Synthesis of Dual-Stimuli-Responsive Microcontainers with Two Payloads in Different Storage Spaces for Preprogrammable Release

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Abstract: Stimuli-responsive microcontainers have become a major topic of interest, from fundamental aspects to applications in materials science. However, microcontainers that enable the loading of multiple species and programmable release are mostly unknown. Herein, we describe the design and synthesis of a dual-responsive organic/inorganic hybrid microcontainer with two payloads in separate storage spaces that is formed by the rapid UV-initiated polymerization of Pickering emulsions. The stellate mesopore silica nanoparticles with poly(N-isopropylacrylamide) grafted inside the mesopores were loaded with one compound (Nile red) and used as Pickering emulsifiers to stabilize oil-in-water droplets. Upon UV irradiation, pH-responsive monomers were polymerized in the presence of 5(6)-carboxyfluorescein diacetate (CFDA) to form hybrid colloidal microcontainers. The release of Nile red and CFDA could be selectively activated by changing the temperature or pH value.

Stimuli-responsive microcontainer systems are of considerable interest in drug delivery, gene delivery, anticorrosive materials, confined-space catalysis, and self-healing materials, among other applications. Although a variety of stimuli-responsive microcontainers have been successfully developed by diverse chemical and physical methods, most can only encapsulate and release one kind of payload. Although a few microcapsules have been synthesized for the encapsulation of two types of target molecules, they usually release these guests simultaneously under one stimulus, which greatly impedes their practical application, especially in some fields, which require more than one payload encapsulated in multiple compartments for programmable release.

The development of containers with multiple storage areas can be independently controlled by different stimuli for programmable release is an important branch of the field of research on next-generation containers. Although the design of such containers usually involves complex parameters owing to their complicated structures, multiresponsive-ness, and multiple loading. However, some enlightening studies based on different preparation methods have been reported. For example, Li et al. fabricated asymmetric single-hole mesoporous nanocages for anisotropic encapsulation. In this nanocarrier system, the big eccentric hole (ca. 25 nm) was functionalized with upconversion nanoparticles and used to store large guest molecules, such as bovine serum albumin (BSA), whereas the uniform mesopores (2–10 nm) in the silica shells could load small guest molecules after azo modification, such as doxorubicin (DOX). The DOX- and BSA-coloaded single-hole nanorattles were then modified with the phase-change material 1-tetradecanol on the surface. Upon heating, the 1-tetradecanol quickly melted, so that most of the encapsulated BSA and a small amount of DOX molecules were released. Then, a combination of heat and NIR light led to the release of the rest of the BSA and DOX. Although this design is rather ingenious, the fabrication process is quite arduous, and there is only one possible order of release.

Xu et al. demonstrated interesting dual-responsive multi-compartmental microcapsules with two different molecular loads. In their study, star block copolymers with dual-responsive blocks formed the shell of microcapsules by a layer-by-layer (LbL) method with a sacrificial template. The release of hydrophobic and hydrophilic molecules from the shell and core of the microcapsules, respectively, could be triggered independently by variations in temperature and the pH value. This approach enabled controllable release by changing the order in which the stimuli were applied. Nevertheless, the preparation process was still complicated and time-consuming, and only a hydrophilic macromolecule could be effectively encapsulated in the core of microcapsules owing to the high permeability of the block-polymer shell for small molecules.

Herein, we report a novel method for the preparation of dual-stimuli-responsive hybrid microcontainers enabling the preprogrammed sequential release of two payloads. In our design strategy, poly(N-isopropylacrylamide) (PNIPAM) surface modified stellate mesopore silica nanoparticles (SMSNPNIpAM) are used as nanocarriers for one guest compound (e.g., Nile red) and simultaneously serve as Pickering emulsifiers to stabilize oil-in-water droplets. These droplets contain pH-responsive monomers and another guest molecule, 5(6)-carboxyfluorescein diacetate (CFDA). Upon UV-irradiation polymerization, the pH-responsive monomers are converted into polymer microcapsules, and dual-stimuli-responsive hybrid microcontainers are quickly obtained, by a method that is totally different from those mentioned above.

In this way, two guests can be independently encapsulated in two different storage spaces—mesoporous silica and polymer microcapsules—and no mutual interference between the two payloads exists, in particular during the release of one payload. More importantly, the obtained hybrid microcontainers can realize preprogrammed and independent release of guest molecules under different stimuli, the order of which...
can be altered. To the best of our knowledge, no dual-responsive microcontainers with dual loading and sequence-controlled release have been prepared previously through simple Pickering emulsion polymerization. This method appears to be of broad interest for the development of programmable release and delivery systems.

Figure 1a briefly describes the preparation process of the dual-stimuli-responsive hybrid microcontainers. SMSNs were prepared, their surface was modified, and PNIPAM was grafted inside their pores (see the Supporting Information). After loading with Nile red, these thermal-responsive hybrid nanoparticles (designated as SMSN-PNIPAM-NR) were dispersed as Pickering emulsifiers with the monomers 2-(N,N-diethylamino)ethyl methacrylate (DEAEMA) and 1,6-hexanediol diacrylate (HDDA), another model molecule CFDA, and water to form stable Pickering emulsions. According to our experiments, Nile red could be replaced with other small hydrophobic molecules, and CFDA could be replaced with other molecules that can dissolve in the mixed liquor of DEAEMA and HDDA.

We used Fourier transform infrared spectroscopy to check the successful surface modification of SMSNs with a Triton X-100 tethered silane coupling agent (m-SMSN) and orderly mesopore grafting with amino groups (SMSN-NH$_2$), an initiator (SMSN-Br), and finally PNIPAM (SMSN-PNIPAM). For bare SMSNs, a Si–O–Si symmetrical stretching vibration at 804 cm$^{-1}$, Si–OH bending vibration at 955 cm$^{-1}$, and Si–O asymmetric stretching vibration at 1080 cm$^{-1}$ were observed (see Figure S3a). An obvious peak at 1512 cm$^{-1}$ corresponding to the C=O stretching of an aromatic ring appeared for the surface of m-SMSN. For SMSN-PNIPAM, a peak was observed at 1653 cm$^{-1}$ due to C=O stretching of the amide I band and another peak at 1538 cm$^{-1}$ due to N–H stretching of the amide II band. Furthermore, two bands appeared at 1367 and 1388 cm$^{-1}$ with similar intensity as a result of deformation of the two methyl groups of the isopropyl group. Weight losses of 10.3 and 31.2% below 800°C occurred for SMSNs and unextracted SMSNs, as shown by thermogravimetric analysis (TGA; see Figure S3b). This difference corresponds to the surfactant cetyltrimethylammonium tosylate, which blocks the pores of unextracted SMSNs. On the basis of the TGA analysis of SMSN-Br and SMSN-PNIPAM, there is 19.3 wt% of PNIPAM in the resultant composite SMSN-PNIPAM. The modification and grafting also changes the z potential: The z potential of bare SMSNs is $-20.4$ mV, whereas that of SMSN-Br and SMSN-PNIPAM is $23.9$ and $2.85$ mV, respect-
Accordingly, electrostatic repulsion between the positively charged polymer amino groups in the polymer shells. pH value to 4 at room temperature leads to protonation of the molecules can rapidly diffuse out of these pores, while the mesopores of SMSN into a collapsed state; thus, the Nile red molecules can rapidly diffuse out of these pores, while the green CFDA is still encapsulated in the polymer microcapsules (Figure 3d). In the second step, a change in the pH value to 4 at room temperature leads to protonation of the amino groups in the polymer shells. Accordingly, electrostatic repulsion between the positively charged polymer

We examined the stimulus response of microcontainers loaded with only Nile red or CFDA and observed a good response to heating or a change in the pH value (see Figures S8 and S9). The hybrid microcontainers contained both Nile red in the SMSN-PNIPAM and green CFDA inside the polymer microcapsules, as measured by a confocal laser scanning microscope (CLSM; Figure 3a–c). The programmable release of the encapsulated guest molecules was achieved by sequential exposure of the hybrid microcontain-

Figure 3. a) CLSM image of the hybrid microcontainers with Nile red encapsulated in the SMSN-PNIPAM and CFDA inside the microcapsules. b) Red channel and c) green channel of the same area in the same sample. The plot below each image is the representative fluorescence intensity profile across the hybrid microcontainers. d) CLSM image of the sample in (a) after heating to 45 °C for 25 min. e) CLSM image of the sample in (d) after the pH value had been changed to 4 for 50 min. f) Transmission-mode image of the area in (e). g) CLSM image of the sample in (a) after the pH value had been changed to 4 for 50 min. h) CLSM image of the sample in (g) after heating to 45 °C for 25 min. i) Transmission-mode image of the area in (h).

After UV irradiation, monomers DEAEMA and HDDA were polymerized along the interior surfaces of the SMSN-PNIPAM-NR nanoparticles as Pickering emulsifiers to create dual-responsive hybrid microcontainers.\[17,46\] As shown in Figure 2e,f, these hybrid spheres with a size distribution of between 1 and 4 μm, which is comparable to that of the oil droplets stabilized by SMSN-PNIPAM-NR (see Figure S7), are clearly covered by a dense layer of mesoporous nanoparticles. The production of these hybrid spheres by photo-initiation polymerization can make the monomers inside these emulsions finish the polymerization reaction instantly without heating. In this way, all the emulsion droplets can smoothly and quickly turn into tight hybrid spheres before the emulsions become unstable. More importantly, without heating, the Nile red molecules preloaded in the mesopores of SMSNs do not escape during polymerization, as verified by fluorescence spectroscopy. Meanwhile, another guest compound, CFDA, can be encapsulated well into the microcapsules to produce hybrid microcontainers with two payloads in two different compartments.

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As shown in Figure S3c). To measure the molecular weight and distribution of the grafted PNIPAM, we treated the as-obtained SMSN-PNIPAM with hydrofluoric acid to remove the SMSNs. The obtained PNIPAM was purified, and its number-average molecular weight was determined by gel-permeation chromatography to be 11400 with a narrow distribution (see Figure S3d).

The SMSNs had the largest BET surface area of 459.7 m²/g⁻¹ and pore size of 11.8 nm, as measured by N₂ adsorption/desorption analysis (see Figure S4), and is consistent with the pore size observed by SEM (Figure 2a). After grafting with PNIPAM, the BET surface area decreased remarkably to 48.5 m²/g⁻¹, and the pores had been blocked, thus further confirming that these mesopores had been grafted with PNIPAM chains. SMSN-PNIPAM can be used as nanocarriers, with Nile red adsorbed in the mesopore channels of SMSN and prevented from diffusing out of the nanoparticles by the PNIPAM chains (see Figure S5). On the basis of the fluorescence intensity of the supernatant of SMSN-PNIPAM, around 93.8% of the Nile red was encapsulated. Lower critical solution temperature (LCST) behavior of SMSN-PNIPAM was also observed (see Figure S6): The nanoparticles, which were well-dispersed at 25 °C, aggregated and precipitated in aqueous media at 40 °C as a result of the transformation of PNIPAM grafted in the pores into the collapsed state. The transformation temperature was a little higher than that for pure PNIPAM (32 °C). Both transformation temperatures were not changed by the pH value of the medium.

Figure 2c,d compares the Pickering emulsions stabilized by bare SMSNs and SMSN-PNIPAM-NR. As expected, the emulsion prepared with SMSN-PNIPAM-NR is homogeneous without any stratification observed, whereas that from unmodified SMSNs showed apparent phase separation. The emulsion stabilized with SMSN-PNIPAM-NR had a much smaller size and size distribution as compared to that stabilized by virgin SMSNs. Owing to the pre-encapsulation of Nile red in the nanoparticles, the emulsion in Figure 2d is pink.

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chains would swell the microcapsules to release the encapsulated green CFDA (Figure 3e,f).

By changing the stimulus sequence, the release order of Nile red and CFDA molecules can be entirely reversed. When the pH value is employed as the first stimulus (a change from pH 7 to 4 at room temperature), complete release of CFDA molecules from the microcapsule interior is observed, whereas Nile red remains inside the mesopores of the SMSNs (Figure 3g). When the hybrid nanoparticles are subjected to increasing temperature, the release of Nile red is activated (Figure 3h,i). All of these hybrid spheres remain stable after the two release steps, and thus might be used for loading and delivery once again.

Quantitative release analysis of Nile red at 45 °C and CFDA at pH 4 from these hybrid microcontainers showed that, on the whole, the CFDA was released at a slower speed than Nile red: The amount of CFDA released was up to 96.1% in 45 min, whereas that of Nile red was up to 94.7% after only 24 min (Figure 4). The release speed based on the pH stimulus could be adjusted by varying the amount of DEAEMA.[17] Furthermore, the use of a temperature above the LCST of PNIPAM also influenced the release speed as a result of the diffusion effect.

Clearly, the reason why the hybrid microcontainers show preprogrammed and independent release of payloads under different stimuli (pH, temperature) without any mutual interference between guest molecules is that the hybrid microcontainers have two separate store spaces: The inorganic mesoporous silica and the hollow core surrounded by a polymer shell can independently encapsulate different payloads and respond to different stimuli.

In summary, we have proposed a viable and relatively simple method to fabricate dual-stimuli-responsive hybrid microcontainers with preprogrammed sequential release of two payloads in different storage spaces through the UV-initiated polymerization of Pickering emulsions. Owing to the encapsulation of one guest compound (e.g., Nile red) in the mesopores of silica nanoparticles and another guest (e.g., CFDA) in the hollow core of the microcapsules, the release of two payloads can be selectively and independently triggered by changes in the temperature or pH value, without any mutual interference or order requirement. This UV-initiated polymerization of Pickering emulsions could be readily extended to the fabrication of other hybrid microcontainers that respond to two or even more stimuli, such as a magnetic field, chemicals, or reducing conditions, and carry multiple payloads. Therefore, this design strategy, the nature of Pickering polymerization, and the preprogrammable sequential release of different payloads of multiresponsive hybrid microcontainers, are of particular fundamental interest and may open interesting perspectives for the application of such preprogrammed functional structures in materials science.

**Experimental Section**

Synthesis of SMSNs: SMSNs were prepared by a modified method based on a previous report.[44] Typically, a mixture of cetyltrimethylammonium tosylate (2.4 g), triethylamine (0.093 g), triethanolamine (0.29 g), and water (125 mL) was stirred at 80 °C for 1 h, then tetraethyl orthosilicate (TEOS; 19.5 mL) was quickly added to the solution, and the mixture was stirred at 80 °C for another 2 h. The obtained SMSNs were washed with water and ethanol and freeze-dried.

Preparation of SMSN-PNIPAM: The external surface of the SMSNs was modified by a Triton X-100 tethered silane coupling agent and their inner surface with an amino group to give SMSN-NH₂, which was further functionalized with 2-bromoisoobutyryl bromide to provide SMSN-Br. Finally, the grafting of PNIPAM in the SMSN pores gave the hybrid nanoparticles SMSN-PNIPAM.

Loading of Nile red: SMSN-PNIPAM (0.062 g) was dispersed in water (36 mL), then a solution of Nile red in methanol (0.5 mg mL⁻¹; 400 μL) was added dropwise under agitation. The mixture was centrifuged, and the precipitate was washed thoroughly with water. The obtained sample was redispersed in water (36 mL) and denoted as SMSN-PNIPAM-NR.

Synthesis of dual-responsive hybrid microcontainers with two payloads: The dispersion of SMSN-PNIPAM-NR (0.172 wt % aqueous dispersion; 36 g) was added to a 150 mL beaker containing DEAEMA (0.1 g), HDDA (0.1 g), 1-hydroxycyclohexyl phenyl ketone (4 mg), and CFDA (0.2 mg), and the mixture was dispersed by ultrasonication in an ice bath for 20 min. The resulting emulsion was transferred to a quartz flask and purged with N₂. Polymerization of the emulsion was carried out under UV irradiation for 30 min.

In vitro release: Briefly, the suspension of the hybrid microcontainers with two payloads was loaded into a dialysis bag, the molecular-weight cutoff of which was much larger than that of the dye molecules. An aliquot of the dialysis fluid was withdrawn, and the fluorescence intensity was measured at different time. For the temperature-induced release of Nile red, the suspension was dialyzed against a bath with temperature of 45 °C. For the pH-induced release of CFDA, the suspension at pH 7 was dialyzed against a bath at pH 4. 100% in the quantitative release curves corresponds to ideal complete release of Nile red or CFDA from the microcontainers.

![Figure 4.](image-url) a) Release of Nile red from the hybrid microcontainers at 45 °C. b) Release of CFDA from the hybrid microcontainers at pH 4.
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Conflict of interest

The authors declare no conflict of interest.

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