide) and Star Shape Oligo (ethylene oxide). Bulletein of Korean Chemical Society, **30,** 1521–1525 (2009).
DOI: 10.5012/bkcs.2009.30.7.1521

[10]   Kwon S., Singh R. K., Perez R. A., Abou Neel E. A., Kim H W., Chrzanowski W.: Silica-based mesoporous nanoparticles for controlled drug delivery. Journal of Tissue Engineering, **4**, 2041731413503357 (2013).
DOI: 10.1177/2041731413503357

[11]   Ugazio E., Gastaldi L., Brunella V., Scalarone D., Jadhav S. A., Oliaro-Bosso S., Zonari D., Berlier G., Miletto I., Sapino S.: Thermoresponsive mesoporous silica nanoparticles as a carrier for skin delivery of quercetin. International journal of Pharmaceutics, **511,** 446–454 (2016).
DOI: 10.1016/j.ijpharm.2016.07.024

[12]   Brunella V., Jadhav S. A., Miletto I., Berlier G., Ugazio E., Sapino S., Scalarone D.: Hybrid drug carriers with temperature-controlled on-off release: A simple and reliable

synthesis of PNIPAM-functionalized mesoporous silica nanoparticles. Reactive and Functional Polymers, **98,** 31–37 (2016).

DOI: 10.1016/j.reactfunctpolym.2015.11.006

[13] Baek S. M., Singh R. K., Kim T. H., Seo J. W., Shin U. S., Chrzanowski W., Kim H. W.: Triple hit with drug carriers: pH- and temperature-responsive theranostics for multimodal chemo- and photothermal-therapy and diagnostic applications. ACS Applied Materials and Interfaces, (2016).

DOI:10.1021/acsami.6b00963

[14] Hong C. Y., Li X., Pan C. Y.: Smart core-shell nanostructure with a mesoporous core and a stimuli-responsive nanoshell synthesized via surface reversible addition-fragmentation chain transfer polymerization. Journal of Physical Chemistry C, **112,** 15320–15324 (2008).

DOI: 10.1021/jp805028z

[15] Hosseinzadeh H., Nassiri-Asl M.: Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. Journal of Endocrinological Investigation, **37,** 783–788 (2014).

DOI: 10.1007/s40618-014-0096-3

[16] Chua L. S.: A review on plant-based rutin extraction methods and its pharmacological activities. Journal of Ethnopharmacology, **150,** 805–817 (2013).

DOI: 10.1016/j.jep.2013.10.036

[17] Eeckman F., Moës A. J., Amighi K.: Synthesis and characterization of thermosensitive copolymers for oral controlled drug delivery. European Polymer Journal, **40,** 873–881 (2004).

DOI: 10.1016/j.eurpolymj.2003.11.010

[18] Furyk S., Zhang Y., Ortiz-Acosta D., Cremer P. S., Bergbreiter D. E.: Effects of end group polarity and molecular weight on the lower critical solution temperature of poly(N-isopropylacrylamide). Journal of Polymer Science Part A Polymer Chemistry, **44,** 1492–1501 (2006).

DOI: 10.1002/pola.21256

[19] Hao L., Yang H., Lei Z.: Synthesis and properties of thermo-responsive macroporous PAM-co-PNIPAM microspheres. Material Letters, **70,** 83–85 (2012).

DOI: 10.1016/j.matlet.2011.11.112

[20] Schönhoff M., Larsson A. Welzel P. B., Kuckling D.: Thermoreversible polymers adsorbed to colloidal silica: A ^{1}H NMR and DSC study of the phase transition in confined geometry. Journal of Physical Chemistry B, **106,** 7800–7808 (2002).
DOI: 10.1021/jp015538l

[21] Sun S., Wu P., Zhang W., Zhang W., Zhu X.: Effect of structural constraint on dynamic self-assembly behavior of PNIPAM-based nonlinear multihydrophilic block copolymers. Soft Matter, **9,** 1807–1816 (2013).
DOI: 10.1039/C2SM27183D

[22] Jadhav S. A., Brunella V., Scalarone D.: Polymerizable ligands as stabilizers for nanoparticles. Particle and Particle Systems Characterization, **32,** 417–428 (2015).
DOI: 10.1002/ppsc.201400074

[23] Jadhav S. A., Miletto I., Brunella V., Berlier G., Scalarone D.: Controlled post-synthesis grafting of thermoresponsive poly(N-isopropylacrylamide) on mesoporous silica nanoparticles. Polymers for Advanced Technologies, **26,** 1070–1075 (2015).
DOI: 10.1002/pat.3534

[24] Li L.L., Sun H., Fang C. J., Xu J., Jin J. Y., Yan C. H.: Optical sensors based on functionalized mesoporous silica SBA-15 for the detection of multianalytes (H+ and Cu2+) in water. Journal of Material Chemistry, **17,** 4492 (2007).
DOI: 10.1039/b708857d

[25] Zhang D., Li J. H.: Ordered SBA-15 mesoporous silica with high amino-functionalization for adsorption of heavy metal ions. Chinese Science Bulletein, **58,** 879–883 (2013).
DOI: 10.1007/s11434-012-5594-0

[26] Borodko Y., Iii J. W. A., Marti G. E., Song H., Niesz K., Somorjai G. A.: Structure Sensitivity of Vibrational Spectra of Mesoporous Silica SBA-15 and Pt / SBA-15. Journal of Physical Chemistry B, **109,** 17386–17390 (2005).
DOI: 10.1021/jp051801x

[27] Chinthamanipeta P. S., Kobukata S., Nakata H., Shipp D. A.: Synthesis of poly(methyl methacrylate)-silica nanocomposites using methacrylate-functionalized silica nanoparticles and RAFT polymerization. Polymer, **49,** 5636–5642 (2008).
DOI: 10.1016/j.polymer.2008.10.018

[28] Chen J., Liu M., Chen C., Gong H., Gao C.: Synthesis and characterization of silica nanoparticles with well-defined thermoresponsive PNIPAM via a combination of RAFT and click chemistry. ACS Applied Materials and Interfaces, **3,** 3215–3223 (2011).

DOI: 10.1021/am2007189

[29] Yang Y., Yan X., Cui Y., He Q., Li D., Wang A., Fei J., Li J.: Preparation of polymer-coated mesoporous silica nanoparticles used for cellular imaging by a 'graft-from' method. Journal of Material Chemistry, **18,** 5731 (2008).
DOI: 10.1039/b811573g

[30] Reichhardt N. V., Giullet-Nicolas R., Thommes M., Klosgen B., Nylander T., Kleitez F., Alfredsson V.: Mapping the location of grafted PNIPAAM in mesoporous SBA-15 silica using gas adsorption analysis. Physical Chemistry and Chemical Physics, **14,** 5651 (2012).
DOI: 10.1039/c2cp22523a

[31] Ritter H., Ramm J. H., Brühwiler D.: Influence of the structural properties of mesoporous silica on the adsorption of guest molecules. Materials, **3,** 4500–4509 (2010).
DOI: 10.3390/ma3084500

[32] Friedman M., Jürgens H. S.: Effect of pH on the stability of plant phenolic compounds. Journal of Agricultural and Food Chemistry, **48,** 2101–2110 (2000).
DOI: 10.1021/jf990489j

[33] Li J., Wang H., Li H., Xu L., Guo Y., Lu F., Pan W., Li S.: Mutual interaction between guest drug molecules and host nanoporous silica xerogel studied using central composite design. International Journal of Pharmaceutics, **498,** 32–39 (2016).
DOI: 10.1016/j.ijpharm.2015.12.005

[34] Malfanti A., Miletto I., Bottinelli E., Zonari D., Blandino G., Berlier G., Arpicco S.: Delivery of Gemcitabine Prodrugs Employing Mesoporous Silica Nanoparticles. *Molecules* **21,** 522 (2016).
DOI: 10.3390/molecules21040522

Captions

Table 1. List of synthesized random copolymers.

Sample	NIPAM fraction (mol %)	AM fraction (mol %)	NIPAM/AIBN (mole ratio)
Copolymer 1	94.0	6.0	10/1
Copolymer 2	94.0	6.0	30/1
Copolymer 3	94.0	6.0	50/1
Copolymer 4	94.0	6.0	100/1
Copolymer 5	88.2	11.8	10/1
Copolymer 6	88.2	11.8	50/1
Copolymer 7	88.2	11.8	100/1

Table 2. Characterization data of copolymers.

Sample	AM fraction (mol %)	M_w (Da)	M_n (Da)	M_w/M_n	Cloud point in water ± 0.2 ℃
Copolymer 1	6.0	107000	37200	2.87	37
Copolymer 2	6.0	108800	46300	2.35	37
Copolymer 3	6.0	112600	51000	2.21	37
Copolymer 4	6.0	164400	62000	2.65	37
Copolymer 5	11.8	97300	29500	3.30	41
Copolymer 6	11.8	162600	67200	2.42	41
Copolymer 7	11.8	172300	69000	2.50	41

Table 3. Drug loading conditions and values.

Sample	Silica:Drug weight ratio, loading conditions	Extraction conditions	Loading by UV-Vis. ± 1 %
SBA-Rutin	1/1, in ethanol, 20 ^{o}C, 24 h	DMSO, 45 ^{o}C, 24 h	35.3
SBA-copolymer-Rutin	1/1, in ethanol, 20 ^{o}C, 24 h	DMSO, 45 ^{o}C, 24 h	27.9