Core Cross-Linked Reverse Micelles from Star-Shaped Polymers

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Core cross-linked reverse polymeric micelles (CCL-RMs) were prepared by an original biphasic approach and their properties compared to their unmodified analogs. The cross-linking reaction did not affect the micelles' sphericity and aggregation number, but it reduced their solvodynamic diameter. Furthermore, while solubilization of model polar dyes in hydrophobic organic media was not markedly affected, this modification allowed for enhanced retention of the encapsulated cargo.

Introduction

Reverse polymeric micelles (RMs) have been obtained from unimolecular dentritic[1–5] or hyperbranched structures[6] as well as from self-assembled amphiphilic macromolecules.[7–9] Earlier work from Forster et al.[10] and Eisenberg et al.[11,12] has indeed provided extensive insight as to the micellization of ionizable poly(styrene)-*b*-poly(4-vinylpyridine) and poly(styrene)-*b*-poly(acrylic acid) in organic solvents. Recently, it was reported that star-shaped alkylated poly(glycerol methacrylate) ($PG_{OH}MA$) also formed multimolecular RMs in organic solvents[13] and oils.[14] These RMs displayed a high loading capacity for small hydrophilic molecules, making them potentially useful for drug delivery applications or as nanoreactors in organic chemistry.[15] Despite their interesting properties, multimolecular RMs, as other conventional micelles, may release their cargo prematurely due to the rapid diffusion of the encapsulated molecules and/or their disassembly upon dilution. Cross-linking has been repeatedly shown to be an effective method to increase micelle stability by freezing the assemblies into permanent core−shell structures.[16,17] Along with an increased stability, the presence of a network provides a mean to control the escape rate of entrapped molecules.[18,19] This strategy was aptly exploited in the generation of shell cross-linked (SCL) micelles which could sustain dilution below their critical aggregation concentration or environmental changes that would normally lead to their unraveling.[20,21] These stabilized micelles could also be manipulated to yield nanostructures with unique morphologies, including hollow nanoparticules or onion-like micelles.[22–24] Zhao et al.[25] recently reported the synthesis of SCL reverse micelles composed of poly(dimethylaminoethyl methacrylate)-*b*-poly(methyl methacrylate-*co*-coumarin methacrylate), which were then employed as macroinitiators.

Although chemical modification of the shell has been the preferred avenue, the latter must be performed at high polymer dilution in order to limit formation of covalently bonded secondary aggregates.[26] Such undesirable reactions could be prevented by cross-linking the core rather than the corona.[17,27] Different strategies can be employed in the preparation of core cross-linked (CCL) micelles, including

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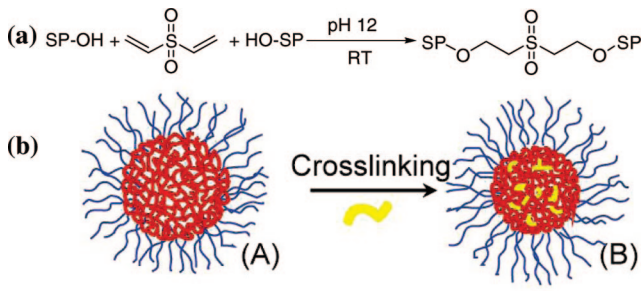
Scheme 1. Core Cross-Linking Reaction (a) and Its Illustration (b)



Note: SP represents star-shaped polymer.

Table 1. Characteristics of RMs

polymer[a]	D_{DCM} (nm) [PI][b]	$D_{pyridine}$ (nm) [PI][c]	loading capacity (mg/g)[d]
$S12C_{40}$	33 [0.16]		106 ± 4
$S12C_{60}$	36 [0.16]		110 ± 5
$S12C_{100}$	9.3 [0.25]	16 [0.32]	3.5 ± 2.7
$S18C_{40}$	46 [0.18]	20 [0.13]	119 ± 6
$S18C_{60}$	50 [0.24]		120 ± 3
$CCLS18C_{40}$	24 [0.18]	77 [0.20]	78.6 ± 3.5

[a] SXC_Y where X and Y stand for the number of C atoms and degree of alkylation, respectively. [b] Mean diameter in DCM. [PI]: polydispersity index calculated from the second moment of the cumulants analysis algorithm. [c] Mean diameter in pyridine. [d] Mean ± SD (n = 3).

polymerization of pendant reactive functionalities[28,29] or introduction of a multifunctional cross-linking agent.[16] The latter approach was also employed by Wooley et al.,[17] who successfully solubilized azide-terminated hydrophobic dendrimers into poly(acrylic acid)-*b*-poly(styrene-*co*-acetylene styrene) micelles to produce CCL nanoparticles. With respect to RMs, the polarity of the core warrants the use of a water-soluble agent that produces little or no side products since their removal may prove difficult. In this work, a one-pot single-step procedure for the cross-linking of RMs core was devised using divinyl sulfone (DVS) (Scheme 1), a hydrophilic agent which seemed to best fulfill these requirements.[22,30] The modified RMs were then characterized to highlight the influence of cross-linking on the physicochemical and loading properties of the micelles.

Results and Discussion

RMs were prepared from a polyol scaffold that was synthesized using a cellobiose-derived initiator (Supporting Information). Modification of the hydrophilic $PG_{OH}MA$ backbone with either lauroyl or stearoyl chloride (40 or 60 mol % compared to −OH content) yielded 30−50 nm particles in dichloromethane (DCM) as determined by dynamic light scattering (DLS) (Table 1). In comparison, fully modified $PG_{OH}MA$ stars (ca. degree of alkylation of 100%), produced ∼9 nm particles, thus sustaining the premise that the presence of free −OH groups is paramount to polymer aggregation.[13] Transport properties of RMs were examined through extraction studies conducted with Congo red, a water-soluble dye. Prior to cross-linking, the RM preparations demonstrated high loading capacities with approximately 105−120 mg of dye solubilized per gram of polymer (Table 1). Increasing the degree of alkylation from 40 to 60 mol % did not significantly affect size or loading, as previously noted for RMs composed of 4- to 6-arm star polymers.[13] Maximum loading was achieved with $S18C_{60}$ (ca. 120 mg/g). Previously, the best results were obtained for the 6-arm polymer and stood at ∼97 mg/g, confirming the determining role of the degree of branching on extraction capacity.[13] Although $S18C_{40}$ RMs demonstrated a similar efficiency as $S18C_{60}$ RMs, $S18C_{40}$ micelles were selected for the cross-linking experiments since a higher amount of residual free −OH groups is available for reaction with DVS.[22]

CCL micelles were produced by an original method based on encapsulation of DVS in the hydrophilic micelle core and subsequent cross-linking of the $PG_{OH}MA$ chains via Michael addition (Scheme 1). DVS was extracted from water into the DCM phase containing the RMs. For the cross-linking reaction to occur, the micelle core was alkalinized by increasing the pH of the aqueous phase. It was verified that the pH of the RM core could be modulated using an indirect spectroscopic assay (Supporting Information). The cross-linking reaction was confirmed by Fourier transform IR-photoacoustic spectroscopy (FTIR-PAS), Raman (Figure 1), and ^{1}H NMR (Figure 2) spectroscopies where new peaks corresponding to the sulfone group were observed. Sulfur content analysis revealed that 12% of −OH groups were actually modified instead of the 50% expected from the feed ratio. The incomplete extraction of DVS (75%) along with its partial hydrolysis may have contributed to reduce the observed yield.[22]

All polymers were examined by differential scanning calorimetry (DSC) (Figure 3), as their thermal properties may be distorted by a change in molecular packing. It is well known that crystallization of branched polyols may be induced by adjunction of long alkyl chains (>12 carbon atoms).[31,32] Here, the T_m of the cross-linked polymer (23.1 °C) was slightly lower than that of the unmodified polymers (29.7 °C). Also, the melting enthalpies (ΔH) changed from 33.4 to 25.3 J/g for RMs before and after cross-linking, respectively. The presence of diethyl sulfone groups may have introduced defects within the structure. Moreover, these groups may also lead to crystals of smaller dimension and a lower degree of crystallinity, which reduced T_m and ΔH accordingly.[33,34]

Particle size analysis revealed that following core modification the RMs collapsed as shown by a sharp decrease in the mean diameter from 46 to 24 nm in DCM. Since both types of RMs exhibited similar aggregation numbers (∼12−13

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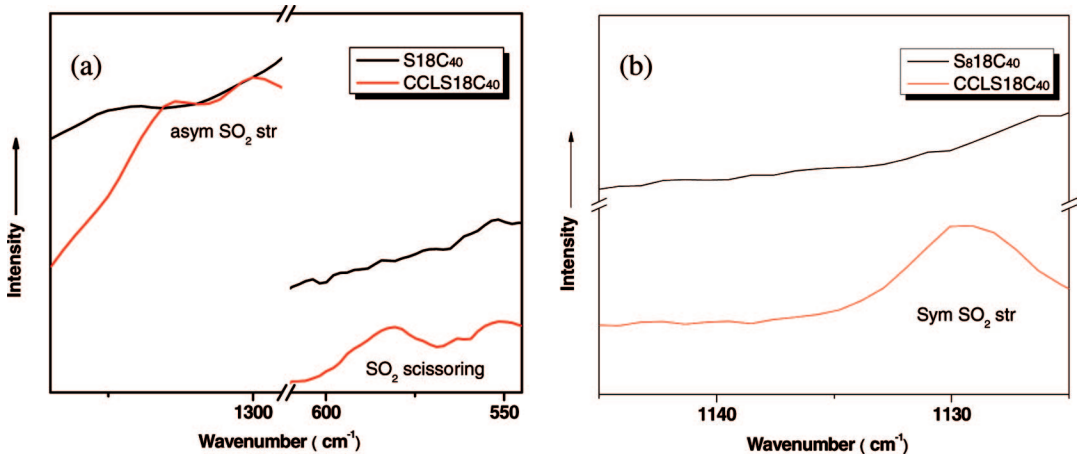

Figure 1. FTIR-PAS (a) and Raman (b) spectra of $S18C_{40}$ and $CCLS18C_{40}$.

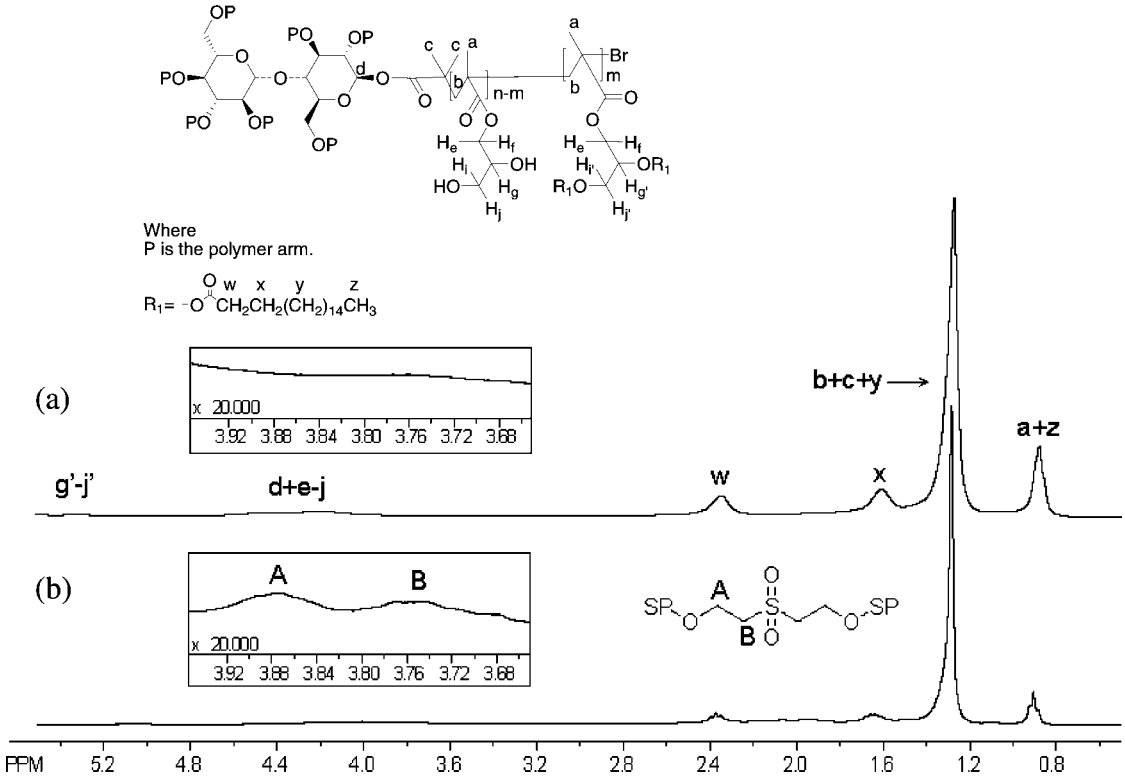

Figure 2. ^1H NMR spectra of $S18C_{40}$ (a) and $CCLS18C_{40}$ (b) in $CDCl_3$/pyridine-d_5 (1:1, v/v). Introduction of DVS was confirmed by the appearance of peaks corresponding to the sulfone groups at 3.87 and 3.74 ppm.

polymer chains), this change in diameter was hypothesized to result from a variation of the solvation of the micellar core in DCM.[28,35] DLS measurements were also performed at different scattering angles (60−140°) to assess whether the micelles remained spherical in solution after cross-linking. The diameter of both CCL RMs and non-cross-linked RMs exhibited no angular dependence, suggesting that the associates were spherical in DCM (Supporting Information).[36,37] Size analyses were also performed in pyridine in order to verify that RMs were locked in a core−shell architecture. Contrary to DCM, pyridine is an unselective solvent and should bring about dissociation of the micelles. Here, changing the solvent indeed caused the $S18C_{40}$ micelles to partially unravel while the *z*-average diameter of $CCLS18C_{40}$ micelles more than tripled to attain 77 nm (Table 1). This change in diameter might be due to swelling of the core as well as formation of secondary micellar aggregates. The latter hypothesis was confirmed by the presence of a bimodal size distribution with a large particle population (>500 nm) after DLS data analysis using the CONTIN algorithm. The absence of dissociated CCL RMs confirmed the presence of a flexible permanent network within the micellar structure.[16] Atomic force microscopy (AFM) analysis showed that both micelles before and after cross-linking were spherical in shape (Figure 4). The mean diameter of $S18C_{40}$ RMs was 48 ± 4 nm, close to that determined by

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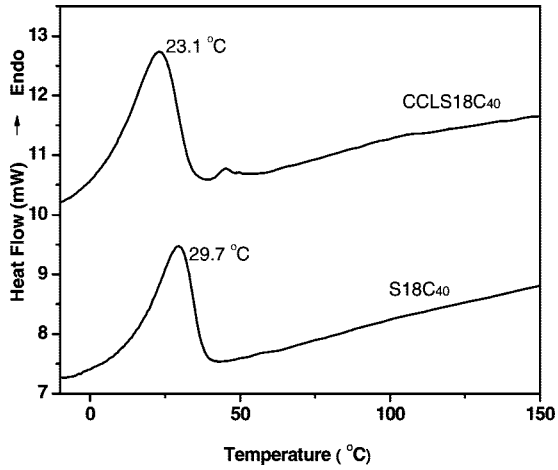

Figure 3. DSC thermograms of $S18C_{40}$ (bottom) and $CCLS18C_{40}$ (top).

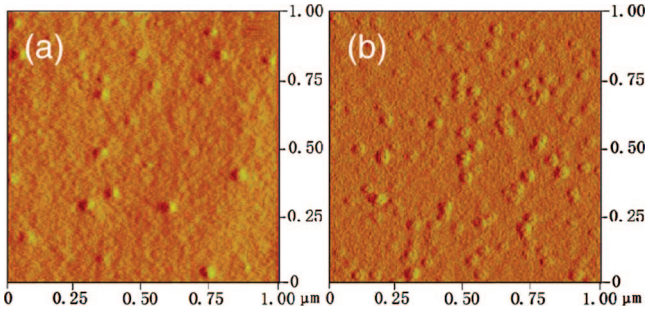

Figure 4. AFM amplitude images of $S18C_{40}$ (a) and $CCLS18C_{40}$ (b) RMs.

DLS (46 nm). After cross-linking, the micelles appeared as aggregates presenting a lateral diameter of 50 ± 3 nm, more than twice the value obtained by DLS (Table 1). Such an increase in size could be caused by flattening of the lightly cross-linked micelle core arising from solvent evaporation and interaction with the mica substrate.[17] Indeed, the AFM phase images of $CCLS18C_{40}$ RMs (Supporting Information) showed micelles with a depressed core.

More than any other, the presence of a cross-linked network may influence both the entrapment efficiency and the release rate.[18] Here, extraction of Congo red from water into DCM was evaluated in order to investigate how cross-linking affected the interaction with the encapsulated dye. Following cross-linking, the loading capacity of $S18C_{40}$ micelles declined from 119 to ~79 mg of dye/g polymer (Table 1). This could be expected as a consequence of the shrinkage of the particles. Release of the encapsulated dye from $S18C_{40}$ and $CCLS18C_{40}$ was then evaluated. For this purpose, the organic phase containing Congo red loaded micelles was isolated following extraction and exposed to an aqueous phase (Figure 5). The amount of dye released was calculated from the optical density of the water phase (λ_{max} = 500 nm). After 1 week, 54.8 ± 3.3% of Congo red was released from $S18C_{40}$ RMs, whereas only 13.4 ± 1.7% of the dye had escaped from $CCLS18C_{40}$ micelles over the same time span, confirming that these micelles better retained their cargo. A similar trend was observed when the experiment was conducted in PBS (see Supporting Information). These findings were reproduced with two other hydrophilic

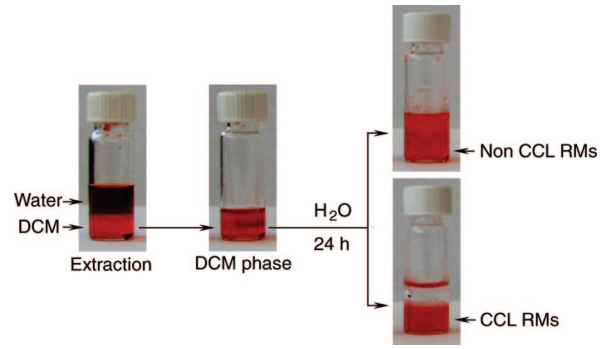

Figure 5. Reverse extraction of Congo red.

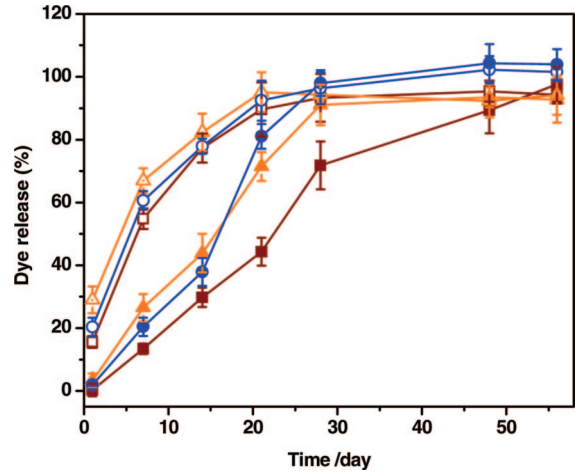

Figure 6. Release of Congo red (red wine squares), methyl orange (orange triangles), and brilliant blue (blue circles) from $CCLS18C_{40}$ RMs (closed symbols) and $S18C_{40}$ RMs (open symbols). The data are mean ± SD (n = 3). This figure shows the decreased burst release and greater dye retention of CCL RMs compared with non-CCL RMs.

dyes (see TOC figure and Figure 6). The greater dye retention of CCL RMs can be rationalized in terms of a decreased core porosity[22,38] and possible affinity of the dyes for the cross-linker's sulfone groups. Cross-linking has been shown to be an effective means to control the release of encapsulated compounds from colloidal particles dispersed in water, as shown for shell cross-linked micelles,[39] CCL polyion complex micelles,[40] and nanogels.[19] However, this study is the first example illustrating how cross-linking the core of RMs can have a profound impact on retention of a hydrophilic molecule within an organic water-immiscible solvent.

Conclusion

Organization of hydrophobically modified $PG_{OH}MA$ in DCM followed by cross-linking of the −OH groups with DVS allowed for preparation of nanoassemblies with sustained release properties. The resulting CCL RMs exhibited a smaller solvodynamic diameter and interacted differently with hydrophilic guest molecules compared to their non-cross-linked analog. Their ability to prevent the fast release

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of their content may be particularly interesting for the design of sustained drug delivery systems and nanoreactors.

Experimental Section

Materials. Glycidyl methacrylate (GMA) was purchased from Polysciences (Warrington, PA). All other reagents were obtained from Aldrich (Oakville, ON, Canada). Chemicals were used without further purification.

Synthesis of Octafunctional Atom Transfer Radical Polymerization (ATRP) Initiator—2,3,4,6,1′,2′,3′,6′-Octa-*O*-isobutyrylbromide Cellobiose. Eight-arm 2,3,4,6,1′,2′,3′,6′-octa-*O*-isobutyrylbromide cellobiose was synthesized from cellobiose (0.014 M) according to a procedure developed for the modification of glucose.[14] Mass and ^{1}H NMR, H−H COSY spectra (Supporting Information) were recorded on a Agilent LC-MSD-TOF (Agilent Technologies, Mississauga, ON, Canada) and Bruker ARX 400 spectrometer (400 MHz, Bruker, Freemont, CA). Yield: 53%. MS $[C_{44}H_{62}O_{19}Br_8Na]^+$: 1556.7125 [Theoretical: 1556.7172].

Synthesis of Poly(glycidyl methacrylate) (PGMA). Star polymers were synthesized by ATRP, as previously described.[14] Briefly, GMA was polymerized in THF using the cellobiose-derived ATRP initiator. Number-(M_n) and weight-average (M_w) molecular weights of PGMA were determined by size exclusion chromatography (SEC) with an Alliance GPCV 2000 system equipped with dual refractive index and viscosity detectors (Waters, Milford, MA) using monodisperse polystyrene standards. SEC analysis was performed in THF at a flow rate of 1 mL/min at 40 °C using three Waters Styragel columns (HT2, HT3, HT5) in series. $M_n = 13.5$ kDa ($M_w/M_n = 1.3$).

Synthesis of Hydrophobically Modified $PG_{OH}MA$. PGMA was hydrolyzed to yield $PG_{OH}MA$. The latter were then partially esterified with either lauroyl or stearoyl chloride (40−60 mol % vs hydroxyl groups). The polymers were characterized by FTIR and ^{1}H NMR spectroscopies and elemental analysis. IR spectra were recorded on a Nicolet FTIR 5 DXB spectrometer (Thermo Scientific, Waltham, MA) using KBr pellets. All amphiphilic star polymers are identified as S*X*C*Y* where *X* and *Y* stand for the number of carbons in the alkyl chain and the degree of alkylation, respectively.

Preparation of CCL Micelles. CCL RMs were prepared by first dissolving $S18C_{40}$ in DCM (20 w/v % solution). DVS (DVS:OH, 1:2 mol/mol) in Milli-Q water was gradually added (DCM/water 5:1 v/v), and the system was equilibrated for 1 h. Then, the aqueous phase was adjusted to pH 12 with NaOH. The two phases were gently agitated on a rocking platform overnight at room temperature. The water-soluble impurities were removed by washing the organic phase with Milli-Q water. The organic phase was dialyzed (Spectra/Por RC, cutoff 25 000) against DCM for 48 h. DCM was then evaporated to isolate the CCL RMs, i.e., $CCLS18C_{40}$. Sulfur elemental analysis (Balzers Omnistar quadrupolar mass spectrometer, Balzers, Liechtenstein) was carried out on CCL micelles both before and after purification by dialysis. Yield: 60−70%.

DSC. The thermal properties of $S18C_{40}$ and $CCLS18C_{40}$ were evaluated on a TA Instruments Differential Scanning Calorimeter (DSC2910; New Castle, DE) at a cooling and heating rate of 10 °C/min ranging from −10 to 150 °C. The DSC thermograms were recorded during the second heating run.

FTIR. FTIR-PAS spectra were recorded by means of a FTS 6000 spectrometer (Bio-Rad Laboratories, Randolph, MA) equipped with a photoacoustic detector.

Raman Spectroscopy. The Raman spectra were recorded on a Renishaw System 2000 (Renishaw, ON, Canada) with an integral microscope using an excitation radiation at 785 nm. All spectra were measured at room temperature and represent the average of 3 scans.

DLS. Mean particle diameter and size distribution were assessed in DCM at room temperature by DLS on a Malvern Autosizer 4800 (Malvern, Worcestershire, U.K.) operating at 90°. All polymer solutions (1 mg/mL) were passed through 0.2 μm filters prior to analysis. DLS measurements were also performed at different scattering angles (60−140°) to examine micelle sphericity. For spherical particles, diameter is independent of detection angle due to the undetectable rotational motion.[30,31]

AFM. Thin films were prepared by first casting solutions of RMs and CCL RMs in DCM at 0.1 mg/mL onto freshly cleaved mica and then air drying at room temperature. A Nanoscope Multimode AFM (Digital Instruments, Santa Barbara, CA), operated in tapping mode, was used to capture images at ambient conditions. Silicon nitride probes with a resonant frequency of 250−300 kHz and spring constant 42 N/m were used.

SLS. An accurately weighed quantity of copolymer was dissolved in DCM. Prior to measurement, all solutions were passed through 0.2 μm filters. The M_w of the micelles (M_{wmic}) was determined by SLS using a Malvern Autosizer 4800 as previously described.[41] Measurements were conducted at 25 °C at various angles ranging from 50° to 140°. Each experiment was performed in duplicate. The aggregation number is the ratio of M_{wmic} over the M_w of a single polymer chain.

Extraction of Congo Red. Aqueous solutions of Congo red at different concentrations (ranging from 0.05 to 5 g/L) were equilibrated with polymer solutions in DCM (5 g/L) for 24 h. After phase separation, the aqueous phase was assayed by spectrophotometry ($\lambda_{max} = 500$ nm) on a BioTek PowerWave X plate reader (BioTek, Winooski, VT). The amount of dye extracted into the organic layer was determined as the difference between the initial and final dye concentrations in water.

Dye Release from RM. Extraction experiments were performed as described above. The organic phase was then carefully transferred to a new vial using a syringe. Dye concentrations were adjusted to account for differences in the loading capacity between CCL and non-cross-linked RMs. Then, 1 mL of the organic phase was sampled and exposed to both 1 mL of either water or phosphate-buffered saline (PBS) (NaCl 75 mM, pH 7.4) containing 0.1% (w/v) sodium azide. The release of the dye in the upper aqueous phase was determined by spectrophotometry ($\lambda_{max} = 500$ nm) at different time points. This reverse extraction procedure was repeated with two other dyes, namely, methyl orange and brilliant blue R. The TOC figure shows that the dye leached in the water phase after 1 day of incubation.

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Supporting Information Available: Synthesis scheme for star-shaped polymers, NMR of 8-arm initiator, qualitative evaluation of the diffusion of acid/base ions into RMs, AFM phase image of $CCLS18C_{40}$, and angular dependence of particle size for RMs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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