DOI 10.5012/bkcs.2011.32.4.1357

Sugar-Induced Release of Guests from Silica Nanocontainer with Cyclodextrin Gatekeepers

Jinwoo Lee, Jeonghun Lee, Saehee Kim, Chang-ju Kim, Sangyong Lee, Byungcheol Min, Yongtae Shin, and Chulhee Kim*

*Department of Polymer Science and Engineering, Inha University, Incheon 402-751, Korea. *E-mail: chk@inha.ac.kr*
Received December 22, 2010, Accepted January 27, 2011

Key Words : Cyclodextrin, Mesoporous silica, Sugar, Stimuli-responsive, Controlled release

Stimuli-responsive silica nanoparticles with porous channels have great potential for useful application in the area of drug and gene delivery because of their unique responsiveness and high stability.[1-3] In particular, these nanoparticles with gatekeepers are numerously attractive as delivery vehicles which have unique properties for the controlled release of guests under specific conditions.[4] Mesoporous silica nanoparticles (Si-MPs) can serve as outstanding host for guest molecules with various sizes, shapes, and functionalites.[5] They are more stable under surrounding conditions like pH, temperature than other nanocarriers such as liposomes, polymer micelles.[6]

A variety of gatekeepers have been used to keep guest molecules in the pore before releasing the guest molecules. Recent reports regarding stimuli-responsive nanocontainers have shown that rotaxane,[7] cyclodextrin (CD),[1,8,9] cucurbituril,[10] polypseudorotaxane,[9] gold nanoparticle,[11] antibody,[12] dendrimer,[13,14] magnetic nanoparticle[15] or anion[16] can be used as the stimuli-responsive gatekeepers on the surface of Si-MPs. There have been various stimuli to remove the gatekeepers such as pH,[9,17] light,[8] temperature,[18] photo,[1,19] competitive binding,[20] redox potential[21] and enzyme.[1]

According to recent reports, boronic acid derivatives are used as fluorescent sugar sensors because of their greater stability.[22] The formation of boronic acid changes due to the concentration of sugar and pH of the surrounding medium.[23,24]

Herein, we report on stimuli-responsive silica nano-container with CD gatekeepers that contain boronic acid linker, calcein as guest molecules and can exhibit sugar responsive characteristics in the medium of pH 9.8. The phenylboronic acid derivatives form stable cyclic esters through the strong binding of the boronic acid functionality with the functionality unit of the sugar.[25] Si-PB can form tetrahedral borate intermediates in the pH level higher than the pK_a of phenylboronic acid to produce Si-PB-CD in which the CD moiety can be a gatekeeper of the silica nanocontainer.[26] In addition, removal of the CD moiety from Si-PB-CD by using other sugar such as D-fructose which can bind more strongly to the boronic acid unit than CD would induce release of the guest molecules in the pore of the silica nanocontainer. The release of the guest molecules can be controlled by pH, nature of the sugar, and the sugar concentration.

For sugar-responsive nanocontainers, we prepared MCM-41 type mesoporous silica nanoparticle, Si-MP, with about 60 nm diameter (pore diameter ~2 nm) and hexagonal porous channels, as shown in Figure 1. As summarized in Scheme 1, we also synthesized aminopropyl-functionalized mesoporous silica nanoparticles (Si-NH_2) which contain amine units on the surface. The surface boronic acid unit was introduced by the reaction of the amine group of Si-NH_2 with *p*-dihydroxyborylbenzoic acid *N*-hydroxysuccinimide ester (BA-NHS) to provide Si-PB. The surfactant template (*n*-cetyltrimethylammonium bromide: CTAB) was removed from Si-PB by refluxing the methanol solution of Si-PB (MeOH/HCl 100:1). The FT-IR spectrum of Si-PB showed the amide absorption band at 1350 cm^{-1}. Si-NH_2 and Si-PB were characterized by transmission electron microscopy (TEM), field emission scanning electron microscope (FE-SEM), powder X-ray diffraction (XRD) and Barret-Joyner-Halenda analysis (BET) (Fig. 1). The ζ-potential values of Si-NH_2, Si-PB and calcein-loaded Si-PB-CD were +14.18 mV, −12.71 mV and −22.54 mV, respectively.

The quantity of the surface amine groups of Si-NH_2 was estimated to be 0.269 mmol per one gram of Si-NH_2 by ninhydrin test.[27] After the reaction of Si-NH_2 with BA-NHS, the remaining unreacted amine groups on Si-PB were estimated to be 0.0944 mmol per one gram of Si-PB by using the same test. Therefore, the penylboronic acid group

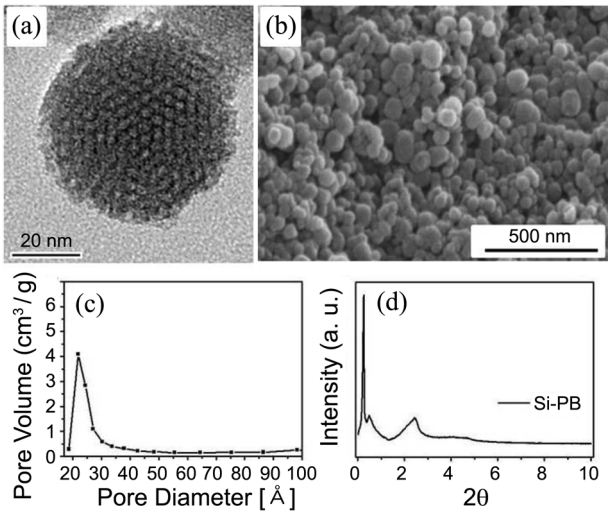
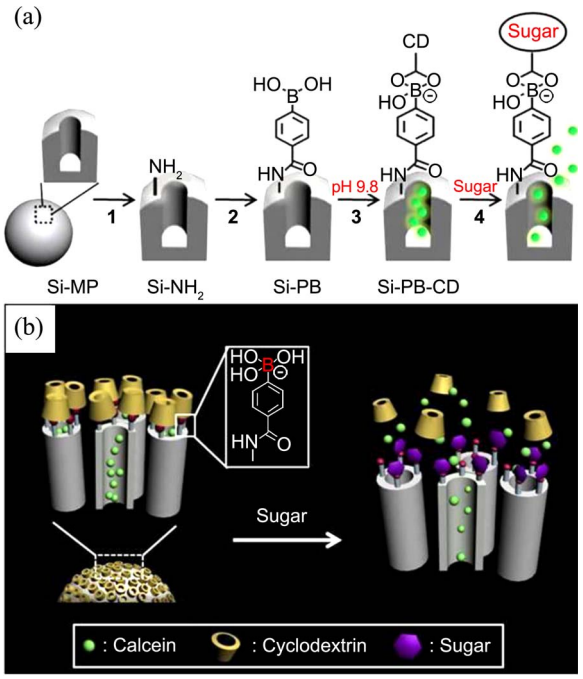


Figure 1. TEM (a) and SEM (b) images of Si-PB. (c) Barret-Joyner-Halenda pore distribution of Si-PB, (d) X-ray diffraction pattern of Si-PB.



Scheme 1. (a) Synthetic procedure for cyclodextrin-covered nanocontainers (Si-PB-CD). 1) APTES; 2) BANHS; 3) removal of CTAB, loading calcein and capping of the pore with β-CD in CHES buffer solution (pH 9.8, adjusted by NaOH with 0.1 M NaCl); 4) addition of sugars such as D-fructose and D-galactose. (b) Schematic illustration of the controlled release of guests from the pore of Si-PB-CD triggered by addition of sugar molecules.

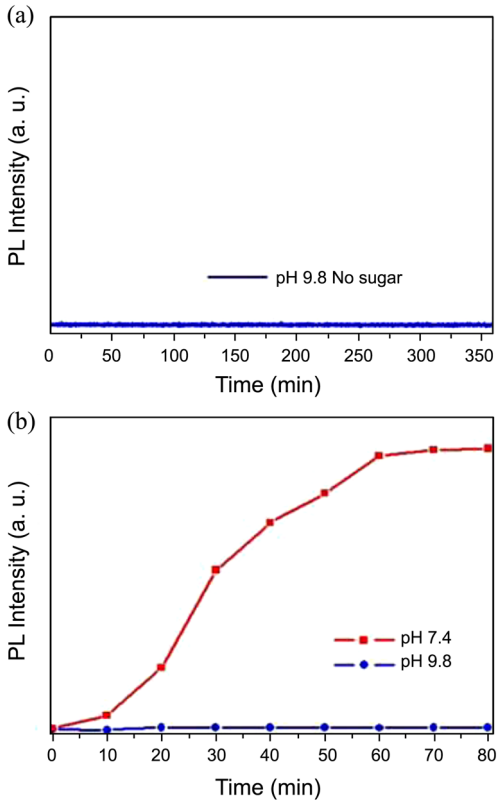


Figure 2. (a) Time-courses of fluorescence intensity of Si-PB-CD in the absence of sugar at pH 9.8. (b) pH-induced release of guest entrapped in Si-PB-CD at pH 7.4 (red dot) and pH 9.8 (blue dot).

was quantified to be around 0.1746 mmol per one gram of Si-PB. The surface coverage of phenylboronic acid moiety on Si-PB was estimated to be about 64.9%. The guest molecules dissolved in the dimethylformamide (DMF) and 10 mM 2-(cyclohexylamino)ethanesulfonic acid (CHES) solution (pH 9.8, adjusted by NaOH with 0.1 M NaCl) were loaded into the pore of silica nanocontainers by soaking method.[28] The pH of the solution was higher than the pK_a value of the boronic acid unit so that addition of the β-CD would induce the diol coupling with Si-PB. In the condition, a tetragonal boronate ester form of β-CD would be an effective gatekeeper of the pore of the nanosilica container.

The weight percentage of β-CD on Si-PB-CD measured by a glucose assay kit (Sigma) was 0.56%. The surface coverage of β-CD on Si-PB-CD, compared with a closely packed surface system, was estimated to be about 10%.[1] The loading percentage of calcein in Si-PB-CD calculated by UV/vis absorption spectra was about 1.8 wt %. The release of the calcein guest molecules from the pore of Si-PB-CD was monitored by fluorescence measurements.

The release property of the guest molecules from the pore of Si-PB-CD was observed by using fluorescence measurements. As shown in Figure 2(a), the calcein guest molecules were not released from the pore of Si-PB-CD more than 6 hours in the absence of D-fructose, indicating that the CD gatekeeper on Si-PB-CD is very effective to keep the calcein molecules in the pore of Si-PB-CD. As shown in Figure 2(b), the change of the pH value of the solution lower than pK_a of the phenylboronic acid unit induced the release of the guest calcein from Si-PB-CD because the CD unit would be dissociated from the borate ester moiety to open the pore of the silica nanocontainer.[26]

Addition of various sugar molecules such as D-fructose and D-galactose to the aqueous suspension of the calcein loaded Si-PB-CD efficiently triggered release of the guest molecules from the pore, because the CD gatekeeper on Si-PB-CD would be removed by the incoming sugar as shown in Scheme 1(a) and Figure 2(a). D-Fructose and D-galactose bind to the boronic acid moiety more strongly than CD as shown in Figure 3(a). Because CDs are cyclic oligosaccharides composed of α-1,4-coupled D-glucose units,[29] D-mannose and D-glucose cannot remove the CD gatekeeper as shown in Figure 3(a). The fluorescence intensity of calcein from Si-PB-CD was effectively increased in the high concentration of D-fructose, as shown in Figure 3(b). With increasing the concentration of D-fructose, we can observe more enhanced release of the calcein molecules from the silica nanocontainer.

We also synthesized and investigated the silica nanocontainers capped with α- or γ-CD. As shown in Figure 4, in the absence of D-fructose, the fluorescence intensities were nearly constant over 2 hours, which indicates that the α- and γ-CD gatekeeper forcibly blocked release of loaded calcein molecules in the channel of Si-PB-CD in basic condition (pH 9.8). In the presence of 70 mM D-fructose, the entrapped guest molecules in the Si-PB-CD using α- or γ-

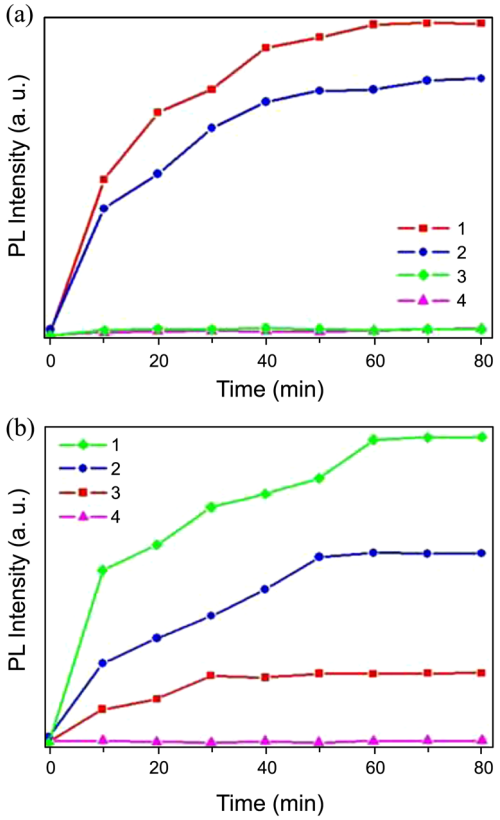


Figure 3. (a) Sugar-induced release of guest from Si-PB-CD; (1) D-fructose, (2) D-galactose, (3) D-mannose, (4) D-glucose. The concentration of monosaccharides was 50 mM. (b) Release profiles of guest loaded in Si-PB-CD induced by D-fructose with different concentration; (1) 100 mM, (2) 50 mM, (3) 10 mM, (4) 0 mM of D-fructose.

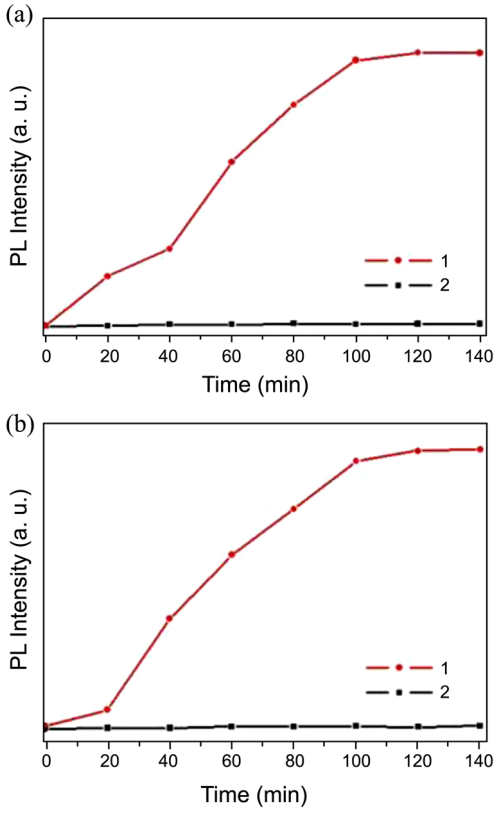


Figure 4. Time courses of fluorescent intensity of Si-PB-CD using α-CD (a) and γ-CD (b) as a gatekeeper in the absence (black) and the presence (red) of D-fructose (70 mM) at pH 9.8.

CD as gatekeepers were released during about 2 hours as shown in Figure 4. These results indicates that the α- and γ-CD are also adequate for gatekeeper of drug carrier to keep guests under control during delivery and replaced by sugar which triggered to release the guest molecules in a controlled manner.

We have demonstrated that the incorporation of the CD gatekeeper on the surface of silica nanocontainers modified with boronic acid is a very efficient approach not only to entrap the guest molecule in the pore reservoir but also to release the guest in response to pH and the sugar molecules such as D-fructose and D-galactose. In such conditions, the CD gatekeeper can be well removed at the phenylboronic acid stalk moiety and then the guest molecules were effectively released from Si-PB-CD. We expect that the Si-MPs systems described here can be used as sugar sensitive nanocontainers and provide diverse applications as an effective delivery system.

Experimental

Materials. Cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), 4-carboxyphenylboronic acid, *N,N*-diisopropylethylamine (DIPEA), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), calcein, ethanol, methanol and dimethylformamide (DMF) were obtained from Aldrich and used as received. D-(+)-galactose, D-(+)-glucose, D-(−)-fructose, D-(+)-mannose, CHES were obtained from Sigma and used as received. β-cyclodextrin (β-CD) was obtained from TCI and used as received.

Synthesis of Si-MP, Si-NH_2. Si-MP and Si-NH_2 were synthesized as described previously.[9]

Synthesis of *p*-Dihydroxyborylbenzoic Acid *N*-Hydroxysuccinimide Ester (BA-NHS). BA-NHS was synthesized using the reference procedure.[31]

Synthesis of Si-PB. Si-NH_2 (100 mg) in DMF (20 mL) was stirred for 48 hours at 60 °C after addition of BA-NHS (200 mg). The resulting particles were separated by centrifugation (9500 rpm, 15 min) and washed with DMF (1 time), methanol (2 times) and distilled water (2 times). Then the particles dried *in vacuo*. To remove CTAB, the resulting particles were stirred for 24 h at 60 °C in the mixture of methanol (16 mL) and concentrated HCl (0.9 mL). The surfactant removed nanoparticles were purified by centrifugation and washed several times with methanol.

Synthesis of Si-PB-CD. Si-PB (30 mg) and calcein (10 mg) suspended in a mixture of CHES buffer (1 mL, pH 9.8) and DMF (1 mL) were stirred for 24 h at room temperature. Then, β-CD (50 mg) was added following to adjust pH of the solution to 9 with NaOH. The mixture was stirred at

room temperature for 3 days. The resulting particles were then purified by centrifugation (9,500 rpm, 20 min) and washed several times with DMF/CHES solution.

Determination of the Surface Coverage of Amine Groups on Si-MPs. The surface amine groups were quantified at 0.269 mmol per one gram of Si-NH_2 by ninhydrin test.[27] In Si-PB, the remaining surface amine groups were quantified at 0.0944 mmol per one gram of Si-PB by ninhydrin test, and surface boronic acid groups were calculated to be around 0.1746 mmol per one gram of Si-PB by subtracting the amount of remaining surface amine groups from that on Si-PB surface. The surface coverage of phenylboronic acid moiety on Si-PB was estimated to be about 64.9%.

Determination of the Surface Coverage of CDs on Si-PB-CD. The quantitative analysis of β-CD on Si-PB-CD was carried out by using glucose assay kit (Sigma) based on the manufacturer's procedure. The weight percentages of β-CD on Si-PB-CD were 0.56 wt %. The surface coverage of β-CD on Si-PB-CD, compared with a closely packed surface system, was estimated to be about 10%.[1]

Instrument.

Fluorescence Measurements. All the fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer. The emission and excitation slit widths were set at 3 nm, and the excitation wavelength was 490 nm (the absorption maximum of calcein).

X-ray Diffraction (XRD) Experiment. XRD patterns were recorded at room temperature on a Rigaku model MAX 2200V counter diffractometer with a CuK_α radiation source (Operated at 60 kV, 60 mA).

Barret-Joyner-Halenda Pore Distribution (BET). The pore size was measured at 77 K on a Quantachrome instrument.

Transmission Electron Microscopy (TEM) Analysis. TEM images were obtained using a Philips CM 200 instrument operated at an acceleration voltage of 120 kV. For the preparation of dispersed samples in water, a drop of sample solution (100 mg/L) was placed onto a 300-mesh copper grid coated with carbon. About 2 min after deposition, the grid was touched with filter paper to remove surface water. The samples were air dried before measurement.

Field Emission Scanning Electron Microscope (FE-SEM) Analysis. FE-SEM analysis was carried out on a field emission gun FE-SEM instrument (Hitachi S-4200) with 10-15 kV of accelerating voltage and 0.8-0.9 Torr of pressure range. The FE-SEM samples were prepared by transferring a drop of sample solution onto a 200 mesh carbon-coated copper grid or a silicon wafer. About 5-10 min after deposition, excess water was removed by touching the edge of the substrate with filter paper. The samples were air dried before measurement.

Zeta-potential Analysis. Zeta-potential values were obtained using OTSUKA Particle Size Analyzer ELS-Z2 by dispersing the sample in distilled water.

Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR spectra were obtained using Perkin-Elmer System 2000 FR-IR spectrophotometer.

UV/vis Spectra. UV/vis spectra were recorded on Hewlett-Packard 8452A spectrophotometer.

Acknowledgments. This work was supported by the Korea Research Foundation (KRF) grant funded by the Korea government (MEST) (No. 2009-0079739).

References

1. Park, C.; Kim, H.; Kim, S.; Kim, C. *J. Am. Chem. Soc.* **2009**, *131*, 16614.
2. Nguyen, T. D.; Leung, K. C.; Liong, M.; Liu, Y.; Stoddart, J. F.; Zink, J. I. *Adv. Funct. Mater.* **2007**, *17*, 2101.
3. Trewyn, B. G.; Slowing, I. I.; Giri, S.; Chen, H.; Lin, V. S.-Y. *Acc. Chem. Res.* **2007**, *40*, 846.
4. Shum, P.; Kim, J.; Thompson, D. H. *Adv. Drug Deliv. Rev.* **2001**, *53*, 273.
5. Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S.-Y. *Adv. Funct. Mater.* **2007**, *17*, 1225.
6. Barbé, C.; Bartlett, J.; Kong, L.; Finnie, K.; Lin, H. Q.; Larkin, M.; Calleja, S.; Bush, A.; Calleja, G. *Adv. Mater.* **2004**, *16*, 1959.
7. Nguyen, T. D.; Liu, Y.; Saha, S.; Leung K. C.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2007**, *129*, 626.
8. Park, C.; Lee, K.; Kim, C. *Angew. Chem. Int. Ed.* **2009**, *48*, 1275.
9. Park, C.; Oh, K.; Lee, S. C.; Kim, C. *Angew. Chem. Int. Ed.* **2007**, *46*, 1455.
10. Angelos, S.; Khashab, N. M.; Yang, Y.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2009**, *131*, 12912.
11. Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Guillem, C. *J. Am. Chem. Soc.* **2009**, *131*, 6833.
12. Climent, E.; Bernardos, A.; Martínez-Máñez, R.; Maquieira, A.; Marcos, M. D.; Pastor-Navarro, N.; Puchades, R.; Sancenón, F.; Soto, J.; Amorós, P. *J. Am. Chem. Soc.* **2009**, *131*, 14075.
13. Radu, D. R.; Lai, C.; Jeftinija, K.; Rowe, E. W.; Jeftinija, S.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2004**, *126*, 13216.
14. Diaz, I.; Garcia, B.; Alonso, B.; Casado, C. M.; Moran, M.; Losada, J.; Perez-Pariente, J. *Chem. Mater.* **2003**, *15*, 1073.
15. Giri, S.; Trewyn, B. G.; Stellmaker, M. P.; Lin, V. S.-Y. *Angew. Chem. Int. Ed.* **2005**, *44*, 5038.
16. Casasus, R.; Climent, E.; Marcos, M. D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Amoros, P.; Cano, J.; Ruiz, E. *J. Am. Chem. Soc.* **2008**, *130*, 1903.
17. Yang, Q.; Wang, S.; Fan, P.; Wang, L.; Di, Y.; Lin, K.; Xiao, F. *Chem. Mater.* **2005**, *17*, 5999.
18. You, Y.; Kalebaila, K. K.; Brock, S. L.; Oqpický, D. *Chem. Mater.* **2008**, *20*, 3354.
19. Patel, K.; Angelos, S.; Dichtel, W. R.; Coskun, A.; Yang, Y.-W.; Zink, J. I.; Stoddart, J. F. *J. Am. Chem. Soc.* **2008**, *130*, 2382.
20. Leung, K. C.; Nguyen, T. D.; Stoddart, F.; Zink, J. I. *Chem. Mater.* **2006**, *18*, 5919.
21. Liu, R.; Zhao, X.; Wu, T.; Feng, P. *J. Am. Chem. Soc.* **2008**, *130*, 14418.
22. Tong, A.; Yamauchi, A.; Hayashita, T.; Zhang, Z.; Smith, B. D.; Teramae, N. *Anal. Chem.* **2001**, *73*, 1530.
23. Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Chem. Commun.* **2008**, *21*, 2477.
24. Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. *Adv. Mater.* **2009**, *21*, 2787.
25. Zhao, Y.; Trewyn, B. G.; Slowing, I. I.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2009**, *131*, 8398.
26. Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Tetrahedron* **2004**, *60*, 11205.
27. Tsai, M.; Tsai, T.; Shieh, D.; Shiu, H.; Lee, C. *Anal. Chem.* **2001**, *73*, 1530.
28. Angelos, S.; Yang, Y.-W.; Patel, K.; Stoddart, J. F.; Zink, J. I. *Angew. Chem. Int. Ed.* **2008**, *47*, 2222.
29. Van, D. M.; Vermonden, T.; van Nostrum, C. F.; Hennink, W. E. *Biomacromolecules* **2009**, *10*, 3157.
30. Armarego, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*, 4th ed.; Butterworth- Heinemann: Oxford, 1996.
31. Masuda, T.; Nagasaki, T.; Tamagaki, S. *Supramolecular Chemistry* **2000**, *11*, 301.