Remotely controllable microcapsules for site-specific delivery of a chemical payload

Jitka Čejková, Viola Tokárová, Anna Pittermannová, František Štěpánek[*]

Chemical Robotics Laboratory, Department of Chemical Engineering, Institute of Chemical Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic
[*]Tel: +420 220 443 236; Fax: +420 220 444 320; E-mail: Frantisek.Stepanek@vscht.cz

Abstract

The problem of targeted delivery and controlled release of a chemical payload in difficult-to-access microstructured environments such as living tissues or porous soil is considered. Composite microcapsules consisting of the thermo-responsive hydrogel poly-N-isopropylacrylamide (PNIPAM) with dispersed iron oxide nanoparticles have been prepared by w/o emulsion polymerization. The microcapsules are able to reversibly change their size and shape, adhesion to substrates under laminar flow conditions, and permeability to diffusing molecules as a result of local temperature change induced by radiofrequency heating of incorporated super-paramagnetic iron oxide nanoparticles. The structural analysis of the composite capsule by x-ray micro-tomography revealed the existence of several internal compartments as well as uniform distribution of iron oxide nanoparticles within the polymer. The thermo-responsive shrinking/swelling behavior of the composite particles around the lower critical solution temperature (LCST) of PNIPAM was verified and the ability to reach the LCST by means of remote induction heating using a 400 kHz alternating magnetic field was demonstrated. The release rate of a model active substance (vitamin B12) from the capsules at two different temperatures was measured, confirming the possibility to influence the release characteristics remotely by the application of a radiofrequency magnetic field. The temperature-dependent adhesion characteristics of the microcapsules towards various substrates were measured in a purpose-build mircofluidic flow cell.

Keywords: thermo-responsive polymer; PNIPAM; iron oxide nanoparticles; magnetic induction heating; controlled release.

1   Introduction

The design and synthesis of stimuli-responsive polymer particles have attracted considerable attention in recent years due to their potential use in areas such as catalysis, separation, encapsulation or controlled release. Stimuli-responsive polymers have the ability to respond to a change in an environmental variable such as temperature, pH, imposed electrical field or ionic strength (i.e., the concentration of salts in the solution) by a change in the conformation of the polymer chain structure, which can manifest itself by a change in hydrophilic/hydrophobic character or density. Depending on the geometry of the object made from the stimuli-responsive polymer, the change in density can lead to a macroscopic change of volume (e.g. spherical particles) or specific deformation (e.g. composite films). Related to the density change is also a change in the diffusion rates of molecules through the polymer structure. The change in volume or shape can be used e.g. in polymeric actuators ("artificial muscles"); the change in diffusivity can be utilised in controlled release applications for drug delivery.

A well-characterized temperature-responsive polymer is poly-*N*-isopropylacrylamide (PNIPAM) [1]. PNIPAM contains both hydrophilic amide and hydrophobic isopropyl groups. At temperatures below the lower critical solution temperature (LCST, around 32 °C) the amide groups interact strongly with water molecules through hydrogen bonding and the polymer swells. At temperatures above the LCST, water is expelled from PNIPAM chains and the polymer shrinks significantly.

By combining PNIPAM with inorganic particles, composite microcapsules with improved properties and/or additional functionality can be obtained [2, 3]. In our recent work [4], composite PNIPAM/SiO$_2$ microcapsules have been prepared via Pickering emulsion polymerization. The core of the prepared capsules was formed by PNIPAM and the surface was covered by a self-assembled layer of silica nanoparticles. By using this approach, not only the chemical stability and mechanical integrity of the hybrid capsules can be enhanced, but also the molecular transport through the shell may be controlled.

In this work we describe the synthesis and properties of composite thermo-responsive PNIPAM capsules containing iron oxide (Fe$_3$O$_4$) nanoparticles instead of silica. These composite capsules will be shown to keep the temperature-responsive swelling/shrinking properties of pure PNIPAM but thanks to the presence of magnetic nanoparticles, the temperature response can be triggered remotely by the application of alternating magnetic field (AMF) in the radiofrequency range [5]. When Fe$_3$O$_4$ nanoparticles of a single-domain size are exposed to AMF, heat can be generated by the combination of Néel and Brownian relaxation, hence Fe$_3$O$_4$ nanoparticles can be used as susceptors directly integrated into the PNIPAM microcapsules. The generated heat causes the thermo-responsive polymer to shrink, which can be used for controlling polymer particle adhesion to surfaces, flow or deposition in porous media or the controlled release of a chemical payload contained in the capsules. The combination of thermo-responsive PNIPAM properties and magnetic Fe$_3$O$_4$ properties has an advantage of remotely triggered release where other trigger mechanisms such as infrared light or ultrasound are not feasible. Alternating magnetic field in the radiofrequency range penetrates a range of materials yet it is considered safe for living tissues [6].
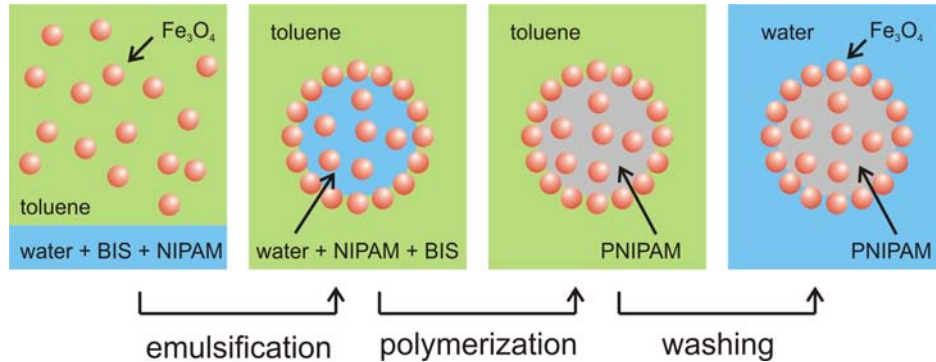

Fig. 1: Schematic illustration of the synthesis of composite PNIPAM/Fe$_3$O$_4$ microcapsules.

2   Materials and methods

The following chemicals were used: *N*-isopropylacrylamide (NIPAM, Sigma), *N,N′*-methylenebisacrylamide (BIS, Fluka), ammonium persulfate (APS, Fluka), Fe$_3$O$_4$ nanoparticle suspension (5 nm average size, 5 mg/ml in toluene, <1% of oleic acid stabilization, Aldrich), ethanol (Penta), and toluene (Lachner). Water was produced by a demineralized water generator Aqual 25.

The diagram of the composite particle fabrication procedure is shown in Fig. 1. Depending on the required final nanoparticle/polymer ratio, 0.3-2 ml of the as-received nanoparticle dispersion was diluted with toluene to 10 g. The monomer NIPAM (0.4 g), cross-linker BIS (0.015 g) and initiator APS (0.02 g) were dissolved in 2 ml of water and mixed with 10 g of the Fe_3O_4 toluene dispersion. The mixture was stirred at 3000 rpm for 3 minutes to obtain a w/o emulsion. The emulsion was deoxygenated by nitrogen bubbling. After 20 minutes, the mixture was heated to 70 °C and the polymerization occurred for 4 hours under nitrogen atmosphere. The final PNIPAM/Fe_3O_4 composite microspheres were washed by ethanol and redispersed in water. The weight percentage of Fe_3O_4 in the final composite capsules was 0.48-1.19%.

The structure of the microcapsules was characterized by optical microscopy (Olympus BX41) in the wet state and by scanning electron microscopy (JEOL CarryScope JCM 5700) and x-ray micro-tomography (SkyScan Micro-CT attachment for SEM) after freeze-drying. The particle size was evaluated by laser diffraction (Horiba Partica LA 950/V2). The thermo-responsive behavior was studied by using optical microscope equipped with a temperature control stage (Linkam PE94). Captured microscope images were processed by image analysis software (NIS-Elements) to evaluate the particle size. Magnetic induction heating studies were carried out using a 400 kHz, 2.5 kW alternating magnetic field generator (PowerCube 32/400, CEIA, Italy) with a 2-loop, 9 mm i.d. water-cooled copper coil. For release experiments the UV/VIS spectrophotometer Specord 205BU (Analytik Jena) was used.
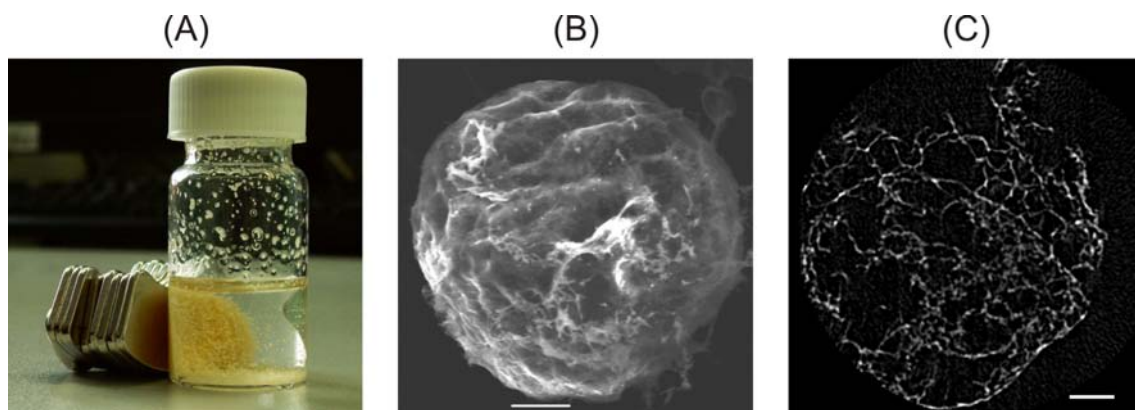

Fig. 2: (A) Suspension of prepared composite PNIPAM/Fe_3O_4 capsules in the presence of a permanent magnet; (B) SEM image of a freeze-dried composite capsule; (C) cross-section of a x-ray micro-tomography image of the capsule. The scale bars represent 50 μm.

3   Results and discussion

The size, morphology and internal structure of the prepared composite PNIPAM/Fe_3O_4 capsules were studied first. At 25 °C the mean diameter was 228 μm for particles with $w_{Fe3O4} =$ 1.19% and 1,243 μm for particles with $w_{Fe3O4} = 0.48\%$. For the intermediate compositions, there was a clear inverse proportionality between capsule size and the weight fraction of iron oxide in the particles. Fig. 2(A) shows an aqueous suspension of the particles and demonstrates the possibility to manipulate them by an external permanent magnet thanks to the presence of magnetic iron oxide nanoparticles in the polymer. A SEM image of a lyophilized particle is shown in Fig. 2(B). Although the macroscopic shape is approximately spherical, it is evident that the surface is locally not smooth. To investigate the internal structure of the capsules, x-ray micro-tomography was used. Fig. 2(C) shows a digital cross-section of a three-dimensional micro-tomography image of one particle. Due to its low density and therefore a low specific absorption coefficient for x-rays, pure PNIPAM is not observable using x-ray micro-

tomography. The measured structures are visible thanks to the Fe_3O_4 dispersed within the polymer and it can be seen that the composite PNIPAM/Fe_3O_4 capsule has a foam-like internal structure with several large cavities separated by walls of the hydrogel. In contrast to PNIPAM/SiO_2 composite capsules described in our recent work [5] where the silica nanoparticles were present only on the outer surface, in this case the iron oxide nanoparticles are dispersed within the polymer both on the external and the internal segments of the capsule.

As was mentioned in the introduction, the main reason for magnetic nanoparticle incorporation into the composite capsules was to enable remote heating by a radiofrequency magnetic field. Fig. 3(A) shows the temperature rise curve of PNIPAM/Fe_3O_4 microcapsules dispersed in water at a concentration of 50 mg/ml, meaning the effective iron oxide concentration was 0.6 mg/ml. Under these conditions the temperature reached the LCST of PNIPAM in less than 10 minutes. The heating curve confirms that *(i)* Fe_3O_4 nanoparticles dispersed within a PNIPAM hydrogel are still capable of dissipating heat by the Néel and Brownian relaxation mechanism, and *(ii)* even at these relatively low concentrations, the rate of heat generation is sufficient to reach the LCST of the polymer necessary for a volume change.
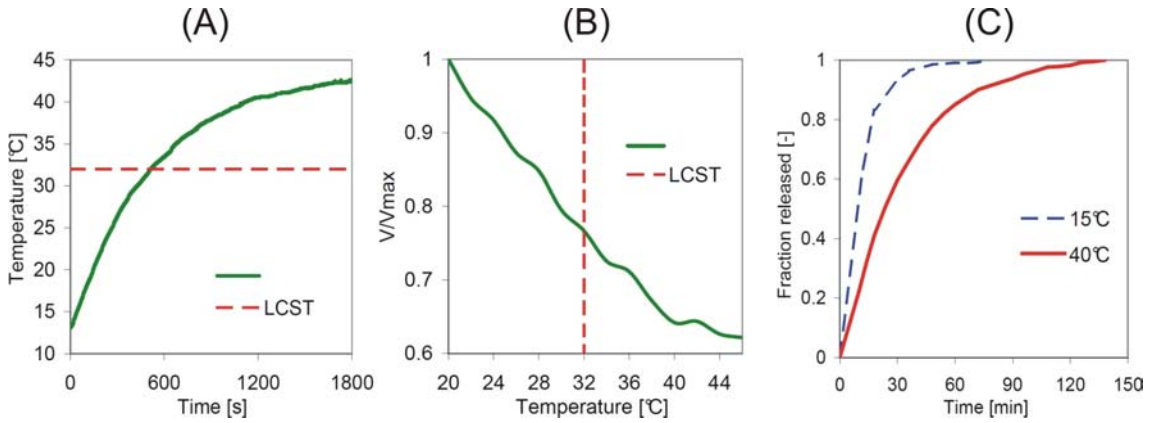

Fig. 3: (A) Heating curve of composite PNIPAM/Fe_3O_4 capsules in 400 kHz alternating magneic field; (B) dependence of the shrinking ratio V/V_{max} on temperature; (C) release curves of vitamin B12 from the capsules at temperatures below and above the LCST of the polymer.

The temperature-sensitive shrinking/swelling behavior of the composite capsules was investigated next. As the observation of volume changes directly in the magnetic field was not technically feasible (metallic parts of the microscope might also heat up by induction), the shrinking/swelling behavior of the microcapsules was observed by a microscope equipped with a temperature control (Peltier) stage. Optical micrographs of the particles at temperatures in the range 20-46°C with 2°C increments were taken and the capsule size evaluated by image analysis. The dependence of the volumetric shrinking ratio V/V_{max} on temperature is shown in Fig. 3(B) for capsules with w_{Fe3O4} = 1.19 %. The composite capsules have shrunk by up to 65% of their original volume and their LCST was comparable with that of the pure PNIPAM, although the volume transition in the vicinity of the LCST was more gradual in this case. Nevertheless, the observed thermo-responsive behavior of composite PNIPAM/Fe_3O_4 particles was similar to that of pure PNIPAM polymer described in literature [1]. This indicates that the presence of dispersed Fe_3O_4 nanoparticles does not suppress the thermo-responsive properties of PNIPAM and the composite microcapsules retained the thermo-responsive capability. There is no *a priori* theoretical limit on the number of shrinking/swelling cycles that a PNIPAM hydrogel can undergo. In a recent work [7] it has been shown that after 20 cycles the behaviour is still fully reversible. When repeated shrinking/swelling cycles were used to expel a chemical payload from the interior of the hydrogel, 10-15 cycles were found to be sufficient to release most of the encapsulated substance.

The combination of results shown in Fig. 3(A) and (B) therefore confirms that a radiofrequency magnetic field is a feasible mechanism for remotely triggering a volume change of up to 65% of the composite PNIPAM/Fe_3O_4 microcapsules. The expanded and collapsed states of the capsules also influence the diffusion rate of a chemical payload from their interior. Fig. 3(C) compares the release curves of vitamin B12 from the capsules at temperatures below (15 °C) and above (40 °C) the LCST. There is a marked difference in the release rates, although it should be noted that the underlying mechanisms are complex, involving the competing effects of changing capsule size, polymer hydrophobicity and the diffusivity of vitamin B12 through the hydrogel network.

For studying the adhesion characteristics of the composite microcapsules, a microfluidic adhesion cell was constructed where it is possible to measure the ratio of adhered microparticles as function of temperature under controlled hydrodynamic conditions (Fig. 4). The bottom section contains a groove for attaching a microscope glass modified by the tested adhesion substrate (bio-polymers, lipid or protein layers, microbial biofilms). A droplet containing suspended microcapsules was first pipetted onto the glass slide, the flowcell was then closed and the number of attached microcapsules with increasing flowrate was recorded using a stereomicroscope equipped with a digital camera and evaluated by automated image analysis. The adhesion strength and therefore the percentage of retained microcapsules can be effectively controlled by temperature, which causes a change in the hydraulic radius and the hydrophilic/hydrophobic character of the microcapsules. Since the drag force acting on a particle in a laminar flow field is $F_d = 6 \pi \eta R u$ (where η is fluid viscosity, R is the particle radius and u is the fluid velocity), the adhesion strength towards each tested surface can be directly calculated.

An example of a cluster of particles adhered to the glass slide is shown in Fig. 5A. During each step increase of flowrate, a certain proportion of particles detached and was removed by the flowing fluid; the percentage of remaining particles, which was stable in time, was then counted. The results, for an untreated glass substrate, are shown in Fig. 5B. It can be seen that at low temperature, when the particles are in the swollen state and their character is hydrophilic, the percentage of remaining particles decreases with increasing flowrate, and above 0.26 ml/s, less than 10 % of the originally present particles remain adhered in the flowcell. On the other hand, at temperature above the LCST of PNIPAM when the particles are hydrophobic and shrunken, the fraction of adhered particles remains high despite the increasing flowrate. Therefore, temperature change (in this case remotely triggered by the RF field) is an effective mechanism for controlling whether the particles attach to a substrate or continue flowing with the carrier fluid.
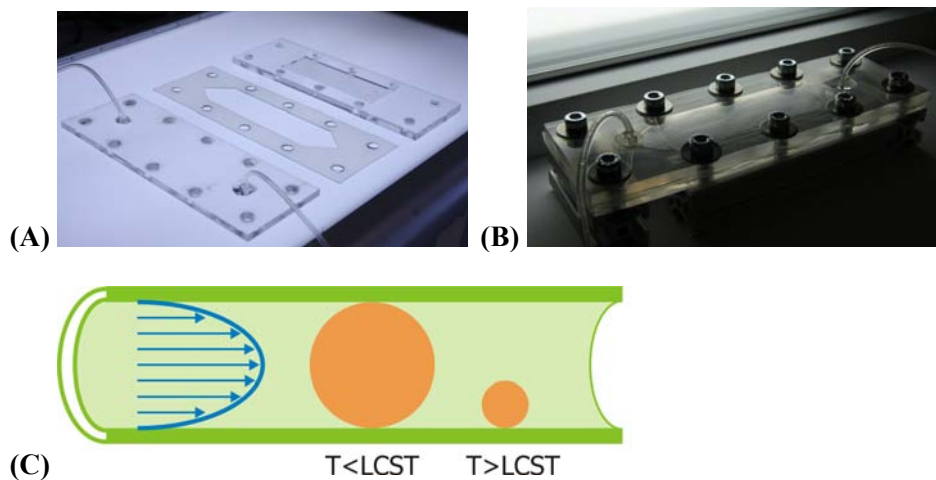

(A)

(B)

(C)   T<LCST   T>LCST

Fig. 4: Laminar flowcell used for adhesion tests (A and B). Principle of thermo-responsive adhesion behaviour in a fluid flow field (C).

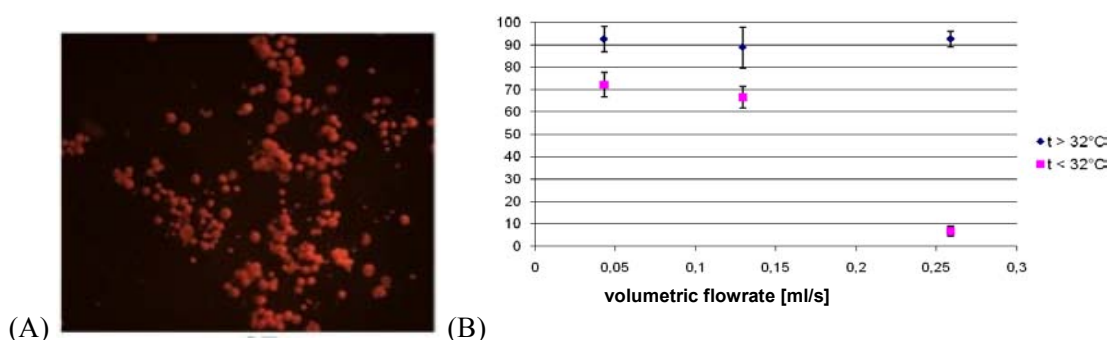(A)   (B)

Fig. 5: (A) Group of particles adhered to a glass substrate at a flowrate of 0.26 ml/s at 40 °C. (B) Dependence of the percentage of adhered particles on flowrate for a glass substrate at temperatures below and above the LSCT of the hydrogel.

4   Conclusions

Composite PNIPAM/Fe$_3$O$_4$ capsules have been prepared. Their size was found to be inversely proportional to the concentration of iron oxide nanoparticles in the starting solution. The internal structure of the capsules was foam-like with macroscopic internal voids (compartments) separated by the PNIPAM/Fe$_3$O$_4$ composite. The capsules exhibited reversible shrinking/swelling behavior as a response to temperature changes, similar to that known from pure PNIPAM. Owing to the presence of iron oxide nanoparticles, it was possible to induce a macroscopic volume change of the capsules as well as their hydrophobic/hydrophilic properties remotely by exposing them to an external alternating magnetic field in the radiofrequency range (400 kHz). The combination of the thermo-responsive behavior of PNIPAM and the induction heating properties of Fe$_3$O$_4$ gives the composite capsules attractive properties such as remote control of their adhesion, aggregation or the diffusion rate of chemical substances.

In relation to the convergence of mechatronic and (bio)chemical-based robotics systems, microcapsules and other objects fabricated from stimuli-responsive polymers can be regarded as one realistic implementation of a system that is able to convert chemical or thermal energy at the micro-scale to macroscopically observable phenomena such as change in size or shape, movement in a flowing fluid (or lack of it due to adhesion), or the change of chemical composition of the local environment. The shape and material substance of a "robot" depends on the task or purpose for which it is built, the nature of the environment in which is has to operate, and on the constraints of the fabrication method by which it is made (mechanical assembly vs. chemical synthesis). If we consider micron-sized robots designed to carry out chemical rather than mechanical tasks in a fluid environment (e.g. targeted delivery of medicines in the human body), it can be expected that these robots resemble internally structured cell-like particles rather than miniature versions of "machines" we know from the macroscopic world. A true convergence between mechatronic and chemically-based robots could be achieved by fabricating hybrid systems. For instance, a stimuli-responsive hydrogel microcapsule carrying a miniature MEMS-based sensor as well as a chemical payload would combine a sensing (diagnostic) capability and the ability to respond by releasing a chemical substance (therapeutic capability). Such hybrid systems could bring novel functionality unattainable by either purely mechatronic or purely (bio)chemical system.

5   References

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