Synthesis of Hyperbranched Multiarm Star Block Copolymers and Their Application as a Drug-Delivery System

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ABSTRACT: A novel hyperbranched multiarm star block copolymers was synthesized. The atom transfer radical polymerization initiators were anchored on the surfaces by the reaction of amino-functionalized silica modified by 3-aminopropyltriethoxysilane with α-bromoisobutyryl bromide. The composites (SNs-g-HPCMS-b-HEMA-b-PNIPAAm) with silica nano particles as core and hyperbranched star block copolymers as arms was prepared by iron(III)-mediated surface-initiated polymerization with FeCl$_3$·6H$_2$O as catalyst, triphenylphosphine as ligand, ascorbic acid as reducing agent, N,N-dimethylformamide as solvent, and 4-chloromethylstyrene, 2-hydroxyethylmethacrylate, and N-isopropylacrylamide as monomers, respectively. The resultant products were characterized by Fourier-transform infrared spectroscopy, N$_2$ adsorption/desorption measurements, thermogravimetric analysis, differential scanning calorimeter, and transmission electron micrographs. With aspirin as the model drug, the drug-release behavior of the hybrid materials was studied. The release behavior could be controlled by the temperature, which will have potential applications in biomedicine and biotechnology. © 2013 Wiley Periodicals, Inc. Adv Polym Technol 2013, 32, 21375; View this article online at wileyonlinelibrary.com. DOI 10.1002/adv.21375

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Introduction

Owing to their unique characters, such as stable pore structure, high surface area, high thermal stability, stable mechanical properties, no biological activity and nontoxicity, silica nanoparticles (SNs) have been widely applied in catalysis, adsorption, separation, sensing, and drug delivery.$^{1,2}$ For the synthesis of novel and high quality inorganic/organic nanocomposites, SNs are usually recognized to be ideal hosting molecules. Pure SNs have faint dispersibility in most organic and aqueous solutions and poor compatibility with chemical and biological systems. To improve dispersibility and compatibility of SNs,$^{2,3}$ surface modification methods with organic groups or polymers have attracted much interest during the past decades. The most extensive synthetic route used for the preparation of such a hybrid material is the “grafting from” approach, which conducts a chain-growth polymerization from initiators attached onto the surface of the inorganic particle. High graft density can be obtained by it.$^4$ Of the many modification techniques, surface-initiated atom transfer radical polymerization (SI-ATRP)$^{5,6}$, one of controlled/“living” radical techniques, has been widely applied to “grafting from” methods. It can prepare the hybrid materials with excellent controllability for the molecular weight, polydispersity, and chain-end functionality. So far, a lot of publications involved in the SNs surface modification with polymers, such as polystyrene,$^7$ poly(lactic acid),$^8$ poly(methyl methacrylate),$^9$ poly(acrylic acid)$^{10,11}$, and poly(N-isopropylacrylamide) (PNIPAAm)$^{12,13}$, via SI-ATRP are available.

In fact, from a scientific point of view, the conventional ATRP still has some shortcomings for the preparation of inorganic/organic nanocomposites,$^{14}$ for example, (1) the catalyst system involves a lower oxidation transition metal, which easily suffers from an irreversible oxidation to a higher oxidation state and then inhibits the polymerization, (2) metal halogen compounds are especially sensitive to moisture, thus the catalysts system must be stored in a sealed drying oxygen-free atmosphere, (3) the initiators are usually toxic halides. To overcome these defects, an improved ATRP method, that is, activators generated by electron transfer ATRP (AGET ATRP),$^{15}$ was developed in 2005. It has all benefits of normal ATRP and remains tolerant to air. Thus, it will be very applicable in the preparation of multifunctional polymers on the surface of SNs.

By AGET ATRP, some linear polymers have been grafted to the SNs surfaces.$^{16}$ In comparison with linear polymers, hyperbranched polymers have much higher density of...
functional groups. Moreover, hyperbranched polymer has many nanocavities, which can be used as efficient carriers and can encapsulate various guest molecules to form complexes. Li et al. reported the graft polymerization of the hyperbranched poly(2-((bromobutryl)oxy)ethyl acrylate) onto the exterior surface of mesoporous silica nanoparticles (MSNs) by the surface-initiated self-condensing atom transfer radical vinyl polymerization. The core-shell nanostructure may have potential applications in biomedicine and biotechnology due to a large amount of the terminal functional groups of the hyperbranched polymers. However, as far as we know, there is no publication about grafting hyperbranched polymers onto SNs via iron-catalyzed AGET ATRP systems.

In this work, we try to modify the SN surfaces with polymers via iron-catalyzed AGET ATRP. A novel organic/inorganic composite material was prepared and its application of drug release was studied. With nontoxic iron as catalyst, the product can be applied to living organisms.

## Experimental

### MATERIALS

Silica nanoparticles (7–40 nm), 2-bromo-isobutryl bromide, iron(III) chloride hexahydrate (FeCl$_3$·6H$_2$O), triphenylphosphine (PPh$_3$), ascorbic acid (VC), aspirin, 3-aminopropyltriethoxysilane (APTES) were obtained from Aladdin Reagent (Shanghai, People’s Republic of China). 4-Chloromethylstyrene (CMS) from J&K Chemical (Tokyo, Japan) was purified by recrystallization with cyclohexane. 4-Chloromethylstyrene (CMS) from J&K Chemical (Tokyo, Japan) was purified by recrystallization with cyclohexane. Toluene from Sinopharm Chemical was dried by CaH$_2$ at room temperature.

Poly(2-(bromobutryl)oxy)ethyl acrylate) onto the exterior surface of silica modified by APTES with α-bromoisobutryl bromide. The resultant product (SNs@Br) was utilized as initiator in the self-condensing atom transfer radical with AGET vinyl polymerization of inimer CMS, resulting in core-shell nanoparticles with SNs core and HPCMS shell (SNs-g-HPCMS). SNs-g-HPCMS was used to initiate the successive polymerization of HEMA by iron (II)-mediated surface-initiated polymerization with FeCl$_3$·6H$_2$O as catalyst, PPh$_3$ as ligand, VC as reducing agent, and DMF as solvent. The product SNs-g-HPCMS-b-PHEMA was utilized as ATRP macroinitiator to prepare the novel organic/inorganic hybrid material SNs-g-HPCMS-b-PHEMA-b-PNIPAAm by reacting with NIPAAm.

### SYNTHETIC PROCESS

The synthesis process of the hyperbranched multiarm block copolymers is shown in Scheme 1. The ATRP initiators were anchored on the surfaces by the reaction of amino-functionalized silica modified by APTES with α-bromoisobutryl bromide.

### SYNTHESIS OF AMINO-FUNCTIONALIZED SILICA NANOPARTICLES (SNs@NH$_2$)

4.0 g of SiO$_2$, 50 mL of freshly distilled toluene, and 10 mL of APTES (0.011 mol) were placed in a 100 mL of flask. After evacuating air with N$_2$ for 30 min, the flask was immersed in an oil bath at 80°C. The mixture was stirred for 24 h, and then the solution was cooled. Amino-functionalized SNs (SNs@NH$_2$) were obtained by centrifugation and then washed with toluene, methanol, and ethanol in ultrasonic. Then it was dried in a vacuum at room temperature.

### SYNTHESIS OF ATRP INITIATOR-IMMOBILIZED SILICA NANOPARTICLES (SNs@Br)

Typically, 3.0 g of amino-functionalized silica nanospheres (SNs@NH$_2$), 40 mL of dry toluene, and 6.0 mL of triethylamine (0.043 mol) was placed in a 100 mL of dried flask and immersed in an ice-water bath, and the contents were gently stirred. When the mixture was cooled to 0°C, a solution of 2-bromoisobutryl bromide (5 mL) in toluene (3 mL) was dropwise added under argon atmosphere. The reaction was then allowed to proceed overnight at room temperature. The amino-functionalized silica nanospheres with 2-bromoisobutryl bromide (SNs@Br) were separated by centrifugation and washed with water and ethanol thoroughly. Then it was dried in a vacuum at room temperature.

### SYNTHESIS OF HYPERBRANCHED CORE-SHELL SILICA NANOPARTICLES (SNs-g-HPCMS)

The surface-initiated AGET ATRP of CMS on the SNs@Br surfaces was accomplished by the following procedure: FeCl$_3$·6H$_2$O (40 mg, 0.14 mmol), PPh$_3$ (74 mg, 0.282 mmol), SNs@Br (0.5 g), DMF (30.0 mL), and CMS (5.0 mL, 35.5 mmol) were placed in a 100 mL of dried three-necked flask. Then, 37.5 mg of VC (0.213 mmol) was added to the solution under nitrogen and the flask was immersed in an oil bath held by a thermostat 95°C to polymerize under stirring. The reaction was kept for 24 h. The...
flask was cooled by immersing it into iced water. The contents were diluted with THF (10 mL). The product (SNs-g-HPCMS) was separated by centrifugation and subjected to intense washing by THF and water under ultrasound. The samples were dried in a vacuum at room temperature.

SYNTHESIS OF HYPERBRANCHED BLOCK COPOLYMER (SNs-g-HPCMS-b-PHEMA)

A representative example was as follows: SNs-g-HPCMS (0.5 g), FeCl₃·6H₂O (40 mg, 0.14 mmol), PPh₃ (74 mg, 0.282 mmol), DMF (30 mL), and HEMA (10 mL, 87.1 mmol) were placed in 100 mL of dried three-necked flask. Then, 37.5 mg of VC (0.213 mmol) was added to the solution mixture under nitrogen and the flask was immersed in an oil bath held by a thermostat 95°C to polymerize under stirring. The reaction was kept for 24 h, the flask was cooled by immersing it into iced water. The crude product was dissolved in THF. Then it was separated by centrifugation and subjected to intense washing by THF and washed with methanol, centrifugation three times. Ultimately the product (SNs-g-HPCMS-b-PHEMA-b-PNIPAAm) was dried in a vacuum at room temperature.

Results and Discussion

The FT-IR spectrum of the samples is shown in Fig. 1. It can be used to observe the results of the amination of pure SiO₂, the amidation of SiO₂@NH₂ with 2-bromoisobutyryl bromide and the surface-initiated AGET ATRP. In Fig. 1a, the peaks at 1092.55 and 1640 cm⁻¹ come from Si-O-Si stretching vibration and N–H deformation vibration, respectively. The peaks at 2800 to 2980 cm⁻¹ correspond to the C–H asymmetry stretching vibration, which may be attributed to the fact that APTES was successfully introduced onto the surface of SNs. In Fig. 1b, the peak at 1648.60 cm⁻¹ is the characteristic peak of C=O of PNIPAAm (2.2 g) were placed in 100 mL of dried three-necked flask. Then, 37.5 mg of VC (0.213 mmol) was added to the mixture under nitrogen and the flask was immersed in an oil bath held by a thermostat 95°C. Polymerization took place under stirring. After the reaction was kept for 24 h, the flask was cooled by immersing it into iced water. The crude product was dissolved in THF. Then it was separated by centrifugation and subjected to intense washing by THF and washed with methanol, centrifugation three times. Ultimately the product (SNs-g-HPCMS-b-PHEMA-b-PNIPAAm) was dried in a vacuum at room temperature.
the bromoisobutyryl unit. Shown in Fig. 1c is the spectra of SNs-g-HPCMS, whose characteristic peaks at 1600–1450 cm\(^{-1}\) are assigned to C=C skeleton vibration of the aromatic ring. Compared to SNs-g-HPCMS, a new characteristic absorbance peak at 1727.56 cm\(^{-1}\) can be observed in Fig. 1d, which is attributed to ester carbonyl (C=O) and demonstrates that HEMA monomers have been grafted to SNs-HPCMS macroinitiators. The graft of PNIPAAm brushes result in a slight change in the spectra, as can be seen in Fig. 1e, which is almost similar to SNs-g-HPCMS-b-PHEMA. The above results indicated that the polymers were successively grafted from the surface of SNs.

Figure 2 shows the TGA curves of the samples. From it, the attachment of 2-bromoisobutyryl moieties can be further proved. There exists 10% of difference in the weight retentions at 800 °C between SNs@NH\(_2\) and SNs@Br. The content of Br grafted onto the SNs was calculated to be 0.55 mmol/g. The initiator density, ID, can be roughly estimated according to the following formula:

$$ID = \frac{N_{Br} \times N_A}{S_{particle} / (\rho_{silica} \times V_{particle})}$$  \hspace{1cm} (1)

where \(N_{Br}\) is the mole number of one gram of SNs@Br, \(S_{particle}\) and \(V_{particle}\) are the surface area and volume of one particle, respectively. \(\rho_{silica}\) is the density of the SiO\(_2\), and \(N_A\) is the Avogadro’s number. If the density and the average diameter of the silica are 2.07 g/cm\(^3\) and 30 nm, respectively, the grafting-initiator density of SNs@Br is about 2.5 initiator/nm\(^2\).

The weight loss of SNs-g-HPCMS is 30.09%. It is more than that of SNs@Br (27.44%), indicating that the HPCMS was successfully grafted. The percentage of grafting (PG, mass ratio of the grafted polymer to SNs) for SNs-g-HPCMS was calculated to be 43%. The total weight loss of SNs-g-HPCMS-b-PHEMA and SNs-g-HPCMS-b-PNIPAAm were about 57.35% and 57.74%, respectively.

The nitrogen adsorption/desorption isotherms of samples are presented in Fig. 3. The BET isotherms of these three materials exhibit the characteristic type IV adsorption/desorption patterns according to the International Union of Pure and Applied Chemistry (IUPAC) classification, with the inflection of the capillary condensation observed at a P/P\(_0\) value of about 0.8 for the adsorption isotherms. The values for the total surface areas, the total pore volume, and the average pore diameters of SNs@Br, SNs-g-HPCMS, and SNs-g-HPCMS-b-PHEMA-b-PNIPAAm are given in Table I. The results show that the surface areas, the pore volume, and the average pore diameters were reduced after functionalization. The reason for this phenomenon is that the polymers were grafted from inner–outer surface of SNs. However, despite the reduction in the adsorbed amount of nitrogen from Fig. 3a to 3c, the type of the hysteresis loop remained unchanged. This means that the pore structure was not significantly changed by the postsynthesis approach. The BJH analysis exhibits that SNs@Br and SNs-g-HPCMS have similar pore size distribution, as shown in the illustration of Fig. 3. It shows that grafting reaction mainly took place on the surface.
TABLE I

<table>
<thead>
<tr>
<th>Materials</th>
<th>BET Surface (m²/g)</th>
<th>BET Pore Volume (cm³/g)</th>
<th>BJH Average Pore Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNs@Br</td>
<td>112.928</td>
<td>0.58</td>
<td>26.223</td>
</tr>
<tr>
<td>SNs-g-HPCMS</td>
<td>112.928</td>
<td>0.58</td>
<td>26.223</td>
</tr>
<tr>
<td>SNs-g-HPCMS-b-PHEMA</td>
<td>20.208</td>
<td>0.11</td>
<td>3.192</td>
</tr>
<tr>
<td>SNs-g-HPCMS-b-PHEMA-PNIPAAm</td>
<td>20.208</td>
<td>0.11</td>
<td>3.192</td>
</tr>
</tbody>
</table>

Because of easy gathering into the second particles, inorganic particles in the organisms are quite difficult to reach homogeneous dispersion in the nanoscale, which will influence the usability of the inorganic nanoparticle. TEM was used to further characterize the functionalized SNs. Figure 4 is the TEM images of SNs@Br, SNs-g-HPCMS, SNs-g-HPCMS-b-PHEMA, and SNs-g-HPCMS-b-PHEMA-b-PNIPAAm. It can be seen from the Fig. 4a, pure SNs@Br was not well dispersible in the ethanol. After coating with polymers, the dispersity is improved. However, there was some nanoaggregation still. It can also be seen that the appearance of nanoparticles has an evolution from irregular shape to an approximately spherical one with increasing grafted content.

Study on Aspirin Loading and In Vitro Drug Release

Drug loading: Typically, 400 mg of sample was suspended in 30 mL ethanol solution of aspirin with a concentration of 40 mg/mL at 45 °C under stirring for 48 h in a sealed flask to prevent the evaporation of the ethanol. Then the sample was collected by centrifugation and washed with ethanol, and the samples were dried in a vacuum at room temperature.

In vitro drug release: 20 mg of samples loaded with aspirin were put into 50 mL of phosphate-buffered saline (pH 7.4) with constant rotation at a speed of 100 rpm. 12 h later, 4 mL of the release medium was removed and the amount of released aspirin was determined by ultraviolet (UV)–visible analysis at 265 nm. The experiment was repeated for several times in different temperatures.

The process of the drug loading and controlled release of the hyperbranched multiarm star block copolymer is shown in Scheme 2. At first, keeping the temperature above lower critical solution temperature (LCST), hydrogen bonds formed between PNIPAAm and H₂O disassembled, the polymer chains swell within the pore network, and the aspirin drugs were loaded into the pores of SNs-g-HPCMS-b-PHEMA-b-PNIPAAm. Then, reducing the temperature below LCST enables the formation of hydrogen bonds between PNIPAAm and water. As a result, PNIPAAm molecular chains expand. Drugs are captured...
SCHEME 2. Schematic representation of the drug loading and controlled release of the hyperbranched multiarm star block copolymer.

FIGURE 5. DSC spectra of samples (a) for SNs-g-HPCMS-b-PHEMA-b-PNIPAAm and drug-release profiles (b) for SNs-g-HPCMS-b-PHENM-b-PNIPAAm measured at different temperatures under 12 h.

in the pores of the delivery system. While raising the temperature above LCST, PNIPAAm solubility decreases rapidly, owing to the disassembly of hydrogen bonds, making the PNIPAAm chains shrink. The polymer chains become dehydrated and swell within the pore, making the pore unobstructed again. Thus, the drug could be released conveniently. It implies that the swelling of the polymer chains in response to the temperature change could be used for controlled release.

Figure 5 shows DSC spectra of sample (A) for SNs-g-HPCMS-b-PHEMA-b-PNIPAAm and the release behavior of aspirin from the samples (B) in different temperatures over a time period of 12 h. The LCST of the grafted polymer was also observed by DSC. Figure 5a shows the DSC thermogram in water for SNs-g-HPCMS-b-PHEMA-b-PNIPAAm. It exhibited an endothermic change at 36.9°C. Drug loading and controlled release were determined with aspirin. The amount of drug released was measured based on UV absorbance at 265 nm. Figure 5b shows that drug release amounts change with the temperature. One can see that the drug release rate is about 48% before 30°C, whereas it is suddenly increased between 30 and 40°C. This section is about 50% of the release quantity. It should attribute to the fact that PNIPAAm and PHEMA are thermoresponsive and they produce a coil-to-globule transition around the LCST. This phenomenon is based on a reversible hydration–dehydration of amide groups in the molecules. At a temperature higher than the LCST, the polymer chain contracts, which helps in the release of drug molecules. This shows that the prepared SNs-g-HPCMS-b-PHEMA-b-PNIPAAm material can be used for temperature control drug release. At the same time, we have studied the release behavior of aspirin at 37°C. Figure 6 shows that the amount of released Aspirin was 75% after 4 h at 37°C, which further...
exhibited that the system could be performed for controlled delivery due to the temperature-responsive pattern.

Conclusions

Surface-initiated AGET ATRP, using FeCl$_3$·6H$_2$O as catalyst, PPh$_3$ as ligand, VC as reducing agent, and DMF as solvent, has been successfully used to prepare hyperbranched multiarm star block copolymers. With nontoxic iron as catalyst, the product can be applied to living organisms. The prepared SNs-g-HPCMS-b-PHEMA-b-PNIPAAm hybrid materials exhibit temperature-sensitivity with a LCST at 36.9°C. Experiments of the drug release show that the hyperbranched multiarm star block copolymers have potential application as drug carriers in biomedicine and biotechnology.

References
