Core-cross-linked polymer micelles via living polymerizations

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ABSTRACT

This work reports a general synthesis to core-cross-linked polymer micelles directly from monomers by two typical living polymerizations, anionic polymerization and atom transfer radical polymerization. The micelle concentrations are hundred times higher than those by traditional synthetic method using selective solvents. The morphologies of polymer micelles can be controlled to be spheres, fibers, and graft-like aggregates by varying the experimental conditions. Micelles with the same polymer in both the core and the shell have also been synthesized by this approach.

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1. Introduction

Polymer micelles have recently attracted increasing attentions from both scientific community and industry due to their promising applications in many fields, e.g., they are proposed as high-quality drug-delivery vehicles to treat cancers [1–12]. Langer et al. reported that after labeled with aptamers on the outer surface of poly(lactic acid)/poly (ethylene glycol) micelles, the engineered nanoparticles can accurately and efficiently target prostate cancer cells [12]. Polymer micelles are widely studied for drug delivery applications because of a number of advantages [13]. First of all, hydrophobic drugs may be physically entrapped in the micelle core and transported at concentrations higher than their solubility in water. The delivery efficiency can be therefore greatly improved. Secondly, for the micelle shell composed of hydrophilic blocks, they often form hydrogen bonds with the surrounding water molecules, producing a tight shell around the core. The drugs in the core are effectively protected from hydrolysis and enzymatic degradation during the delivery. Thirdly, the chemical compositions, molecular weights, and components of polymers can be easily adjusted to synthesize micelles with tunable sizes and morphologies [14,15]. Finally, the core or the shell of polymer micelles can be further cross-linked to make them stable during the transportation as drug-delivery vehicles [16–19].

Polymer micelles are traditionally produced by assembling block copolymers in selective solvents with soluble blocks forming the shell while non-soluble blocks forming the core [14,15]. The other synthetic approaches such as micellizations induced by non-covalent interac-

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polymerization and atom transfer radical polymerization, have been investigated and compared.

2. Experimental

2.1. Materials

Argon (99.9995%) was used for all manipulations and storages of materials. All chemicals used in this study are of analytical grades and were used without further purifications. Styrene, 4-vinylpyridine, and α-bromotoluene were respectively distilled from CaH₂ three times and stored in the freezer. Copper (I) bromide was re-crystallized with glacial acetic acid and washed with ethanol and diethyl ether for three times. Hexane was distilled for two times.

2.2. The general procedures of the anionic living polymerization (Fig. 2)

All purifications of reagents and solvents and polymerizations were performed on a double manifold connected to a high vacuum line and high-purity argon (Fig. 2). Dry tetrahydrofuran was distilled in a flask containing sodium/benzophenone and directly transferred to the reaction flask by a syringe. Styrene in tetrahydrofuran at an atmosphere of argon was initiated with the addition of n-butyllithium. The reaction flask was submerged in a dry ice/acetone cooling bath (−78 °C) before polymerizations. Then the living polystyrene was capped with 1 equivalent of 1,1-diphenylethylene typically after reaction of 30 min. In all cases, a small portion of the living polystyrene was removed and quenched with methanol for the characterization of molecular weights. The second monomer, 4-vinyl pyridine (or styrene), and the cross-linker, divinyl benzene, were then transferred into the reactor and allowed to stir for at least 2 h. The polymer micelles were collected by precipitation of the reaction solution into at least a 10-fold excess of hexane.

2.3. The general procedures of the atomic transfer radical polymerization (ATRP) (Fig. 3)

Copper (I) bromide and 2,2′-bipyridine were added to a reaction flask with a magnetic stir bar, and the flask was sealed and thoroughly flushed with high-purity argon (Fig. 3). Styrene and α-bromotoluene were then transferred to the reaction flask via argon-purged syringes. The sealed flask was submerged in an oil bath at 110 °C for 9 h. The derived polystyrene capped with bromine was analyzed by gel permeation chromatography to determine the molecular weight. Then polystyrene-Br, copper (I) bromide, 2,2′-bipyridine, and benzene ether, were added to a new reaction flask with a magnetic stir bar and flushed with argon again. The second monomer, 4-vinyl pyridine, and cross-linker, divinyl benzene, were transferred to the flask via the syringe. The reaction flask was submerged in the oil bath at 110 °C for 10 h.

2.4. Analysis

A commercial dynamic laser light scattering spectrometer (Malvern Autosizer 4700) equipped with a multi-τ digital time correlation (Malvern PCS7132) and a solid-state laser (ILT 5500QSL, output power = 100 mW at λ₀ = 532 nm) as light source was used to characterize the micelle solutions. The measurements were typically performed at 25 °C and at a scattering angle of 90°. Monomodal was used to analyze the data. Scanning electron microscopy (SEM) observations were conducted on a Philips XL300 electron microscopy at an accelerating voltage of 25 kV. The specimens were prepared by
depositing a drop of the solutions onto a glass slide. The nuclear magnetic resonance measurements were performed on a Bruker DMX500 spectrometer in CDCl3 using TMS as an internal reference.

3. Results and discussion

3.1. Polymer micelles with high concentrations and tunable morphologies synthesized by anionic polymerization

The anionic polymerization has been first studied with two different monomers (Fig. 2). Styrene (ST) is used as monomer 1, 4-vinyl pyridine (4VP) is used as monomer 2, and divinyl benzene (DVB) is used as the cross-linker. We choose the above monomers and cross-linker because they have been widely studied for living polymerizations. In addition, they are commercially available and easy to be purified.

Stable polymer micelle solutions with concentrations up to 200 g/L have been synthesized, and the derived micelles are uniform in size. As shown in Fig. 4a, for the polymer micelles synthesized with PS molecular weight (Mw,PS) of 114,520, the molar ratio between 4VP and ST (N4VP/NS) of 1/1, and the molar ratio between DVB and 4VP (NDVB/N4VP) of 1/9, dynamic light scattering (DLS) measurements showed that the polydispersity values are lower than 0.1 at 100 g/L and the hydrodynamic diameters kept at around 223 nm under different scattering angles. This result suggests that the synthesized micelles are spherical and uniform. Scanning electron microscopy (SEM) observations further confirm the above conclusions. Uniform and spherical micelles with average diameter of ~170 nm were observed under SEM (Fig. 4b). The core-shell structure of the above micelles with PS in the shell and P4VP in the core has been confirmed by X-ray photoelectron spectroscopy (XPS) as almost no nitrogen has been detected by XPS [30].

By tuning the experimental conditions, we can also control the morphologies of the derived polymer micelles. Fibers and graft-like aggregates have been prepared besides spheres by varying the molecular weight of PS and the molar ratio between two monomers. Fig. 5 shows the representative SEM images of the fibers and graft-like aggregates. The fibers are synthesized with Mw,PS of ~84,244, N4VP/NS of 1/1, and NDVB/N4VP of 1/9, while the graft-like aggregates are synthesized with Mw,PS of ~21,486, N4VP/NS of 1/1, and NDVB/N4VP of 3/17. Note that spherical micelles are observed with Mw,PS of ~114,520, N4VP/NS of 1/1, and NDVB/N4VP of 1/9. So the high molecular weight of PS favors the formation of spheres, while the low molecular weight of PS produces fibers or graft-like aggregates. In addition, low N4VP/NS favors the formation of spheres, while high N4VP/NS produces fibers or graft-like aggregates. Three samples with similar Mw,PS of ~10,000, the same NDVB/N4VP and different N4VP/NS values (1/3, 1/1, and 3/1) were investigated. SEM images showed that spheres with diameters of 170–280 nm, fibers (diameter of 90 nm and length of 100–1000 nm) with a few spheres (similar diameter to fibers), and graft-like aggregates with diameter of 100 nm, were obtained, respectively.

A possible mechanism for the formation of above tunable morphologies of spheres, fibers, and graft-like aggregates is summarized as below. For spherical core-shell micelles, when their shells are thick enough and the densities of the shells are high, the possibility for the contacts among their cores is little. Therefore, the cross-linking reactions among the cores can be prohibited, and spherical micelles are stabilized as the final products. However, when their shells are relatively thin and/or their densities are low, and once two core-shell Fig. 4. The core-cross-linked micelles with the core of poly (4-vinyl pyridine) (P4VP) and the shell of polystyrene (PS) synthesized by anionic polymerization. (a) Dynamic light scattering (DLS) characterization. (b) Scanning electron microscopy (SEM) image. The molecular weight of the PS macroinitiator is 114,520 with polydispersity of 1.23, the molar ratio of 4VP to ST is 1/1, the molar ratio of divinylbenzene (DVB) to 4VP is 1/9, and the micelle concentration is 100 g/L.

Fig. 5. SEM images of the core-cross-linked polymer micelles with morphologies of (a) fibers and (b) graft-like aggregates. The shell is composed of PS, and the core is composed of P4VP cross-linked by DVB.
spheres collide together, the fluctuations of the shell densities may lead to the contacts between two cores and the following reactions between them. After this fusion to form a cylinder, the shell density around the fusion region becomes higher, and the two ends of the cylinder are relatively thinner. Obviously, it is easier for the third sphere to attach the two ends and so on and so forth to form a fiber. In the case that the shell density around the fusion point is not high enough, the other spheres or fibers may still have chances to contact the core of a fiber, graft-like aggregates will be then produced. For both fibers and graft-like aggregates, the PS shells are not considerably wrapped under TEM, i.e., no segmented morphology has been observed, so the shells have been re-distributed after the coupling among micelles.

If ST was used to replace 4VP at the second step, polymer micelles with PS both as the core and the shell had been prepared. For example, we studied a spherical PS/PS micelle with PS molecular weight of 9785 (macroinitiator), the molar ratio of ST in the core to that in the shell is 1/1, the molar ratio of DVB to ST in the core is 1/9, and the concentration is 100 g/L.

3.2. Polymer micelles with high concentrations synthesized by atom transfer radical polymerization

Atom transfer radical polymerization (ATRP) represents another living polymerization to synthesize polymer materials. It was discovered by Wang and Matyjaszewski in 1995 [31] and has been widely explored recently [32–35]. Compared with anionic polymerizations which require strictly oxygen-free and anhydrous conditions, the synthesis by ATRP is much milder. Here we also choose ST as monomer 1, 4VP as monomer 2, and DVB as the cross-linker.

Higher concentrations can be achieved by ATRP than anionic polymerization. For a sample prepared with $M_{w,PS}$ of ~10,000, $N_{4VP}/N_{ST}$ of 1/1, and $N_{DVB}/N_{4VP}$ of 1/5, the stabilized micelles was synthesized at concentrations up to 300 g/L. DLS measurements show the average hydrodynamic diameter of 102 nm and the polydispersity higher than 0.7 at the scattering angle of 90° (Fig. 7a). SEM image in Fig. 7b demonstrates that the resulting micelles are spherical and not uniform in size (diameter of 35–243 nm). In this case, anionic polymerization is better to synthesize uniform micelles than ATRP. The much higher polydispersity of the micelles synthesized by ATRP may be due to the much longer reaction time at the second step. The polymerization and cross-linking reaction at different living sites by ATRP may proceed with more varied speeds and degrees compared to anionic polymerization. Similar to anionic polymerization, if 4VP was replaced by ST, micelles with PS both as the core and the shell were prepared. Interestingly, no fibers or graft-like aggregates were observed for the synthesis of ATRP by varying experimental conditions. This may be due to the relatively low reaction rate of ATRP. Two colliding spheres could not fuse into an aggregate when the reactions between them are not fast enough to stabilize them, i.e., the spheres after collision separate. Therefore, only spherical micelles were synthesized by ATRP.

4. Conclusions

To summarize, we have developed a general synthetic approach to core-cross-linked polymer micelles with much higher concentrations...
than the traditional method by assembling block copolymers in selective solvents. This new synthesis can be easily scaled up and shows promising and real applications in the near future. Polymer micelles in which the core and the shell are composed of the same polymer have been first discovered in this work.

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