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“Breathing” Unimolecular Micelles Based on Novel Star-like Amphiphilic Hybrid Copolymer

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Presented in this paper is a novel unimolecular micelle that possesses a pH-induced “breathing” feature. The hybrid copolymer POSS-(PAA-(PLLA-PEG))₃, in which eight linear-dendritic like arms poly(acrylic acid)-(poly(L-lactide)-poly(ethylene glycol))₄ (PAA-(PLLA-PEG))₄ are grafted onto an oligomeric silsequioxane core, was synthesized via the combination of SET-LRP, ROP and thiokbromo “click” reaction. ¹H NMR confirmed that the formed micelles was composed of a biocompatible PEG outer corona, biodegradable hydrophobic PLA layer in the middle and inner hydrophilic PAA cavities. And, TEM result revealed that the morphology of the self-assembled micelles takes rod-like. Interestingly, the formed micelles can “breathe”, that is, size of the micelles changed as pH values varying. Doxorubicin (DOX), an anticancer drug, was encapsulated into the micelles formed by POSS-(PAA-(PLLA-PEG))₃ to invaculate drug release profile. Due to the unique architecture and properties, this novel amphiphilic hybrid copolymer can be considered a good candidate for controlled drug delivery.

Introduction

Nano-sized polymeric micelles self-assembled from amphiphilic block copolymers have received tremendous attention due to their great promising applications in drug delivery.¹⁻⁷ As drug carrier, the inner hydrophobic core can solubilize the hydrophobic drugs, whereas the outer shell protects the loaded drugs from the uptake by the reticuloendothelial system (RES) or premature degradation and prolongs their circulation time in vivo.⁸⁻⁹ As a result, polymeric micelles can be used as efficient carrier to enhance the therapeutic efficacy and reduce side effects of therapeutic drugs, especially those with poor solubility.¹⁰ However, classical polymeric micelles represent thermodynamic aggregations of amphiphilic macromolecules above their critical micelle concentration (CMC), and they will disassemble into free polymeric chains once the polymer concentration is below CMC. This drawback will heavily hinder their in vivo drug delivery application.¹¹ In sharp contrast to multimolecular micelles, polymeric micelles from unimolecular amphiphilic copolymers can maintain excellent micellar stability, in which the hydrophilic and hydrophobic segments are covalently linked together. So, well-defined unimolecular amphiphilic block copolymers will be of great interest for the drug delivery applications.

Rapid development of diverse controlled living polymerization techniques¹²⁻¹⁶ and emergence of high efficient coupling techniques¹⁷⁻²² have open pathways to a library of complicated polymers with different compositions and architectures. Among them, star-like (co)polymers is a special class of complex polymers, in which a number of polymer arms are linked covalently to a central core.²³⁻²⁸ In recent years, multi-arm star-like amphiphilic block copolymers have attracted special interest from researcher due to the fact that they can self-assemble into mono-disperse and stable unimolecular micelles in aqueous solution.²⁹⁻³⁶ For example, Song and his colleagues have prepared amphiphilic star-like block copolymer with multi poly(lactide-b-poly(ethylene glycol) arms using hyperbranched polyester as core.³⁰ Lin et al reported the synthesis of amphiphilic star-like block copolymer with multi poly(acrylic acid)-b-polystyrene arms based on β-cyclodextrin.³¹ However, micelles from amphiphilic multi-arm star-like block copolymers are relatively less studied because of limited model copolymers. Due to their unique architecture and properties, design and synthesis of well-defined amphiphilic multi-arm star-like block copolymers will still be a fascinating subject for polymeric chemists.

Herein, a novel star-like amphiphilic hybrid copolymer has been reported for the first time, in which the arms of linear-dendritic like amphiphilic block copolymers PAA-b-(PLLA-b-PEG)₄ are linked to an inorganic polyhedral oligomeric silsequioxane (POSS) core, as shown in Scheme 1. Due to its unique architecture, this copolymer can self-assemble into unimolecular micelles. The biocompatible PEG chain is designed as the hydrophilic outer corona to obtain “stealth micelles”. The middle hydrophobic membrane is composed of biodegradable PLA. And the pH-responsive PAA is designed as the inner corona, offering the loading and stabilization of hydrophilic drugs (such as doxorubicin hydrochloride (DOX.HCl)) via electrostatic interaction.³⁷⁻⁴⁰ Like conventional polymeric micelles, this kind of micelles can be explored for controlled release of hydrophilic and hydrophobic drugs. However, it should be noted that the unique micelles based on POSS-(PAA-(PLLA-PEG))₃ copolymer is more stable in vivo as compared with micelles derived from conventional di- or tri-block copolymer.
Scheme 1. Synthetic route to a novel amphiphilic multi-arm star-like block copolymer POSSPAAk-(PLLAk-PEG)k.

Experimental Section

Materials

tert-Butyl acrylate (t-BA) (98%, Sigma) was purified by passing through a short column with neutral alumina oxide just before use to remove the inhibitor. Octa-(3-hydroxy-3-methylbutyldimethylsiloxy)-POSS was purchased from Hybrid Plastics. L-lactide monomers (99.5%) were purchased from Purac and recrystallized in ethyl acetate before use. Poly(ethylene glycol) methyl ether (Mn = 5000) was purchased from Sigma. 2-Bromoiso-butyryl bromide (98%, Aldrich), 2-bromopropionyl bromide (97%, Aldrich), N, N, N', N'', N''-pentamethyldiethylenetriamine (PMDETA), copper powder (~625 mesh, Alfa Aesar), thioglycerol (≥ 97%, Aldrich), Tin(II) 2-ethylhexanoate (Sn(Oct)2, 92.5-100%, Aldrich), 4-dimethylamino pyridine (DMAP) (>99%, Aldrich), dicyclohexylcarbodiimide (DCC) (99%, Alfa Aesar) and trifluoroacetic acid (TFA, 99%, Sigma) were used as received. Triethylamine, pyridine and chloroform were dried with calcium hydride and distilled before use. Initiator POSS-Br8 was synthesized according to the literature procedure. 4

Characterization

1H nuclear magnetic resonance (1H NMR) spectra were recorded on a Bruker Ultrashield 600 MHz/54 mm NMR spectrometer at room temperature using CDCl3 or DMSO-d6 as the solvent. Gel permeation chromatographic (GPC) analysis was performed on a Waters 2690 equipped with an evaporative light scattering detector (Waters 2420) and three phenomenex linear 5 mm styragel columns (500, 104 and 106 Å). DMF was used as eluent at a rate of 1.0 mL min⁻¹. Monodispersed poly(methyl methacrylate) (PMMA) was used as standards. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer spectrum 2000 spectrometer at a resolution of 1 cm⁻¹. Transmission electron microscopy (TEM) images were obtained on a JEOL JEM 2100F field emission electron microscope operating at an acceleration voltage of 200 kV. The TEM samples were prepared by dropping the polymer solution onto the surface of the carbon-coated 200 mesh copper grid and stained with phosphotungstic acid. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Brookhaven BI-900AT Digital Autocorrelator.

Synthesis of star-like POSS-(PtBA-Br)8 via SET-LRP (2)

(Scheme 1) The synthesis process of POSS-(PtBA-Br)8 was conducted under the mild conditions of SET-LRP. Typically, POSS-Br8 (0.656 g, containing Br 1.88 mmol), PMDETA (0.40 mL, 1.88 mmol), tBA (15 mL) and acetone (6 mL) were added to a 50 mL Schlenk flask. The reaction system was degassed by bubbling with nitrogen for 15 min. After Cu(0) powder (0.119 g, 1.88 mmol) was introduced into the flask under nitrogen atmosphere, the mixture was purged by nitrogen for another 5 min. Then the flask was immersed into an oil bath maintained at 30 °C. After 1 h, the polymerization was stopped by diluting with THF. The THF solution was passed a short neutral alumina column to remove copper salts. After concentration on rotary evaporator, the residue was precipitated in the mixture of methanol/water (v/v 1:1) twice times. The product POSS-(PtBA-Br)8 was dried under vacuum at 40 °C until a constant weight. (Mn,NMR = 37,610 g/mol; Mn,GPC = 27,150 g/mol, Mw/Mn = 1.46)
Synthesis of POSS-(PtBA-OH)$_n$ (3) (Scheme 1) Through three steps involving thio-bromo nucleophilic “click” reaction and acylation of hydroxyl groups, POSS-(PtBA-OH)$_n$ with multi surface hydroxyl groups was obtained from POSS-(PtBA-Br)$_n$. The detail procedure as following: POSS-(PtBA-Br)$_n$ (1.154 g, 32.45 µmol) were charged into the flask. After the solution was cooled to 0 °C by ice/water bath, Et$_3$N (0.18 mL, 1.30 mmol) was added dropwise via syringe. The reaction mixture was continuously stirred overnight at room temperature. POSS-(PtBA-2OH)$_n$ was obtained by precipitating the solution in the excessive mixture of methanol/H$_2$O (v/v 1:1) two times and dried to constant weight under vacuum. POSS-(PtBA-Br)$_n$. POSS-(PtBA-OH)$_n$ (0.577 g, 15.84 µmol) were dried by azetrope distillation with toluene, then 3 mL dry THF and pyridine (0.20 mL, 2.53 mmol) were charged into the flask. After the solution was cooled to 0 °C, 2-bromopropionyl bromide (0.27 mL, 2.53 mmol) was added. After the solution was cooled to 0 °C, and another 24 h at room temperature. The solution was precipitated in the excessive mixture of methanol/H$_2$O (v/v 1:1) two times to provide the polymer POSS-(PtBA-Br)$_n$. POSS-(PtBA-OH)$_n$ was obtained via once thio-bromo “click” reaction of POSS-(PtBA-Br)$_n$ with thioglycerol.

Synthesis of POSS-(PtBA-PLLA-PEG)$_n$ (4) (Scheme 1) POSS-(PtBA-OH)$_n$ (0.464 g, 12.09 µmol) was dissolved in 20 mL dry toluene and dried by distilling portion of toluene. Under nitrogen atmosphere, L-lactide (1.038 g, 9.11 mmol) and the 1 mL toluene solution of Sn(Oct)$_2$ (0.016 g, 38.70 µmol) were added to the system. The reaction was carried out at 125 °C for 24 h. POSS-(PtBA-(PLLA-OH)$_n$ was obtained by pouring the solution into excessive ethanol and dried under vacuum at 45 °C. (M$_n$/NMCH = 120,750 g/mol, M$_G$/GPC = 78,560 g/mol, M$_w$/M$_n$ = 1.21).

Preparation of carboxyl-terminated mPEG (mPEG-COOH) mPEG$_n$-COOH (6.420 g, 1.28 mmol), succinic anhydride (0.257 g, 2.56 mmol), DMAP (0.313 g, 2.56 mmol) were dissolved in 200 mL dry chloroform, and the reaction was performed at 80 °C overnight. The solution was washed with a diluted HCl solution and water successively, then dried over anhydrous MgSO$_4$. The filtrate was concentrated on rotary evaporator and precipitated in diethyl ether two times.

Synthesis of POSS-(PAA-(PLLA-PEG)$_n$ (5) (Scheme 1) The target amphiphilic copolymer POSS-(PAA-(PLLA-PEG)$_n$ was obtained by coupling POSS-(PtBA-PLLA-OH)$_n$ with mPEG-COOH, followed by selective hydrolysis of PtBA blocks using TFA. First, POSS-(PtBA-PLLA-OH)$_n$ (0.412 g, 3.56 µmol) and mPEG-COOH (0.925 g, 0.19 mmol) were charged into a flask and dried by azetropdistillation. After 20 mL dry chloroform was added, the mixture was violently stirred until the solid disappeared completely. Then, DCC (0.074 g, 0.37 mmol) and DMAP (0.045 g, 0.37 mmol) were added to the reaction system and the reaction was allowed to proceed for 24 h under nitrogen atmosphere. The insoluble byproduct dicyclohexylcarbodiurea was removed by filtration. The crude product was purified by slowy adding hexane to the filtered solution until the precipitate appeared and this procedure was repeated two times. The product POSS-(PtBA-(PLLA-OH)$_n$ was dried under vacuum at 40 °C until a constant weight. (M$_n$/NMCH = 250,750 g/mol; M$_G$/GPC = 131,670 g/mol, M$_w$/M$_n$ = 1.29).

The copolymer POSS-(PtBA-4-(PLLA-PEG)$_n$ (0.501 g, containing 0.49 mmol tert-butyl ester group) was dissolved in 10 mL CH$_2$Cl$_2$, then trifluoroacetic acid (0.94 mL, 12.25 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. After the hydrolysis was completed, the resulting star-like copolymer, POSS-(PAA-4-(PLLA-PEG)$_n$ was purified by precipitating in excessive hexane two times.

Preparation of DOX- loaded micelles from POSS-(PAA-(PLLA-PEG)$_n$ star-shaped micelles The blank and DOX-loaded POSS-(PAA-(PLLA-PEG)$_n$ micelles were prepared according to the emulsion method. In a typical experiment, POSS-(PAA-(PLLA-PEG)$_n$ (40 mg) were dissolved in 40 mL of deionized water, while 8 mg DOX were dissolved in chloroform/TEA mixture and stirred for 4 h at room temperature until fully dissolved. Then the DOX/ chloroform solution was added dropwise into the polymer solution and subsequently stirred at room temperature for 48 hours to vaporize chloroform fully. The remaining DOX residuals, together with blank and DOX-loaded micelles were centrifuged to remove excess DOX residuals, filtered using a membrane filter (80 µm pore) to remove aggregated particles and subsequently freeze-dried to obtain the final product which was stored at −20 °C until further experiments. The DOX loading content (LC) and entrapment efficiency (EE) were determined by fluorescence. One milligram of the DOX-loaded micelle powder was dissolved in 10 mL of DMSO. The concentration of DOX at 590 nm was recorded with reference to a calibration curve of pure DOX/DMSO solution. The LC and EE of DOX were calculated using the following formulas, respectively.

\[
LC(\%) = \frac{mass of DOX in micelles}{mass of drug loaded micelles} \times 100 \%
\]

\[
EE(\%) = \frac{mass of DOX in micelles}{mass of drug initially added} \times 100 \%
\]

In Vitro Release of DOX from Micelles

The in vitro DOX release properties from the POSS-(PAA-(PLLA-PEG)$_n$ self-assembled micelles were determined as follows: 2 mg of DOX-entrapped micelle was suspended in 1 mL of PBS solvent and then placed in a pre-swollen cellulose dialysis membrane tube (MWCO 5000). The dialysis tube was then immersed into 40 mL of PBS buffer at pH 7, and kept in a 37 °C water bath. At specific time intervals, a 2 mL sample of the release media was taken out and replaced by 2 mL fresh buffer (with different pH) to maintain the total volume. The concentrations of DOX in different samples were determined using fluorescence. The cumulative drug release percent ($E_r$) was calculated based on the equation

\[
E_r(\%) = \frac{V_e \sum C_n \times 100}{m_{DOX}}
\]

where $m_{DOX}$ represents the amount of DOX in the micelle (mg), $V_e$ is the whole volume of the release media ($V_e = 40$ mL), and $C_n$ represents the concentration of DOX in the nth sample (mg). The in vitro experiments were repeated three times, and all samples were analyzed in triplicate to get the final release curves.

Result and discussion

Synthesis and Characterization of star-like amphiphilic copolymer POSS-(PAA-4-(PLLA-PEG)$_n$ The star-like amphiphilic copolymer with POSS core and linear-dendritic like
PAA- (PLLA-PEG)$_4$ arms was successfully synthesized according to the steps shown in Scheme 1. The star-like polymer POSS-(PtBA-Br)$_8$ was first synthesized by SET-LRP of t-BA monomers using POSS-Br$_8$ as initiator and Cu(0)/PMDETA as catalyst. Figure 2A shows the GPC trace of POSS-(PtBA-Br)$_8$, which is a monomodal peak, indicating that POSS-Br$_8$ successfully initiated polymerization of t-BA monomer. $^1$H NMR spectrum of the obtained POSS-(PtBA-Br)$_8$ is shown in Fig 1A. The peaks at about 0.10 (a) and 2.23 (c) ppm are attributed to methyl protons of the initiator adjacent to the core and methine protons in PtBA arms, respectively. The molecular weight of POSS-(PtBA-Br)$_8$ is derived from the $^1$H NMR spectrum following the formula (1):

$$M_{n,NMR} = \frac{6A_c}{A_a} \times 128 \times 8 + 2795 \quad (1)$$

where $A_a$ and $A_c$ stand for the integral areas of the peaks (a) and (c). The values of 128 and 2795 stand for the molecular weight of tBA repeating units and the initiator, respectively. It should be noted that the molecular weight of POSS-(PtBA-Br)$_8$ based on GPC is different from that of $^1$H NMR (Table 1). This is because that the structure of POSS-(PtBA-Br)$_8$ is star-like, however, the standard of GPC is the linear PMMA.

**Figure 1.** $^1$H NMR spectra of POSS-(PtBA-Br)$_8$ (A), POSS-(PtBA-(PLLA-OH)$_4$)$_8$ (B) and POSS-(PtBA-(PLLA-PEG)$_4$)$_8$ (C) in CDCl$_3$.

**Figure 2.** GPC traces of POSS-(PtBA-Br)$_8$ (A), POSS-(PtBA-(PLLA-OH)$_4$)$_8$ (B) and POSS-(PtBA-(PLLA-PEG)$_4$)$_8$ (C) with DMF as eluent.

Thio-bromo nucleophilic substitution reaction reported by Percec was rapid and proceeded with near-quantitative conversion, possessing characteristics of “click” reactions.$^{42-45}$ Meanwhile, this reaction was shown to be compatible with a range of functional thiols. Through iterative three steps involving thio-bromo “click” reaction of thioglycerol with α-bromooester and acylation of hydroxyl groups with 2-bromopropionyl bromide, POSS-(PtBA-OH)$_8$ with multi surface hydroxyl groups was obtained from POSS-(PtBA-Br)$_8$. $^1$H NMR technique was used to analyze the end group transformation processes. As compared to that of POSS-(PtBA-Br)$_8$ (Figure 3A), the signal at 4.15 ppm assigned to methine protons neighboring to the terminal bromide groups disappeared and the characteristic signals of thioglycerol at 3.56–3.73 ppm could be observed in the spectrum of POSS-(PtBA-OH)$_8$ (Figure 3B), confirming that the thio-bromo reaction was performed completely. After acylation of –OH on POSS-(PtBA-OH)$_8$, the signals at 3.56–3.73 ppm belonging to thioglycerol shifted entirely to lower resonance field (4.40 ppm (g) and 5.03 ppm (f)), indicating that this reaction was complete (Figure 3C). From Figure 3D, it can be seen that the signal ascribed to methine proton of 2-bromo-propanoyl groups disappeared completely and the characteristic signals of thioglycerol at 3.56–3.73 ppm could be observed again after another thio-bromo “click” reaction of POSS-(PtBA-Br)$_8$ with thioglycerol. Based on the results above, it can be concluded that POSS-(PtBA-OH)$_8$ was successfully obtained.

**Figure 3.** $^1$H NMR spectra of POSS-(PtBA-Br)$_8$ (A), POSS-(PtBA-OH)$_8$ (B), POSS-(PtBA-Br)$_8$ (C) and POSS-(PtBA-OH)$_8$ (D) in CDCl$_3$. 
Using POSS-(PtBA-OH)₈ as macroinitiator initiated ring-opening polymerization (ROP) of L-lactide to provide the copolymer POSS-(PtBA-(PLLA-OH))₈. The GPC trace of POSS-(PtBA-(PLLA-OH))₈ is shown in Figure 2B, which was monomodal and clearly shifted toward the higher molecular region as compared with the precursor POSS-(PtBA-Br)₈. The FT-IR spectrum of POSS-(PtBA-(PLLA-OH))₈ is given in Figure 4B, the characteristic absorption at 1756 cm⁻¹ corresponds to the ester carbonyl of PLLA chains. Meanwhile, the peaks (d) at 5.14 and (e) at 1.56 ppm assigned to the methine and methyl protons of the PLA block could be observed clearly in the ¹H NMR spectrum of POSS-(PtBA-(PLLA-OH))₈ (Figure 1B). These results confirmed the successful synthesis of POSS-(PtBA-(PLLA-OH))₈. The repeat units of the PLLA chains were derived from the intensity ratio of the peaks (b) at 2.23 to (d) at 5.14 ppm. And, the molecular weight of POSS-(PtBA-b-(PLLA-OH))₈ was calculated according to the following formula (2):

\[ M'_{n,NMR} = M_{n,NMR} + \frac{34 A_d}{A_b} \times 32 \times 72 \]

where \( M'_{n,NMR} \) and \( M_{n,NMR} \) stand for the molecular weight of POSS-(PtBA-b-(PLLA-OH))₈ and POSS-(PtBA-PLLA-OH))₈, respectively; \( A_d \) and \( A_b \) stand for the integral area of the peaks (d) and (b), respectively; The value 32 stands for the number of PLLA chains and 72 stands for the molecular weight of the repeat unit of PLLA. The result is listed in Table 1.

Table 1. Characterization of the star-like amphiphilic copolymer POSS-(PAA-(PLLA-PEG))₈.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Length of Arms</th>
<th>Coupling Efficiency (%)</th>
<th>( M_{n,NMR} )</th>
<th>( M_{n,GPC} )</th>
<th>( M_n/M_d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSS-(PtBA-Br)₈</td>
<td>4,350</td>
<td>PLLA²</td>
<td>PEG</td>
<td>37,610</td>
<td>27,150</td>
</tr>
<tr>
<td>POSS-(PtBA-(PLLA-OH))₈b</td>
<td>4,350</td>
<td>2,510</td>
<td>5,000</td>
<td>120,750</td>
<td>78,560</td>
</tr>
<tr>
<td>POSS-(PtBA-(PLLA-PEG))₈b</td>
<td>4,350</td>
<td>2,510</td>
<td>5,000</td>
<td>250,750</td>
<td>131,670</td>
</tr>
</tbody>
</table>

\( a \) Determined by \(^1H\) NMR spectroscopy.  
\( b \) Coupling efficiency between POSS-(PtBA-(PLLA-OH))₈ and mPEG-COOH, determined by \(^1H\) NMR spectroscopy.  
\( c \) Calculated according to the formulas (1), (2) and (3), respectively.  
\( d \) Determined by GPC analysis with PMMA as standard.

Properties of unimolecular micelles formed by star-like amphiphilic copolymer POSS-(PAA-b-(PLLA-PEG))₈

The morphology and size of the formed micelles were analyzed using TEM and DLS. The TEM image (Figure 6A) shows that this unimolecular amphiphilic copolymer self-assembled into well-dispersed rod-like micelles with a size about 100 nm in 1.0 mg mL⁻¹ aqueous solution and this value is basically consistent.
with the theoretical size (110 nm). The average size of the micelles determined by DLS was about 130 nm. It should be noted that the size measured by DLS was bigger than that measured by TEM because DLS measures the hydrodynamic size of the nanoparticles, while TEM measures the size of the dried nanoparticles. At the same time, we investigated the properties of the micelles as the concentration of the solution decreased from 1.0 mg mL\(^{-1}\) to 0.05 mg mL\(^{-1}\). From Figure 6B, it can be seen that the morphology and size of the micelles didn’t change as the solution was diluted 20 times. Meanwhile, the size of the micelle POSS-(PAA-(PLLA-PEG))\(_{4}\) was also characterized in THF. Due to the fact that THF is a good solvent for POSS-(PAA-(PLLA-PEG))\(_{4}\), it should exist as separate unimolecules. The obtained average size is about 154 nm, which is bigger than that (~ 130 nm) determined in aqueous solution. The reason is that the middle PLLA chains are stretching in THF good solvent, however, they aggregate in aqueous solution. So, it can be basically concluded that the formed micelles were unimolecular. According to the unique structure, the amphiphilic copolymer POSS-(PAA-(PLLA-PEG))\(_{4}\) could self-assemble into the micelles having a biodegradable PLA membrane with the biocompatible PEG outer corona and PAA hydrophilic cavity. Its structure was confirmed by the \(^{1}\)H NMR technique. From Figure 5, it can be seen that the characteristic signals belonging to PLA and PAA segments can be observed clearly in DMSO-\(d_{6}\), however, they disappeared completely in D\(_{2}\)O. This phenomenon demonstrated the formation of the micelles with the PEG chains stretching on the exterior of the PLA membrane and PAA chains on its interior. Otherwise, the signal of PAA should be observed in D\(_{2}\)O media.

It is well known that PAA is one of the most studied pH-responsive polymers, and micelles with hydrophilic PAA outer corona displayed size-change behaviors in the presence of pH change. So, it can be hypothesized that micelles with inner hydrophilic PAA cavity should display swelling-deswelling behavior with varying of the system pH. Swelling the formed micelles as a function of pH is shown in Figure 7. It is apparent from the graph that the micelles were minimum at pH above 4 when the PAA was not charged. As pH increased, the micelles progressively swelled due to the increasing amount of ionized carboxylic groups. At pH about 7 the swelling reached a maximum since the carboxylic groups were completely deprotonated. However, the micelles began to deswell at pH above 7, which might be contributed to the electrostatic attraction exerted from the charged carboxylate and counter ions. The fact indicated that the micelles can “breathe” with variation of the pH.

**Figure 6.** TEM images of the micelles formed from star-like amphiphilic copolymer POSS-(PAA-(PLLA-PEG))\(_{4}\) with different concentrations (A) 1 mg/mL and (B) 0.05 mg/mL.

**Figure 7.** pH-dependent size of the micelles formed by the hybrid copolymer POSS-(PAA-(PLLA-OH))\(_{4}\).

**Figure 8.** Release Curve of DOX from POSS-(PAA-(PLLA-PEG))\(_{4}\) Star-Shaped Micelles.

### Conclusion

In summary, a novel unimolecular star-like amphiphilic copolymer POSS-(PAA-(PLLA-PEG))\(_{4}\) has been developed for the first time via the combination of SET-LRP, ROP and thio-bromo “click” reaction. Due to its unique architecture, this copolymer self-assembled into special nano-sized unimolecular micelles, which possesses a PLA hydrophobic membrane with the PEG corona and interior PAA hydrophilic cavities. This kind of micelles based on POSS-(PAA-(PLLA-PEG))\(_{4}\) copolymer...
holds great promising applications in controlled drug delivery due to the following advantages: 1) They possess better stability in vivo than the conventional multimolecular micelles. 2) Their PLA membrane of the micelles can encapsulate some hydrophobic drugs. 3) Their inner PAA hydrophilic cavities can load some hydrophobic drugs via electrostatic interactions, such as DOX.HCl. Moreover, this micelle can be further explored to prepare a range of biomedical materials. For example, it can be used for cancer theranostics as superparamagnetic iron oxide nanoparticles are precipitated in the inner PAA cavities. In one word, the unique architecture and features make this novel amphiphilic copolymer promising materials in the biomedical area.

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References:
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