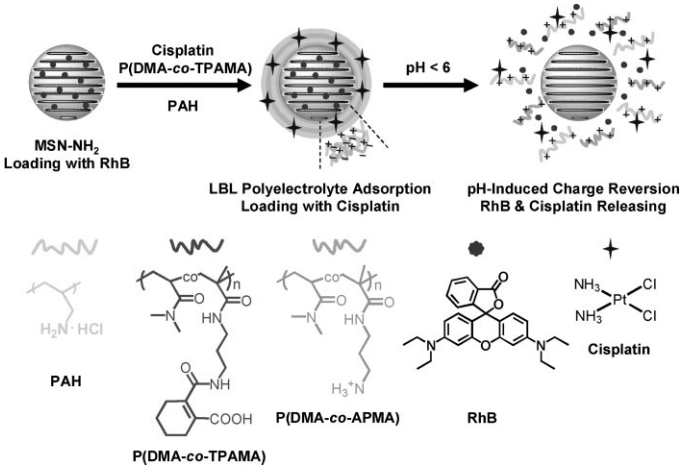
pH-Disintegrable Polyelectrolyte Multilayer-Coated Mesoporous Silica Nanoparticles Exhibiting Triggered Co-Release of Cisplatin and Model Drug Molecules[a]

Xuejuan Wan, Guoying Zhang, Shiyong Liu*

We report on the fabrication of pH-disintegrable polyelectrolyte multilayer-coated mesoporous silica nanoparticles (MSN) capable of triggered co-release of cisplatin and model drug molecules. The outer polyelectrolyte multilayer was assembled from permanently cationic polyelectrolyte, poly(allyl amine hydrochloride) (PAH), and negatively charged polyelectrolyte, P(DMA-*co*-TPAMA), consisting of *N*,*N*-dimethylacrylamide (DMA) and 3,4,5,6-tetrahydrophthalic anhydride-functionalized *N*-(3-aminopropyl)methacrylamide (TPAMA) monomer units, which exhibits pH-induced charge conversion characteristics. Thus, the subtle alteration of solution pH from 7.4 to ≈5–6 can lead to the disintegration of outer polyelectrolyte multilayers, accompanied with the co-release of cisplatin and RhB.



Introduction

Mesoporous silica nanoparticles (MSN) possess tremendous advantages such as high stability, large surface areas, tunable pore sizes, and abundant surface functionalization sites. They have been extensively explored as smart drug nanocarriers integrated with ingeniously designed off/on switches for the effective blockage of nanopore entrances after loading of drug molecules and on-demand uncapping to trigger payload release under a specific external stimulus or combined stimuli.[1] Relevant research in the field of MSN-based nanocarriers has focused on the development of new strategies for the off/on switching of nanopore entrances for effective loading and triggered-release. Previously, various non-covalent interactions and (dynamic) covalent linkages have been employed to attach capping agents, such as nanoparticles,[2] organic molecules,[3] biomacromolecules,[4] and polymer brushes[5] at the outer surface of MSNs to prevent the premature release of payload from interior mesopores; when a specific stimuli was applied, such as photo irradiation,[6] pH,[7] redox,[8] and

X. Wan, G. Zhang, S. Liu
CAS Key Laboratory of Soft Matter Chemistry, Hefei National Laboratory for Physical Sciences at the Microscale, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China
Fax: (+86) 551 3607 348; E-mail: sliu@ustc.edu.cn

[a] **Supporting Information** for this article is available at Wiley Online Library or from the author.

DOI: 10.1002/marc.201100198

the addition of enzymes[9] and competitive recognition molecules,[10] the capping agent can be removed to trigger the release of loaded guest molecules.

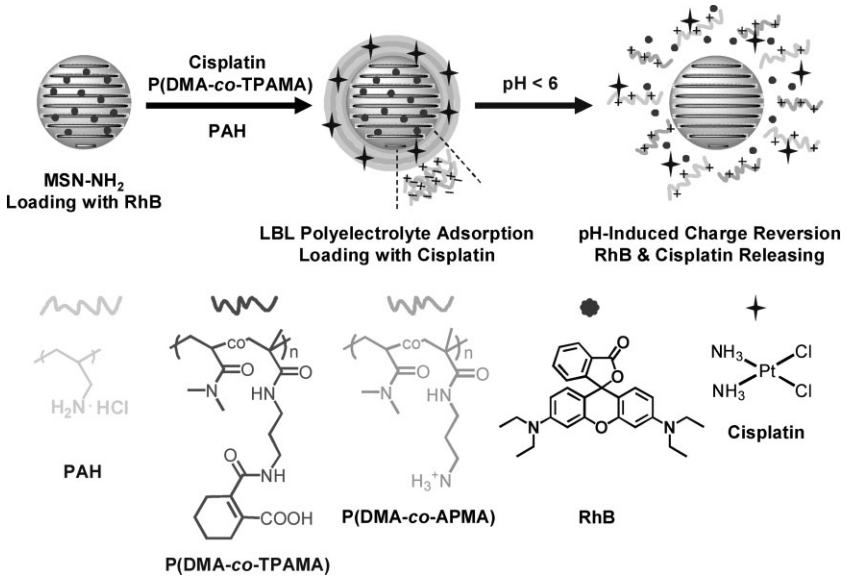
On the other hand, layer-by-layer (LBL) deposition of oppositely charged polyelectrolyte at the outer surface of MSNs can provide an alternate approach for the payload encapsulation;[11] upon properly incorporating responsive moieties into the polyelectrolyte, the permeability and/or structural stability of the outer polyelectrolyte multilayer can be tuned to allow controlled release of guest molecules. In this context, Shi et al.[11a] deposited multilayers of poly(allylamine hydrochloride) (PAH) and sodium salt of poly(styrene sulfonate) (PSSNa) at the surface of ibuprofen-loaded MCM-41, the release of payload can be achieved under extremely acidic pH (≈1.4) or high ionic strength. Recently, Wang et al.[11b] sequentially deposited poly-(vinylpyrrolidone) (PVP)/thiolated poly(methacrylic acid) (PMA_{SH}) multilayers onto doxorubicin (Dox)-loaded MSNs, the subsequent oxidation of thiol functionalities led to the formation of disulfide moieties and the cross-linking of polyelectrolyte multilayers. It is quite expected that this new type of nanocarrier system can be applied for intracellular drug delivery and triggered release by taking advantage of the reductive microenvironment within cells to enhance the permeability of outer multilayers. However, only when combined with suitable targeting moieties at the nanocarrier surface, they can serve as effective smart drug nanocarriers due to that normal cells also possess relatively high intracellular thiol levels. Considering the current limitations encountered in the in vivo applications of multilayer-coated hybrid MSN nanocarriers, it is highly desirable to integrate new stimuli-triggered multilayer-disintegrating mechanism into the current design.

We expect that polyelectrolyte multilayers coated at the surface of MSNs exhibiting complete rupture under mildly acidic pH conditions might be quite advantageous to be integrated into the design of novel type of MSN-based drug nanocarriers. It is well known that in living organisms, intracellular pH plays key roles in enzyme, cell, and tissue activities.[12] Microenvironments in various intracellular organelles, such as endosomes (pH 5.5–6.0) and lysosomes (pH 4.5–5.0) are mildly acidic.[13] Besides, certain tumor tissues possess lower extracellular pH (≈6.0) as compared to normal ones (pH 7.2–7.4). Previously, Lo et al.[14] covalently attached Dox onto the interior surface of MSN nanopores via pH-labile hydrazone linkages; under intracellular pH conditions, the triggered-release of Dox can be achieved. Zink and Nel et al.[7e] fabricated drug-loaded MSNs surface functionalized with 1-methyl-1*H*-benzimidazole (MBI) moieties, and the close and opening of mesopore entrances can be facilely achieved via the pH-switchable molecular recognition between MBI and β-cyclodextrin.

While seeking for mildly acidic pH-disintegrable multilayer for the coating and encapsulating of MSN surfaces, we figured out that the recently emerged novel type of charge-conversion polymers, as initially proposed by the Kataoka research group,[15] can be involved in the construction of polyelectrolyte multilayers possessing pH-triggered rupture characteristics. pH-triggerable charge-conversion polymers typically possess amide functionalities linked with β-carboxylic acid moieties via α,β-unsaturated bond. In the original works by Kataoka et al.,[15a] water-soluble block copolymers consisting of poly(ethylene oxide) and polyamine segments were reacted with maleic anhydride or citraconic anhydride or *cis*-aconitic anhydride, and the negatively charged moieties, α-amide-β-carboxyl unsaturated bond, are stable under physiological conditions (pH 7.4) and hydrolyzed into positively charged primary amines under mildly acidic pH conditions. They have employed the pH-induced charge conversion diblock copolymers for highly efficient protein and gene delivery. In another report by Shen and coworkers,[16] they functionalized polylysine-drug conjugates with 3,4,5,6-tetrahydrophthalic anhydride (THPA) and endowed them with pH-induced charge conversion characteristics. They found that 3,4,5,6-tetrahydrophthalic amide moieties are more stable at physiological pH and possess appropriate charge-conversion rates under mildly acidic media. We can expect that when drug-loaded MSNs were surface coated with multilayers of permanently positively charged polyelectrolyte and initially anionic polyelectrolyte possessing pH-triggered charge-conversion properties, disintegration of the outer polyelectrolyte multilayer will occur under mildly acidic pH media and the triggered-release of payload can be achieved.

Furthermore, for the efficient in vivo chemotherapy, single drug-based delivery often suffered from intrinsic drug resistance and failed to achieve the best performance. In this context, the strategy of co-delivery of multiple drugs and synergistically enhancing agents has been proposed.[17] Zhang and coworkers[17a] fabricated chitosan-based micellar nanoparticles encapsulated with Dox and pyrrolidinedithiocarbamate (PDTC), the latter can serve as sensitizer of anticancer drug and the effective elimination of multidrug resistance (MDR) was demonstrated. Nguyen and coworkers[17b] reported the co-delivery of two types of chemotherapeutic drugs, Dox, and cisplatin, based on poly(acrylic acid) protected liposomes. Starting from MSNs, Lin et al.[10a] reported a novel type of glucose-sensitive delivery system for the triggered co-release of insulin and cyclic AMP.

In this work, we attempted to integrate the concept of charge conversion polymers into the construction of pH-disintegrable polyelectrolyte multilayers at the surface of MSNs with the mesopore interior loaded with model drug molecules, rhodamine (RhB). During the LBL deposition process, cisplatin was also incorporated into the multilayers by forming complexes at first with the negatively



Scheme 1. Schematic illustration for the fabrication of organic/inorganic hybrid MSN coated with P(DMA-*co*-TPAMA)/PAH polyelectrolyte multilayers via the LBL technique, and the pH-triggered dual release of cisplatin adsorbed within multilayers and model drug molecules, RhB, encapsulated within mesopores of MSN via the mechanism of pH-induced charge conversion of P(DMA-*co*-TPAMA) into positively charged P(DMA-*co*-APMA), leading to the disintegration of polyelectrolyte multilayers.

charged polyelectrolyte possessing charge conversion characteristics (Scheme 1). The outer polyelectrolyte multilayer was assembled from permanently cationic polyelectrolyte, PAH, and negatively charged polyelectrolyte, P(DMA-*co*-TPAMA), consisting of *N*,*N*-dimethylacrylamide (DMA) and THPA- functionalized *N*-(3-aminopropyl)methacrylamide (TPAMA) monomer units. We then investigated the co-release profiles of cisplatin and RhB under different pH conditions (from physiological pH to mildly acidic) to verify the proposed pH-induced multilayer disintegration and co-release mechanism. It was found that the pH-triggered co-release of cisplatin and RhB can be achieved at ≈pH 5–6, whereas the release of them at pH 7.4 is quite slow. These results augur well for the application of this novel type of pH-disintegrable multilayer-coated hybrid MSNs as smart drug nanocarriers with intracellular and tumor tissue-specific triggered release characteristics.

Experimental Section

Materials

N,*N*-Dimethylacrylamide (DMA, TCI) was purified by distillation under reduced pressure just prior to use. Poly(allylamine hydrochloride) (PAH, $\overline{M}_w$ ≈60 000), *N*-(3-aminopropyl)methacrylamide hydrochloride (APMA · HCl), 3,4,5,6-tetrahydrophthalic anhydride (THPA), cisplatin, and rhodamine B (RhB) were purchased from Aldrich and used as received. Tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTS) were purchased from Silicone Materials Co. (Wuhan Univ.) and distilled under reduced pressure just prior to use. 2,2′-Azoisobutyronitrile (AIBN, Fluka) was recrystallized from 95% ethanol prior to use. All other chemicals were purchased from Shanghai Chemical Reagent Co. and used as received. Amine-functionalized mesoporous silica MCM-41 (MSN-NH_2, ≈1.5 mmol amine moieties per gram of MSN) was synthesized according to literature procedures.[7d,10a]

Sample Preparation

General approaches employed for the preparation of pH-triggerable charge conversion polymer, P(DMA-*co*-TPAMA), and the fabrication of pH-disintegrable polyelectrolyte multilayer-coated organic/inorganic hybrid MSN are shown in Scheme S1 and Scheme 1, respectively.

Synthesis of pH-Triggerable Charge Conversion Polymer (Scheme S1, see Supporting Information)

DMA (0.30 g, 3 mmol), APMA · HCl (0.36 g, 2 mmol), AIBN (4 mg), 0.9 mL 1,4-dioxane, and 0.3 mL deionized water were introduced into a glass ampoule. The ampoule was degassed through three freeze–thaw cycles, sealed under vacuum, and kept in an oil bath thermostated at 70 °C to start the polymerization. After 2 h, the ampoule was soaked into liquid nitrogen to quench the polymerization. The copolymer was dialyzed against deionized water for 2 d (pH 4–5; MW cutoff, 3.0 kDa) and then dried via lyophilization to afford a white powder (0.45 g, yield: 68.2%; $\overline{M}_n$ = 73.8 kDa, PDI = 1.67). ^{1}H NMR analysis of P(DMA-*co*-APMA) (Figure S1) revealed a DMA/APMA molar ratio of ≈2:1. Thus, the copolymer was denoted as P($DMA_{0.67}$-*co*-$APMA_{0.33}$).

The obtained P($DMA_{0.67}$-*co*-$APMA_{0.33}$) (0.4 g, 1.08 mmol APMA) was dissolved in a mixture of 30 mL dry DMF and 0.78 mL dry triethylamine (5.4 mmol), and then THPA (0.20 g, 1.3 mmol, 1.2 equiv.) in 5 mL dry DMF was added. The reaction mixture was allowed to stir at room temperature for 4 h. After removing all the solvents under reduced pressure, the residues were dissolved in THF and precipitated into an excess of anhydrous ethyl ether. The sediments were collected by filtration, and then dried to constant weight in a vacuum oven at room temperature to obtain the target charge conversion polymer P($DMA_{0.67}$-*co*-$TPAMA_{0.33}$) (0.49 g, 81.7% yield).

Preparation of pH-Disintegrable Polyelectrolyte Multilayer-Coated MSN and their Loading with RhB and Cisplatin

Typically, 20 mg of MSN-NH_2 and RhB (10 mg) in 10 mL PBS buffer (10×10^{-3} M, pH 9) were stirred at room temperature for 5 h, and then centrifuged (10 000 rpm, 15 min) and washed with deionized

water for two times in order to remove the excess RhB dyes. As-prepared MSN-NH_2 or RhB-loaded MSN-NH_2 was then alternately deposited with P($DMA_{0.67}$-*co*-$TPAMA_{0.33}$) (10.0 mL, 5.0 g · L^{-1}, pH 8) in the absence or presence of 1.0 g · L^{-1} cisplatin and PAH (10.0 mL, 5.0 g · L^{-1}, pH 6.8) via the LBL assembly process at 25 °C. After the deposition of each polyelectrolyte layer, the dispersion was centrifuged to collect MSNs for the next cycle of deposition (10 000 rpm, 15 min). After completing the multilayer adsorption process, the obtained organic/inorganic hybrid MSN coated with polyelectrolyte multilayers was extensively purified by repeated cycles of centrifugation and washing with deionized water. Supernatant solutions from the above all centrifugation processes were combined and the RhB content was determined by fluorescence spectroscopy ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 585$ nm) against the standard calibration curve. The RhB loading content was calculated to be ≈6.7 wt.-% (≈0.14 mmol RhB/g) relative to MSN-NH_2. For the determination of cisplatin loading content, hybrid MSN coated with polyelectrolyte multilayers was first treated with hydrofluoric acid, and the residues were subjected with ICP-AES analysis. The cisplatin loading content was calculated to be ≈2.9 wt.-% (≈0.096 mmol · g^{-1}) relative to MSN-NH_2. For TGA and FT-IR measurements, the assembly of P(DMA-*co*-TPAMA)/PAH multilayers at the surface of MSN was conducted without the loading of RhB and cisplatin.

pH-Regulated charge-conversion and co-release of loaded RhB and cisplatin from organic/inorganic hybrid MSN coated with P(DMA-co-TPAMA)/PAH multilayers. In vitro release experiments were performed in aqueous media buffered at pH values of 7.4, 6.0, and 5.0 using 20×10^{-3} M Na_2HPO_4-NaH_2PO_4 and citric acid-Na_2HPO_4, respectively. Hybrid MSN (4.0 mg) coated with P(DMA-co-TPAMA)/PAH multilayers and encapsulated with RhB and cisplatin was dispersed in 10 mL buffer media. Parallel experiments were conducted simultaneously. At different time intervals, the dispersion was centrifuged (10 000 rpm, 15 min) and the supernatant was further subjected to fluorescence measurement ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 585$ nm) and ICP-AES analysis to determine the amount of released RhB and cisplatin, respectively.

Characterization

^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer using D_2O as the solvent. Fourier transform infrared spectra (FT-IR) were recorded on a Bruker VECTOR-22 IR spectrometer. Thermogravimetric analysis (TGA) was performed in air using a Perkin-Elmer Diamond TG/DTA at a heating rate of 10 °C · min^{-1} over the temperature range from room temperature to 800 °C. High-resolution transmission electron microscopy (HRTEM) observations were conducted on a JEOL2010 electron microscope. The sample for HRTEM observations was prepared by placing 10 μL MSN dispersion on copper grids successively coated with thin films of Formvar and carbon. No staining was required. X-ray diffraction (XRD) patterns were conducted on a Japan Rigaku D/max rA X-ray diffractometer. N_2 adsorption measurements were performed using Micromeritics ASAP 2000 instrument using nitrogen at 77 K as the standard adsorptive gas. The surface area was obtained by the Brunauer–Emmett–Teller (BET) method and the pore size distribution was calculated from adsorption branch of the isotherm using the Barrett–Joyner–Halenda (BJH) method. Fluorescence spectra were recorded using a RF-5301/PC (Shimadzu) spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The slit widths were set at 5 nm for excitation and 5 nm for emission. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) measurements were conducted on an Atomscan Advantage spectrometer (Thermal Jarrel Ash Corp.). The Malvern Zetasizer Nano ZS is being used to characterize the zeta potential of hybrid MSNs during the LBL assembly process.

Results and Discussion

General approaches employed for the synthesis of organic/inorganic hybrid MSN coated with P(DMA-*co*-TPAMA)/PAH polyelectrolyte multilayers via the LBL self-assembly approach are shown in Scheme 1. The encapsulation of RhB within the mesopores of amine-functionalized MSNs was conducted prior to the LBL deposition and cisplatin/P(DMA-*co*-TPAMA) complexes was utilized in the LBL process for cisplatin loading. PAH is permanently positively charged under the current experimental condition and the specially designed water-soluble anionic polyelectrolyte, P(DMA-*co*-TPAMA), exhibits highly pH-dependent structural stability, which is quite stable under physiological conditions (pH 7.4) and transforms into positively charged polymers under mildly acidic conditions, i.e., pH-induced charge conversion.[15,16] This process was accompanied with the disintegration of polyelectrolyte multilayers and the co-release of cisplatin and RhB as model drug molecules.

Synthesis of pH-Triggerable Charge Conversion Copolymer, P(DMA-co-TPAMA)

The water-soluble pH-triggerable charge conversion copolymer, P(DMA-*co*-TPAMA), was synthesized in two steps as illustrated in Scheme S1. To ensure sufficient water solubility of P(DMA-*co*-TPAMA), we opted to copolymerize hydrophilic DMA monomer with APMA at first. The copolymerization of DMA and APMA was conducted at 70 °C with AIBN as the initiator. After thorough dialysis and lyophilization, the obtained P(DMA-*co*-APMA) copolymer was reacted with THPA, and the amidation of APMA moieties with THPA affords the pH-triggerable charge conversion copolymer, P(DMA-*co*-TPAMA). ^{1}H NMR spectra of P(DMA-*co*-APMA) and P(DMA-*co*-TPAMA) are shown in Figure S1, together with the peak assignments. On the basis of integral ratios of peaks at ≈2.4–3.3 ppm (*a, b, d*) to peaks at ≈0.9–2.0 ppm (*c, e, f*) in Figure S1a, the molar ratio of DMA/APMA was calculated to be ≈2:1, which is quite close to the feed molar ratio. After the amidation reaction with THPA, we can clearly observe the appearance of new resonance signals at 2.06–2.25 ppm (peaks *e* and *h*, Figure S1b), which are ascribed to methylene protons neighboring to the carbonyl group in 3,4,5,6-tetrahydrophthalic amide moieties. On the basis of the integral ratio between peaks *e*

and *f* (2.06–2.25 ppm) and peaks *a, b,* and *d* (2.4–3.3 ppm) in Figure S1b, the degree of amidation reaction for primary amine moieties was determined to be ≈100%, i.e., quantitative pendent group transformation. Thus, the obtained pH-triggerable charge conversion polymer, initially negatively charged, was denoted as P(DMA$_{0.67}$-*co*-TPAMA$_{0.33}$).

Fabrication of Organic/Inorganic Hybrid MSN Coated with pH-Disintegrable P(DMA-co-TPAMA)/PAH Polyelectrolyte Multilayers

MCM-41 with relatively narrow size and homogeneous pore distribution was prepared according to established literature procedures.[7d] The obtained MCM-41 was then treated with APTS to introduce amine functionalities at the surface, followed by removing CTAB templates by refluxing overnight in methanolic solution of HCl (1.5 M).[7d] Pure MCM-41 was prepared in the absence of APTS. The ordered internal mesoporous structure and particle morphology were examined by HRTEM (Figure 1), X-ray diffraction (XRD, Figure S2), and N_2 adsorption measurements (Figure S3). The diameter of the amine-functionalized MSN is ≈150 nm with BET surfaces of ≈1 012 $m^2 \cdot g^{-1}$ and a pore size of ≈2.6 nm, which are quite comparable to those previously reported.[5a] It should be noted that amine-modified MSN (MSN-NH_2) can facilitate the subsequent alternate deposition of negatively charged P(DMA-*co*-TPAMA) and cationic PAH polyelectrolyte. The electrostatic complexation between surface primary amines of MSN-NH_2 with the first layer of P(DMA-*co*-TPAMA) can ensure tight attachment of polyelectrolyte multilayers at the outer surface of hybrid MSNs. It was expected that under mildly acidic pH conditions, the charge conversion of P(DMA-*co*-TPAMA) into positively charged P(DMA-*co*-APMA) will lead to the disruption and complete detachment of outer polyelectrolyte multilayers (Scheme 1).

Compared to amine-functionalized MSN, highly ordered parallel cylindrical pore channels were well kept after surface coating with nine bilayers PAH/P(DMA-*co*-TPAMA), as evidence by HRTEM observations (Figure 1). Moreover, we can clearly observe the appearance of an outer polymer layer with thickness of ≈10–15 nm after LBL polyelectrolyte deposition. TGA results indicated that the weight retention of organic/inorganic hybrid MSN decreased from 96.0% for amine-functionalized MSN to 70.6% for polyelectrolyte multilayer-coated hybrid MSNs (Figure 2). Using amine-functionalized MSN as a reference, the weight fraction of PAH/P(DMA-*co*-TPAMA) multilayers in organic/inorganic hybrid MSN is calculated to be ≈26.5%. FT-IR spectra of amine-functionalized MCM-41 and hybrid MSN coated with polyelectrolyte multilayers are shown in Figure 2. As compared to the FT-IR spectrum of amine-functionalized MSN, new absorption peaks at 1 710 and 1 640 cm^{-1}

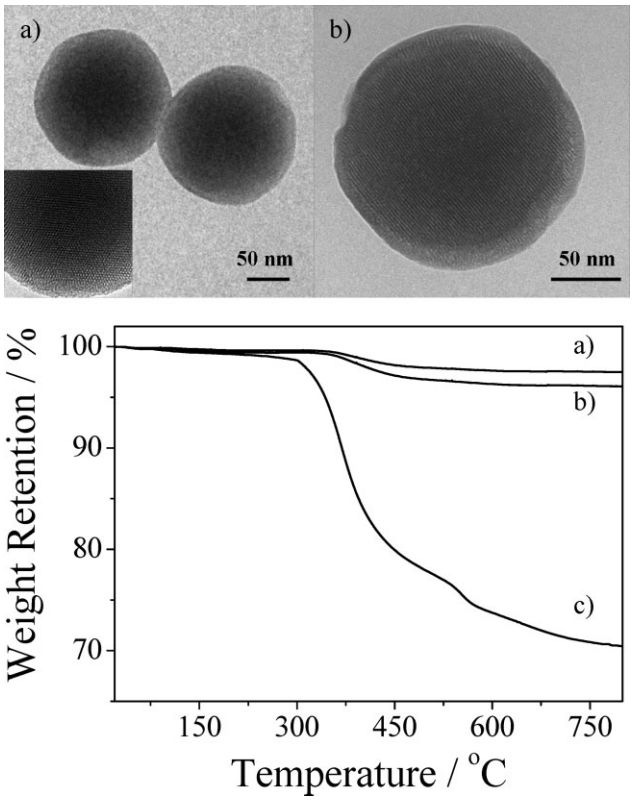


Figure 1. Characterization of MSN nanoparticles coated with and without polyelectrolyte multilayers: (top) TEM images of (a) amine-functionalized mesoporous silica MCM-41 and (b) organic/inorganic hybrid MSN coated with nine bilayers of P(DMA-*co*-TPAMA)/PAH; (bottom) TGA curves recorded for (a) pure mesoporous silica MCM-41, (b) amine-functionalized MSN (MSN-NH_2), and (c) hybrid MSN coated with nine bilayers of P(DMA-*co*-TPAMA)/PAH.

appeared in multilayer-coated hybrid MSN, which are characteristic of carboxyl and amide moieties of P(DMA-*co*-TPAMA).

During the process of sequential adsorption of P(DMA-*co*-TPAMA)/PAH multilayers at the surface of hybrid MSN, the ζ-potential of the resultant hybrid nanoparticles were monitored after each deposition step (Figure 2). The original amine-functionalized MSN possesses a ζ-potential of ca. +23 mV, which exhibits a reversal to negative value, i.e., ca. −18 mV, after coating of first P(DMA-*co*-TPAMA) layer. The subsequent alternate depositions of PAH polycations and P(DMA-*co*-TPAMA) polyanions clearly resulted in the oscillation of ζ-potential values between ca. +50 and −30 mV, which agrees quite well with previous reports concerning LBL assembly processes at the surface of spherical inorganic nanoparticles.[11c] The successful LBL polyelectrolyte deposition can be further supported by the decrease of XRD characteristic peak intensities as shown in Figure S2b, which should be ascribed to the multilayer deposition-induced pore-filling effect. Variations in pore

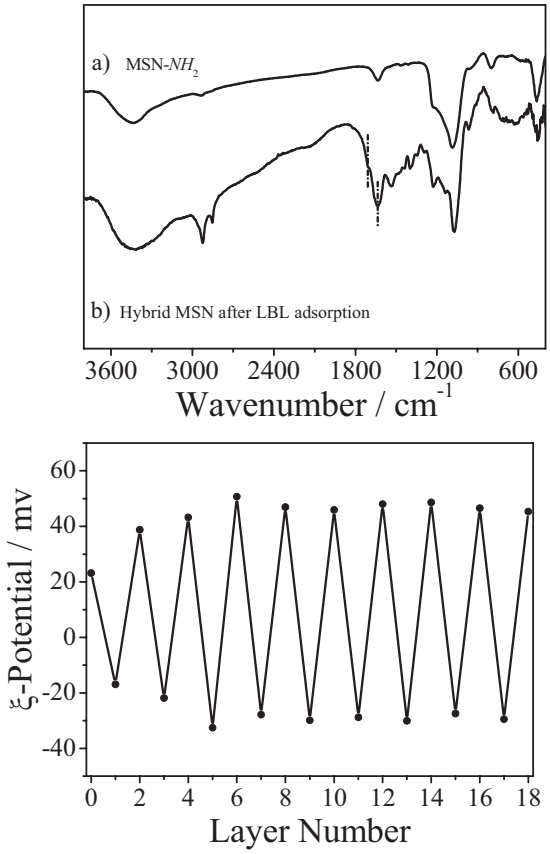


Figure 2. (Top) FT-IR spectra of (a) amine-functionalized MSN (MSN-NH_2) and (b) organic/inorganic hybrid MSN coated with nine bilayers of P(DMA-*co*-TPAMA)/PAH. (Bottom) Variation in the ζ-potential for the LBL deposition of P(DMA-*co*-TPAMA)/PAH multilayers on MSN. Layer 0 corresponds to amine-functionalized MSN. The odd numbers correspond to P(DMA-*co*-THPAMA) deposition, while even numbers correspond to PAH deposition.

volume and diameter were also investigated by nitrogen adsorption–desorption experiments (Figure S3). The transformation from typical isotherm of mesoporous materials to that characteristic of nonporous materials is clearly evident after the P(DMA-*co*-TPAMA)/PAH multilayer deposition at the surface of amine-functionalized MSN. Besides, both of the nitrogen-accessible nanopore volume and the pore size distribution decreased after LBL assembly, demonstrating the successful deposition of P(DMA-*co*-TPAMA)/PAH polyelectrolyte multilayers and the effective capping of mesopores entrances.

pH-Regulated release of embedded RhB and adsorbed cisplatin from hybrid MSN coated with pH-disintegrable polyelectrolyte multilayers

A key issue in designing novel MSN-based smart nanocarriers lies in the exploration of new off/on external stimuli-switchable capping/decapping strategies. In previous reports, Caruso et al.[11c] immobilized enzymes within large-pore MSNs and then capped them with multilayers of PSSNa/poly(diallyldimethylammonium chloride) (PDDA) or PSSNa/silica nanoparticles, which can effectively retain the catalytic activity of encapsulated enzymes and protect them from external enzyme-degrading proteases. Shi et al.[11a] and Wang et al.[11b] fabricated polyelectrolyte multilayer-coated hybrid MSNs loaded with guest molecules, and the release of them was achieved by lowering solution pH (≈1.4) or increasing the ionic strength to disrupt the outer multilayers in the former case, and by the addition of thiol compounds to de-crosslink the multilayer in the latter case, respectively. Previously, Thierry et al.[18] coated magnetic nanoparticles with PAH/PAA polyelectrolyte multilayers via LBL electrostatic self-assembly, this was followed by the subsequent loading of cisplatin within multilayers. The release of up to ≈40% loaded cisplatin over ≈72 h was observed.

In the current work, we attempted to integrate the concept of charge conversion polymers into the construction of mildly acidic pH-disintegrable polyelectrolyte multilayers at the surface of MSNs with the mesopore interior loaded with model drug molecules, rhodamine (RhB); during the LBL deposition process, cisplatin was also incorporated into the multilayers by forming complexes with the charge conversion polymer, P(DMA-*co*-TPAMA), at first, which was then followed by sequential LBL deposition with PAH (Scheme 1). Since the outer polyelectrolyte multilayer was assembled from permanently cationic polyelectrolyte, PAH, and negatively charged P(DMA-*co*-TPAMA) exhibiting pH-triggerable charge conversion characteristics, we can envisage that under mildly acidic pH conditions, the transformation of P(DMA-*co*-TPAMA) layers into positively charged ones, P(DMA-*co*-APMA),[15,16] will lead to the disruption of polyelectrolyte multilayers and the simultaneous co-release of RhB and cisplatin.

pH-Triggered release profiles of encapsulated RhB and adsorbed cisplatin from hybrid MSNs coated with P(DMA-*co*-APMA)/PAH polyelectrolyte multilayers were then measured and the results are shown in Figure 3. Under physiological pH conditions, the release of both RhB and cisplatin are very slow (less than 10% over 12 h), indicating that the surface coated polyelectrolyte multilayers are quite stable. Thus, they can effectively block the nanopore outlets and prevent the premature leakage of the RhB and cisplatin molecules. This is understandable considering that P(DMA-*co*-APMA) does not undergo effective charge conversion at pH 7.4, as previously reported by Kataoka[15] and Shen research groups.[16] On the other hand, upon decreasing solution pH, considerable enhancement of RhB and cisplatin release rates is clearly evident. In the pH range of 5–6, the lower the solution pH, the faster the release rate of RhB and cisplatin. This fact can be ascribed to the faster

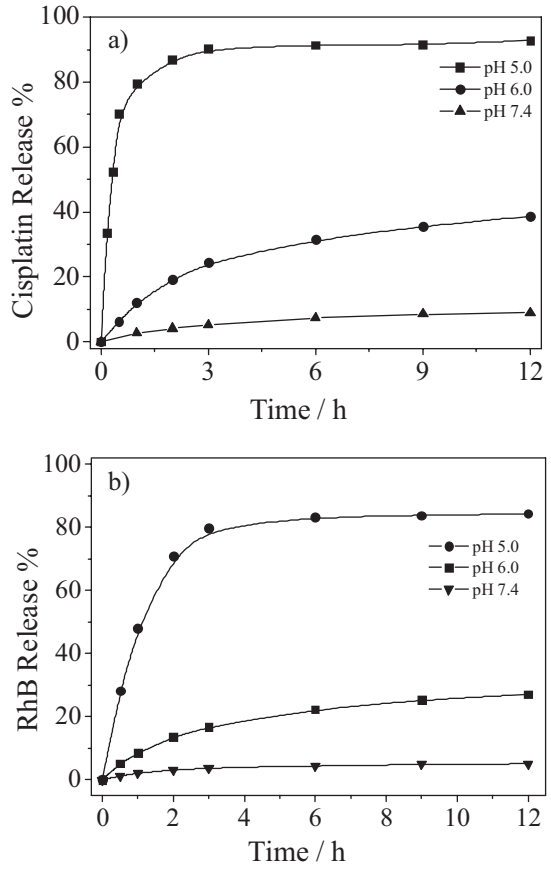


Figure 3. Time evolution of pH-triggered release of (a) cisplatin and (b) RhB from organic/inorganic hybrid MSN coated with nine bilayers of P(DMA-*co*-TPAMA)/PAH, RhB, and cisplatin were encapsulated within the mesopores of MSN and outer polyelectrolyte multilayers, respectively.

hydrolysis of P(DMA-*co*-TPAMA) into P(DMA-*co*-APMA) at lower pH conditions, i.e., pH-triggered charge conversion. This will lead to the disintegration of polyelectrolyte multilayers coated at the surface of silica nanoparticles and the opening of nanopore entrances. A closer examination of Figure 3 can further reveal that at pH 5.0, ≈80% RhB and ≈90% cisplatin can be release over the time period of ≈4 h. In principle, RhB can be replaced with well-known anticancer drugs such as doxorubicin or paclitaxel, for which MSNs have been well-known to serve as nanocarriers.[2–10] The surface functionalization of the reported MSN system with cancer cell targeting moieties, pH-modulated co-release of cisplatin and paclitaxel, and further in vitro cell viability investigations are currently underway.

Conclusion

In conclusion, we report on the facile fabrication organic/inorganic hybrid MSN coated with mildly acidic pH-disintegrable P(DMA-*co*-TPAMA)/PAH polyelectrolyte multilayers, which exhibits pH-triggered co-release of two types of guest molecules. Model drug molecule, RhB, was first loaded into the interior mesopores of amine-functionalized MSN. During the following LBL deposition process to effectively block the mesopores entrance of MSN, cisplatin was incorporated into P(DMA-*co*-TPAMA)/PAH multilayers by forming complexes with the negatively charged polyelectrolyte at first. The TPAMA moieties in P(DMA-*co*-TPAMA) is highly pH-dependent, i.e., stable under neutral media and hydrolyzed into positively charged APMA moieties in weakly acidic media, leading to the disintegration of the polyelectrolyte multilayers coated at the surface of MSN. Thus, the off/on switching of nanopore gates of MSN can be conveniently achieved, resulting in the controlled co-release of cisplatin and RhB molecules. It was found that the co-release of cisplatin and RhB can be well-triggered at ≈pH 5–6, whereas the release of them at pH 7.4 is quite slow. This work represents the first example of employing mildly acidic pH-disintegrable multilayer as a new strategy to cap MSN nanopore entrances for switchable encapsulation, and we envisage that the reported smart nanocarrier system might be utilized for intracellular and tumor tissue-specific multicomponent chemotherapy and its further integration with clinical diagnosis and imaging capabilities.

Acknowledgements: The financial support from National Natural Scientific Foundation of China (NNSFC) Project (20874092, 91027026, and 51033005), Fundamental Research Funds for the Central Universities, and Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP) is gratefully acknowledged.

Received: March 28, 2011; Revised: April 20, 2011; Published online: May 25, 2011; DOI: 10.1002/marc.201100198

Keywords: charge conversion polymers; drug delivery systems; mesoporous silica nanoparticles (MSN); polyelectrolytes; layer-by-layer (LBL) deposition

[1] [1a] B. G. Trewyn, I. I. Slowing, S. Giri, H. T. Chen, V. S. Y. Lin, *Acc. Chem. Res.* **2007**, *40*, 846; [1b] S. Saha, K. C. F. Leung, T. D. Nguyen, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 685; [1c] M. Vallet-Regi, F. Balas, D. Arcos, *Angew. Chem., Int. Ed.* **2007**, *46*, 7548; [1d] J. L. Vivero-Escoto, I. I. Slowing, B. G. Trewyn, V. S. Y. Lin, *Small* **2010**, *6*, 1952; [1e] I. I. Slowing, J. L. Vivero-Escoto, B. G. Trewyn, V. S. Y. Lin, *J. Mater. Chem.* **2010**, *20*, 7924.

[2] [2a] C. Y. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija, V. S. Y. Lin, *J. Am. Chem. Soc.* **2003**, *125*, 4451; [2b] J. E. Lee, N. Lee, H. Kim, J. Kim, S. H. Choi, J. H. Kim, T. Kim, I. C.

Song, S. P. Park, K. M. Moon, T. Hyeon, *J. Am. Chem. Soc.* **2010**, *132*, 552.
[3] [3a] N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350; [3b] Y. Yang, W. X. Song, A. H. Wang, P. L. Zhu, J. B. Fei, J. B. Li, *Phys. Chem. Chem. Phys.* **2010**, *12*, 4418; [3c] D. Brevet, M. Gary-Bobo, L. Raehm, S. Richeter, O. Hocine, K. Amro, B. Loock, P. Couleaud, C. Frochot, A. Morere, P. Maillard, M. Garcia, J. O. Durand, *Chem. Commun.* **2009**, 1475.
[4] [4a] F. Torney, B. G. Trewyn, V. S. Y. Lin, K. Wang, *Nat. Nanotechnol.* **2007**, *2*, 295; [4b] C. E. Chen, J. Geng, F. Pu, X. J. Yang, J. S. Ren, X. G. Qu, *Angew. Chem., Int. Ed.* **2011**, *50*, 882; [4c] E. Climent, R. Martinez-Manez, F. Sancenon, M. D. Marcos, J. Soto, A. Maquieira, P. Amoros, *Angew. Chem., Int. Ed.* **2010**, *49*, 7281.
[5] [5a] X. J. Wan, D. Wang, S. Y. Liu, *Langmuir* **2010**, *26*, 15574; [5b] R. Liu, Y. Zhang, P. Y. Feng, *J. Am. Chem. Soc.* **2009**, *131*, 15128; [5c] Q. Gao, Y. Xu, D. Wu, Y. H. Sun, X. A. Li, *J. Phys. Chem. C* **2009**, *113*, 12753; [5d] C. Y. Liu, J. Guo, W. L. Yang, J. H. Hu, C. C. Wang, S. K. Fu, *J. Mater. Chem.* **2009**, *19*, 4764; [5e] T. Wu, Z. S. Ge, S. Y. Liu, *Chem. Mater.* **2011**, *23*. DOI: 10.1021/cm200102g; [5f] T. Wu, G. Zou, J. M. Hu, S. Y. Liu, *Chem. Mater.* **2009**, *21*, 3788; [5g] T. Wu, Y. F. Zhang, X. F. Wang, S. Y. Liu, *Chem. Mater.* **2008**, *20*, 101; [5h] J. M. Hu, S. Y. Liu, *Macromolecules*, **2010**, *43*, 8315.
[6] [6a] J. L. Vivero-Escoto, I. I. Slowing, C. W. Wu, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 3462; [6b] C. Park, K. Lee, C. Kim, *Angew. Chem., Int. Ed.* **2009**, *48*, 1275.
[7] [7a] R. Liu, Y. Zhang, X. Zhao, A. Agarwal, L. J. Muller, P. Y. Feng, *J. Am. Chem. Soc.* **2010**, *132*, 1500; [7b] C. Park, K. Oh, S. C. Lee, C. Kim, *Angew. Chem., Int. Ed.* **2007**, *46*, 1455; [7c] E. Aznar, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, P. Amoros, C. Guillem, *J. Am. Chem. Soc.* **2009**, *131*, 6833; [7d] S. Angelos, Y. W. Yang, K. Patel, J. F. Stoddart, J. I. Zink, *Angew. Chem., Int. Ed.* **2008**, *47*, 2222; [7e] H. A. Meng, M. Xue, T. A. Xia, Y. L. Zhao, F. Tamanoi, J. F. Stoddart, J. I. Zink, A. E. Nel, *J. Am. Chem. Soc.* **2010**, *132*, 12690.
[8] [8a] Z. Luo, K. Y. Cai, Y. Hu, L. Zhao, P. Liu, L. Duan, W. H. Yang, *Angew. Chem., Int. Ed.* **2011**, *50*, 640; [8b] T. D. Nguyen, Y. Liu, S. Saha, K. C. F. Leung, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2007**, *129*, 626.
[9] [9a] K. Patel, S. Angelos, W. R. Dichtel, A. Coskun, Y. W. Yang, J. I. Zink, J. F. Stoddart, *J. Am. Chem. Soc.* **2008**, *130*, 2382; [9b] A. Bernardos, E. Aznar, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, J. M. Barat, P. Amoros, *Angew. Chem., Int. Ed.* **2009**, *48*, 5884; [9c] A. Schlossbauer, J. Kecht, T. Bein, *Angew. Chem., Int. Ed.* **2009**, *48*, 3092; [9d] J. S. Liu, X. Z. Du, X. F. Zhang, *Chem. -Eur. J.* **2011**, *17*, 810.
[10] [10a] Y. N. Zhao, B. G. Trewyn, I. I. Slowing, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 8398; [10b] E. Climent, A. Bernardos, R. Martinez-Manez, A. Maquieira, M. D. Marcos, N. Pastor-Navarro, R. Puchades, F. Sancenon, J. Soto, P. Amoros, *J. Am. Chem. Soc.* **2009**, *131*, 14075.
[11] [11a] Y. F. Zhu, J. L. Shi, W. H. Shen, X. P. Dong, J. W. Feng, M. L. Ruan, Y. S. Li, *Angew. Chem., Int. Ed.* **2005**, *44*, 5083; [11b] C. L. Zhu, X. Y. Song, W. H. Zhou, H. H. Yang, Y. H. Wen, X. R. Wang, *J. Mater. Chem.* **2009**, *19*, 7765; [11c] Y. J. Wang, F. Caruso, *Chem. Mater.* **2005**, *17*, 953; [11d] Y. Hu, K. Y. Cai, Z. Luo, K. D. Jandt, *Adv. Mater.* **2010**, *22*, 4146.
[12] [12a] I. Yuli, A. Oplatka, *Science* **1987**, *235*, 340; [12b] V. A. Golovina, M. P. Blaustein, *Science* **1997**, *275*, 1643; [12c] R. A. Gottlieb, A. Dosanjh, *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 3587.
[13] [13a] L. E. Gerweck, K. Seetharaman, *Cancer Res.* **1996**, *56*, 1194; [13b] A. S. E. Ojugo, P. M. J. McSheehy, D. J. O. McIntyre, C. McCoy, M. Stubbs, M. O. Leach, I. R. Judson, J. R. Griffiths, *NMR Biomed.* **1999**, *12*, 495.
[14] C. H. Lee, S. H. Cheng, I. P. Huang, J. S. Souris, C. S. Yang, C. Y. Mou, L. W. Lo, *Angew. Chem., Int. Ed.* **2010**, *49*, 8214.
[15] [15a] Y. Lee, S. Fukushima, Y. Bae, S. Hiki, T. Ishii, K. Kataoka, *J. Am. Chem. Soc.* **2007**, *129*, 5362; [15b] Y. Lee, K. Miyata, M. Oba, T. Ishii, S. Fukushima, M. Han, H. Koyama, N. Nishiyama, K. Kataoka, *Angew. Chem., Int. Ed.* **2008**, *47*, 5163; [15c] M. Sanjoh, S. Hiki, Y. Lee, M. Oba, K. Miyata, T. Ishii, K. Kataoka, *Macromol. Rapid Commun.* **2010**, *31*, 1181.
[16] Z. X. Zhou, Y. Q. Shen, J. B. Tang, M. H. Fan, E. A. Van Kirk, W. J. Murdoch, M. Radosz, *Adv. Funct. Mater.* **2009**, *19*, 3580.
[17] [17a] L. Fan, F. Li, H. T. Zhang, Y. K. Wang, C. Cheng, X. Y. Li, C. H. Gu, Q. A. Yang, H. Wu, S. Y. Zhang, *Biomaterials* **2010**, *31*, 5634; [17b] S. M. Lee, T. V. O'Halloran, S. T. Nguyen, *J. Am. Chem. Soc.* **2010**, *132*, 17130–17138; [17c] J. A. Johnson, Y. Y. Lu, A. O. Burts, Y. Xia, A. C. Durrell, D. A. Tirrell, R. H. Grubbs, *Macromolecules* **2010**, *43*, 10326; [17d] A. Khdair, D. Chen, Y. Patil, L. N. Ma, Q. P. Dou, M. P. V. Shekhar, J. Panyam, *J. Controlled Release* **2010**, *141*, 137.
[18] B. Thierry, F. Al-Ejeh, A. Khatri, Z. Yuan, P. J. Russell, S. Ping, M. P. Brown, P. Majewski, *Chem. Commun.* **2009**, 7348.