Synthesis and Characterization of Starch Piperinic Ester and Its Self-Assembly of Nanospheres

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ABSTRACT: A novel amphiphilic starch piperinic ester (SPE) has been synthesized by coupling a carboxyl group on the piperic acid and a hydroxyl group on the starch backbone. The synthetic process includes three steps. Firstly, piperic acid was obtained by hydrolyzing piperine that was extracted from seeds of *Piper longum* L (a kind of traditional Mongolian medicine). Then, piperic acid was reacted with carbonyl diimidazole (CDI) under N_2 at 70°C for 4 h to form an activated piperic acid. Finally, starch piperinic ester was obtained by the reaction of the activated piperic acid with water-soluble starch at 80°C under N_2 for 24 h. The product was characterized by FTIR, NMR. It was found that the starch piperinic ester could easily form stable micelles in aqueous solution, and the spherical morphology of micelle was observed by ESEM. The results of DLS analysis proved that the micelle size depends on the composition of the amphiphilic polymer. Our results demonstrate that SPE has great potential in the drug controlled release delivery. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 523–528, 2008

Key words: polysaccharide; starch; *piper longum* L; nanosphere; self-assembly

INTRODUCTION

With the development of new type of medical agents like peptides, protein and DNA for different new therapies, such as vaccines and gene therapy, increasing attention has been paid to designing polymeric drug carriers which are degradable, nontoxic, and tissue-compatible. Polysaccharide is one of the candidates, which can meet certain requirements.

Polysaccharides have been studied extensively for applications as biomaterials and biomedicines for their unique physicochemical properties and excellent biocompatibility. It is well known that polysaccharides are not only natural polymers, which are produced annually in huge amount by plants and micro-organisms, but also the structural and superstructural diversity make them become potential starting materials for defined modification and specific applications. For the practical applications, it is necessary to modify polysaccharides by chemical reactions in order to have some functionalities and properties. Ruxandra et al.[1] prepared amphiphilic polysaccharides by polysaccharides grafted with polyesters and found that the amphiphilic polysaccharide derivatives can be made into nanopartices. In our case, we synthesized amphiphilic starch piperinic ester, which can self-assemble into micelles. The micelles have unique characteristics such as nanosize, core–shell architecture, and a good thermodynamic stability in physiological condition because of their low critical micelle concentration (cmc).[2–6] This provides a useful basis for industrial, pharmaceutical, and biomedical applications.[7–9]

Since many important therapeutic agents have poor solubility in aqueous solution, it is quite a challenge to develop effective systems for delivery of these agents. Micelle-like aggregates of amphiphilic copolymers have been recently receiving much attention as carriers for hydrophobic drug.[10–13] During the micellization process, their hydrophobic segments form the core of micelles as an incorporation site of lipophilic drugs, whereas the hydrophilic corona or outer shell plays a role in avoiding the uptake by reticuloendothelial system. This unique architecture enables polymeric micelles to serve as stabilizers for poorly water-soluble drugs.

Starch, a kind of a natural polysaccharide with natural abundance and low cost as it is obtained

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from plants, has full biodegradability, water-soluble properties, and lack of toxicity; moreover, the hydrophilic backbone is desired for drug delivery carriers. Starch bound form of nictonic acid has been previously synthesized and pharmacologically tested on rats.[14] This derivative was found to combine prolonged antilipolytic activity with absence of rebound in free fatty acid (FFA) plasma levels. Later, Luisa et al. prepared a series of starch–nicotinic acid copolymers with a different degree of esterification, and examined variation of some physicochemical characteristics in the series.[15] Recently, the grafted starches have been prepared as new biodegradable plastics[16] and drug delivery carriers.[17]

Piperine, a major hydrophobic alkaloid of *piper longum* L and *piper nigrum*, has been reported to have pharmacological/toxicological effects.[18–20] For long time, much more attention has been paid to studying natural piperidine and its derivatives have been extracted from plants for chemistry and pharmacology.[21–23] There are no reports involving investigation on polymer derivatives of piperine. In our lab, the amphiphilic starch piperinic ester composed of polysaccharide (water-soluble starch) backbone grafted with hydrophobic piperic acids has been synthesized by means of ester bridges, which had antihyperlipidemic activity, and had no obvious toxicity for oral administration to Wistar rats.[24] In this article, the amphiphilic starch–piperic acid grafted copolymers with controlled structures were prepared by coupling a carboxyl group on the piperic acid and a hydroxyl group on the starch backbone. The resulting amphiphilic starch piperinic ester was characterized by various means. The stable self-aggregates were prepared by a dialysis method and observed with dynamic light scattering (DLS) and environmental scanning electron microscopy (ESEM).

EXPERIMENTAL

Materials and general methods

Soluble Starch (α-Amylose, AR) was purchased from Tianjin Chemical Ragent Plant, China. Carbonyldiimidazole (CDI) was the commercial product supplied by Aldrich Chemical Company (USA). Dimethylsulfoxide (DMSO) was from Arcos Organics. DMSO was distilled after drying with calcium hydride for 24 h under a nitrogen atmosphere. Water-soluble starch with a medium molecular weight (M_n = 12000, M_w/M_n = 1.38) was prepared by hydrolysis of commercial starch in the presence of H_2SO_4 as catalyst, according to the method reported previously.[25] Molecular weight of the water-soluble starch was determined by means of GPC in pH = 6.8 phosphate buffer solution at 25°C. Other reagents were commercially available and used as received.

Preparation of piperine

Piper longum L fruit powder (1000 g) was extracted with 95% ethanol (1000 mL) and refluxed for 4 h for three times. The supernatant collected by centrifugation at 3600 rpm/h was dried in a vacuum and designated as a crude piperine. The crude piperine was further purified by recrystallization in ethanol. Powdery, yellow piperine (10.05 g; mp: 129–130°C) was obtained.

Preparation of piperic acid

Piperine (10 g) was dissolved in 300 mL of anhydrous ethanol containing KOH (20 wt %) in a 500-mL reaction flask equipped with a reflux condenser. The mixture was heated at reflux with stirring for 10 h to give the precipitate of potassium piperate. After filtration, the precipitate of potassium piperate was washed three times with anhydrous ethanol. The precipitate was dissolved in distilled water, and then purified by precipitating piperic acid in water by adding HCl solution (0.1*M*). The yellow precipitate of piperic acid was filtered, washed with distilled water (200 mL) three times to produce 8.66 g of powdery yellow piperic acid (mp: 206–208°C, yield 86.6%) by freeze-drying from water.

Synthesis of amphiphilic starch piperinic ester

To synthesize starch piperinic ester, a two-step reaction was carried out according to the method reported in the literature.[1] For example, 1.090 g (5 mmol) of piperic acid and 0.907 g of carbonyldiimidazole (5.5 mmol) were dissolved in 6 mL of anhydrous DMSO in a 25-mL round-bottomed reaction flask equipped with a reflux condenser and connected to the N_2 line. The flask was heated at 70°C under N_2; CO_2 evolution was observed. The reaction was not stopped until CO_2 was no longer released in 4 h, and the activated polymer formed. Then 1.238 g of water-soluble starch dissolved in 6 mL of anhydrous DMSO was added. The mixture was stirred for 24 h at 80°C under N_2. The reaction mixture was dropped into 50 mL of hot anhydrous acetone, and the precipitated polymer was isolated by centrifugation. The supernatant was discarded and the precipitate was washed twice by ethanol. The crude product was further purified by dialysis. The precipitate was dissolved in DMSO completely, and was dialyzed for 24 h against DMSO and then for 48 h against distilled water using a dialysis membrane (MWCO = 2000–3000 g/mol). Slight yellow, powdery starch piperinic ester (1.36 g) was obtained by freeze-drying from water.

Characterization of starch piperinic ester

The starch piperinic esters were determined by ^1H NMR, ^3C NMR, and The Fourier transform infra-

red (FTIR). FTIR spectrum of starch piperinic ester was recorded using Bio-Rad FTS 6000 spectrometer at room temperature using KBr pallet. ^{13}C NMR and ^{1}H NMR spectra were recorded on a Bruker AV 400 MHz in DMSO-d_6 at 25°C; chemical shifts were given in parts per million from tetramethylsilane. The percentage of piperic acid group was calculated from the peak intensities of ethylene proton signal (6.042 ppm) of grafted piperic acid and the ethylene proton signal (5.490 ppm) of starch backbone in the ^{1}H NMR spectrum of starch piperinic ester.

Preparation of self-assembled micelles

The self-assembled nanoparticles were prepared using a solvent displacement method with a dimethyl sulfoxide/water (DMSO/H_2O) system by a dialysis process.[26] First, the conjugate (0.05 g) was dissolved in a common solvent (DMSO, 10 mL) for both segments and then water (2–4 mL), which is a precipitant for piperic acid segments but a good solvent for water-soluble segments, was added to induce the aggregation of graft copolymers. The mixed solution of graft copolymer was stirred and then dialyzed for 2 days against distilled water using a dialysis membrane (MWCO = 2000–3000 g/mol).

Dynamic light scattering

Size distribution of micelles was determined by dynamic light scattering (DLS) with a vertically polarized He–Ne laser (DAWN EOS, Wyatt technology). The scattering angle was fixed at 90°C, and the measurement was carried out at a constant temperature of 25°C. The sample solution was diluted in filtered double-distilled water prior to analysis.

Environmental scanning electron microscopy

The morphology of the micelles was investigated by environmental scanning electron microscopy (ESEM). ESEM was performed on an XL 30 ESEM FEG Scanning Electron Microscope (Micrion FEI PHILIPS). A drop of micelle solution was deposited onto a silicon chip mounted on an aluminum stub. The sample was air-dried and covered with gold before measurement.

RESULTS AND DISCUSSION

Synthesis

Starch piperinic ester was prepared by a synthetic route as illustrated in Figure 1. In the first step, piperic acid was obtained by hydrolyzing natural piperine. In the second step, *N,N′*-carbonyldiimidazole

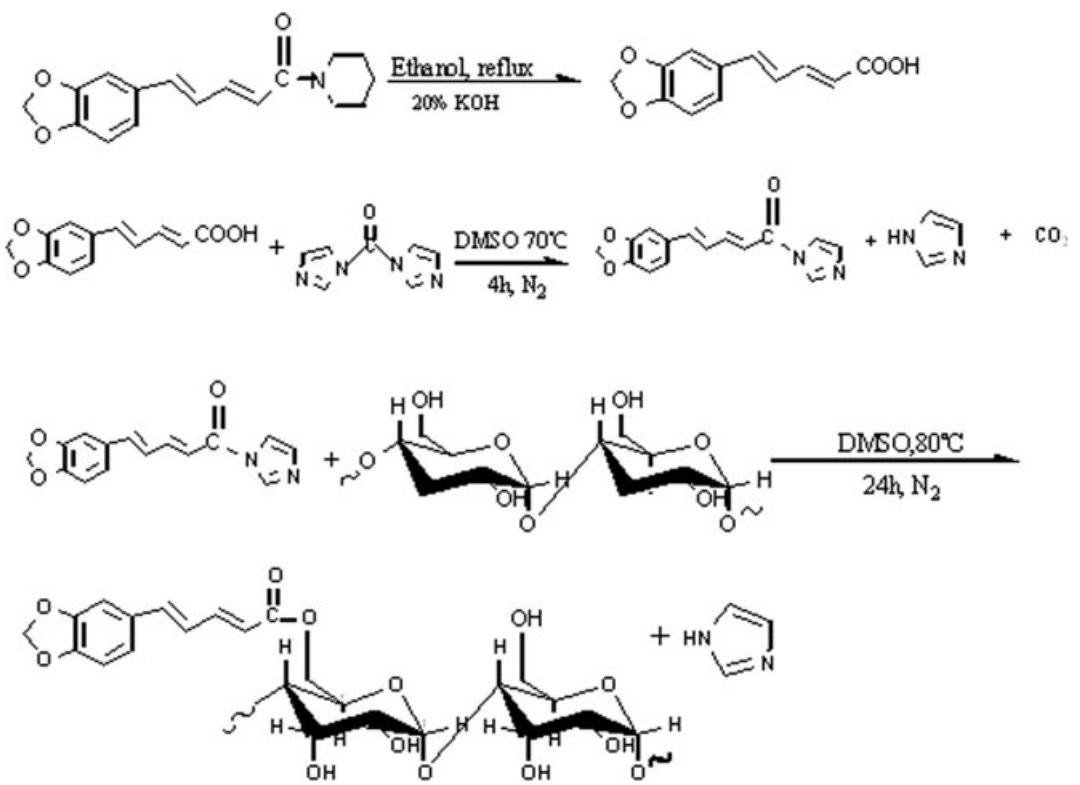

Figure 1 Synthetic route of starch piperinic ester.

TABLE I
The Results for the Esterification of Water-Soluble Starch with Piperic Acid in the Presence of CDI in DMSO for 24h

Reagent (mmol)			Product (SPE)	
Piperic acid	Starch[a]	CDI	DS[b] (mol %)	Yield[c] (%)
1.20	5.56	1.44	15.1	97.6
2.40	5.56	2.44	42.3	98.3
3.60	5.56	4.32	57.9	90.9

[a] Molar was mole of repeating unit of D-glucose (GLU) in starch.
[b] Degree of substitution determined by ^{1}H NMR of products.
[c] Yield of starch piperinic ester calculated in ration of starch of reagent.

(CDI) in excess was used for conversion of carboxylic groups of piperic acid into a imidazolide-activated piperic acid. In the third step, starch dissolved in DMSO was added to the not isolated mixture of the activated piperic acid containing imidazole. The esterification was achieved by homogeneous reaction of the starch with a piperic acid imidazolide, which was prepared *in situ* by conversion of piperic acid with *N*,*N*-carbonyldiimidazole (CDI).

For the coupling reaction of piperic acid and starch, different strategies were tried. For example, the carboxylic group of piperic acid can be activated with DCC/DMAP, but coupling with polysaccharide needed several days and the yield did not exceed 60% in our case. In contrast to DCC/DMAP, the activation of piperic acid with *N*,*N*′-carbonyldiimidazole (CDI) was more efficient and convenient at the mild reaction condition. The activated carboxylic acid can react with the hydroxyl group of polysaccharide to produce starch esters in high yield (up to 98.3%), and the byproducts (imidazole) were nontoxic and completely removable. During conversion with CDI, only carbon dioxide and imidazole are formed. Thus, pure starch piperinic ester with high degree of the substitution was obtained simply by precipitation in ethanol and washing with ethanol.

It is obvious that the *N*,*N*′-carbonyldiimidazole (CDI) in excess was used for conversion of carboxylic groups of piperic acid into a imidazolide-activated piperic acid. The result shows that the degree of the substitution (DS) of the starch piperinic esters is up to 57.9% in presence of suitable CDI as catalyst in DMSO. The data of reaction are shown in Table I. The activation of carboxylic group shows more advantages when CDI is used as acylating agent, such as in short reaction time, in good yield. The results demonstrated that CDI is quite effective agent for the piperic acid activation and coupling reaction with starch, and that DMSO is the best solvent in this case.

Characterization of starch piperinic ester

We found that SPE has good solubility only in DMSO, although starch (M_n = 12,000 Dlt) is easily soluble in both H_2O and DMSO, and piperic acid is hydrophobic and soluble in DMSO, ethanol, and acetone. The structure of the starch piperinic ester was analyzed by ^{1}H NMR, ^{13}C NMR, and FTIR. The degree of substitution (DS) was calculated from the ratio of the integral peak H_{18}(δ = 6.046) of piperic acid and H_1(δ = 5.490) of starch in the ^{1}H NMR spectrum of SPE. The results of calculation are shown in Table I.

The IR spectra of starch (a), SPE (b), and piperic acid (c) are shown in Figure 2. It is obvious that the structural features of SPE are different from that of starch and piperic acid. Comparing the spectrum of SPE (b) with the starch(a), new strong absorptions appear at 1703 and 1255 cm^{-1}, assigned to carbonyl (C=O) of ester and the vibration peak of ester group(CO—O), respectively. Meanwhile, two sharp new peaks appear at 1608 and 1504 cm^{-1} for the backbone vibrations of benzene ring. The vibration peak of carbonyl (=CO) for carboxylic function is at 1677 cm^{-1} in the spectrum of piperic acid (c), whereas this is at 1703 cm^{-1} in the spectrum of SPE (b). A red shift from 1677 to 1703 cm^{-1} takes place. The result indicates the formation of ester groups (—COO—).

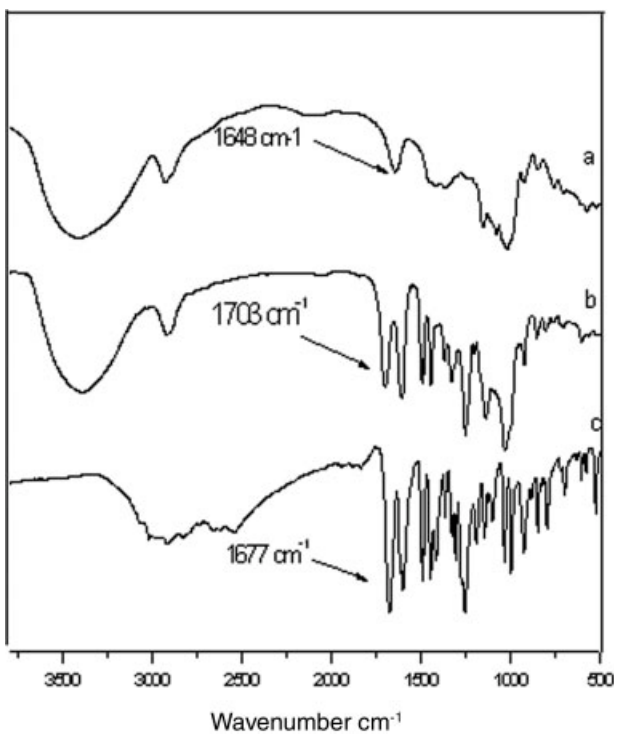

Figure 2 FTIR spectra of starch (a), SPE (b), and piperic acid (c).

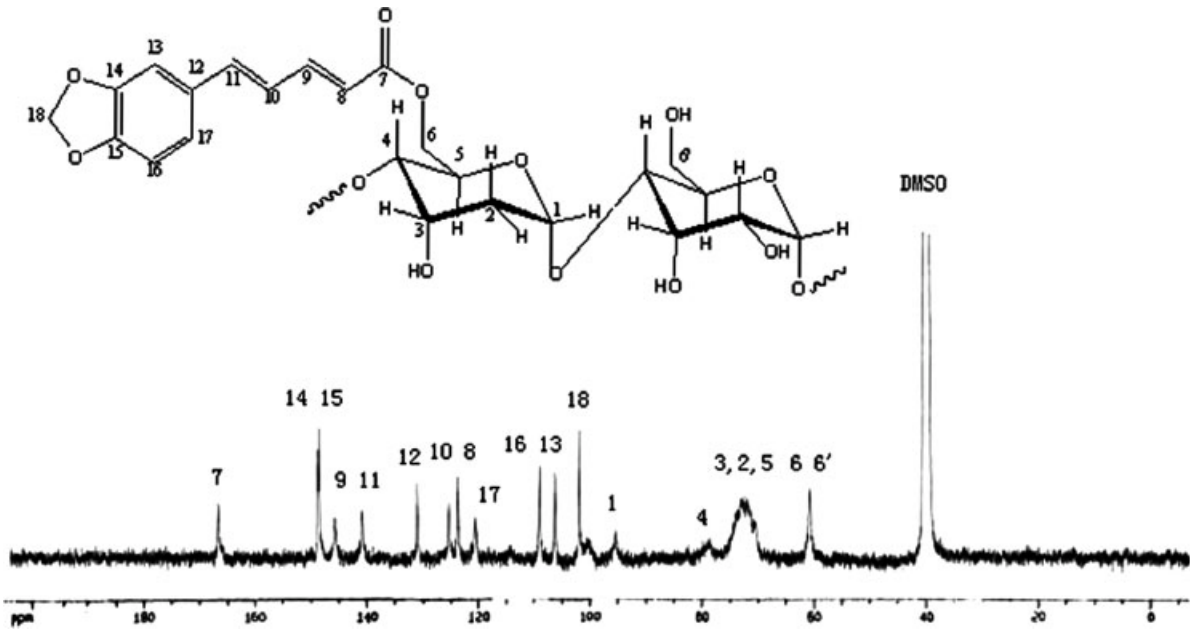

Figure 3 ^{13}C NMR spectrum of starch piperinic ester with DMSO-d_6 as solvent at 25°C.

The chemical structure of starch piperinic ester was confirmed by ^{13}C NMR. All signal peaks were assigned as Figure 3. The signals of the starch backbone chain are all obvious, indicating that the structure of the original polysaccharide remained; the peaks due to piperic acid are all detectable, as evidence of the formation of starch piperinic ester in agreement with the abovementioned FTIR results. The resonance signal at 166 ppm is ascribed to carbonyl carbon of starch piperinic ester, and the peak of carbonyl carbon of starch piperinic ester appeared at 168 ppm, which demonstrates the complete removal of unreacted piperic acid. All the evidences from the spectra of FTIR and NMR indicate that starch piperinic ester has been synthesized successfully.

Formation of self-assembled micelles

Since the starch piperinic ester possesses amphiphilic nature, micelles can form easily in water. It was suggested that the hydrophilic shell of the micelles consists of the water-soluble starch chains and the hydrophobic core consist of piperic ester moieties. The formation of the micelles was confirmed by dynamic light scattering (DLS) and environmental scanning electron microscopy (ESEM). It was observed that the size distribution is narrow and the size of micelle relates to the degree of substitution (DS) of piperic acid on starch. For example, the average diameter is about 200 nm when DS of piperic acid is 31.1% in the starch piperic ester. However, the average diameter increases from 250 to 335 nm

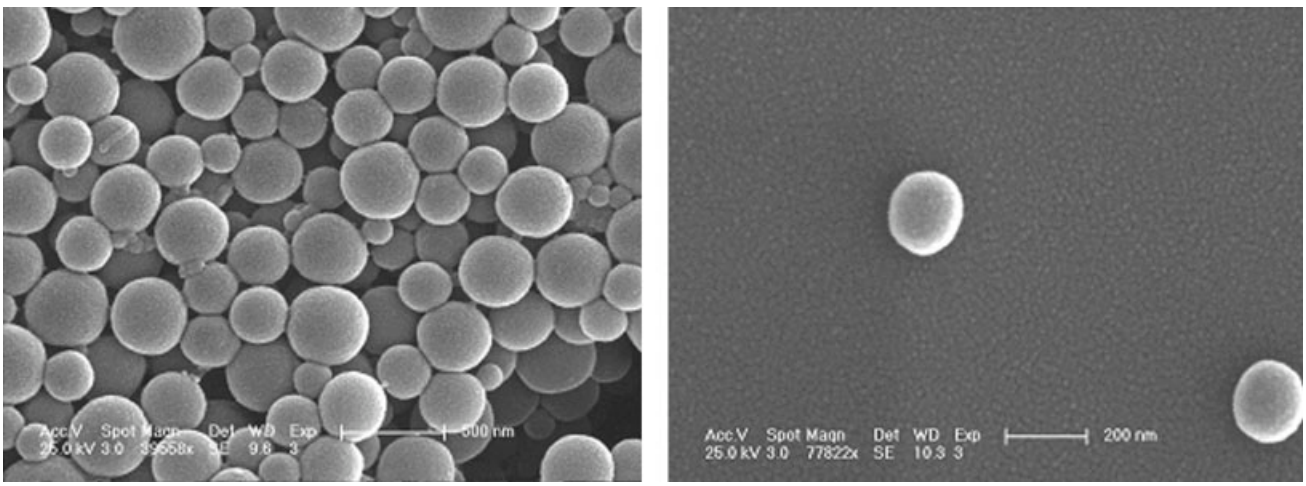

Figure 4 Typical ESEM micrograph of starch piperic ester micelles.

with the increase in DS of piperic acid from 42.3 to 57.9%. In short, SPE with high DS of piperic acid can form larger micellar particles.

Figure 4 presents the ESEM photos of the micelles prepared from DMSO/H_2O system. The micelle size observed by ESEM is about 170 nm, which is a little smaller than that determined by DLS, because the micelle diameter determined by DLS presents their hydrodynamics diameter but that obtained by ESEM is related to the collapsed micelles after water evaporation.

CONCLUSIONS

The amphiphilic starch piperinic ester has been synthesized successfully from nature piperine and starch. The copolymer shows excellent amphiphilicity, and can be formed into micelles easily in the system of DMSO/H_2O by a dialysis process. Furthermore, the micelle nanospheres have narrow size distribution, and their average diameters can be tuned within the range of several hundred nanometers by changing the degree of the substitution of hydrophobic group of piperinic ester. It is expected that the newly developed copolymer of amphiphilic starch piperinic ester could be potentially used as drug vehicles for drug delivery uses.

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