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Letter

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Sulfonato-β-Cyclodextrin Mediated Supramolecular Nanoparticle for Controlled Release of Berberine

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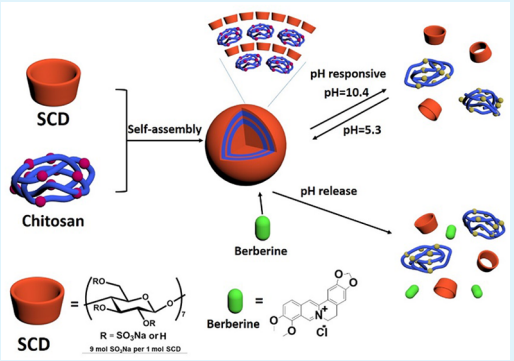
Supporting Information

ABSTRACT: A kind of supramolecular assemblies was constructed from two water-soluble and biocompatible saccharides, sulfonato-β-cyclodextrin (SCD) and chitosan, and characterized by dynamic light scattering (DLS), UV−vis, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The results showed that such nanoparticles presented good stability and controlled loading/release property, which enabled them as good drug carrier for berberine chloride (BE), a representative drug from traditional Chinese herbs. That is, the nanoparticles can load BE with high stability in a low-pH environment like that of the stomach but released BE when moved to a high-pH environment like that of the intestine.



KEYWORDS: *supramolecular nanoparticles, cyclodextrin, chitosan, drug loaded, pH-controlled release*

Reversible stimuli-responsive assemblies have been a popular research interest during the recent few decades.[1−4] Because of their special properties, these assemblies give various applications in the fields of chemistry, materials, medical science, etc.[5,6] By controlling the condition of assembly, the progress of formation and disaggregation can be smartly and reversibly switched.[7−9] Therefore, the reversible and stimuli-responsive supra-amphiphile is expected to be able to provide a promising way to construct smart and recyclable nanomaterials for the delivery of biologically important matter. During recent years, some water-soluble macrocyclic compounds have been widely used in constructing stimuli-responsive supra-amphiphiles.[10−19] For example, we have reported a supramolecular nanorods constructed by tetrasulfonato dinaphtho-32-crown-8 and an amphiphilic viologen, which can be dissociated by adding α-cyclodextrin and reversibly controlled by light after adding 4,4′-azodibenzoic acid.[20]

Chitosan is a natural cationic saccharide, which can provide cationic polyelectrolyte from the protonated amino groups produced by the deacetylation of chitin. Zhang[21] and Xu[22] reported chitosan-based nanomaterials for assembling with ATP and immobilize concanavalin A, respectively. Zhou and co-workers developed the chitosan-grafted β-cyclodextrin to improve the load and release of doxorubicin in the nanoparticles.[23] More recently, we reported a supramolecular assembly through the incorporation of sulfonatocalixarene and chitosan,[24] which showed the good response to the pH change and the addition of competitive guest. In addition, we also reported a supramolecular assembly by the associating sulfato-β-cyclodextrin with protamine.[25] In this work, we use two kinds of ionic polysaccharides, i.e., chitosan and sulfonate-β-cyclodextrin (SCD) (Scheme 1), as building blocks to construct a new reversible and stimuli-responsive supramolecular assembly. The inherent advantages of such supramolecular assembly are (1) both chitosan and SCD are biocompatible, water-soluble, nontoxic and commercially low cost;[26] (2) as compared with other anionic macrocycles including sulfonato crown ethers, sulfonatocalixarenes and pillararenes, SCD possesses the higher negative charge density, which is more efficient to induce the aggregation of cationic chitosan; (3) different from the irreversible assembly constructed by SC4A and chitosan, this supramolecular assembly can reversibly respond to pH. It is our special interest to produce a new biocompatible supramolecular assembly employing biocompatible polysaccharides as building blocks and different physiological pH as controlling method and to explore its potential application in the loading and controlled release of drugs.

Herein, we use two chitosans in different degrees of deacetylation to construct assemblies with SCD. (Chitosan-0.95 represents chitosan with 95% deacetylation degree and chitosan-0.6 represents chitosan with 60% deacetylation degree.) A mixture of SCD with chitosan-0.95 or chitosan-0.6 in pH 5.3 aqueous solution shows Tyndall effect, which indicates that supramolecular nanoparticles are formed in

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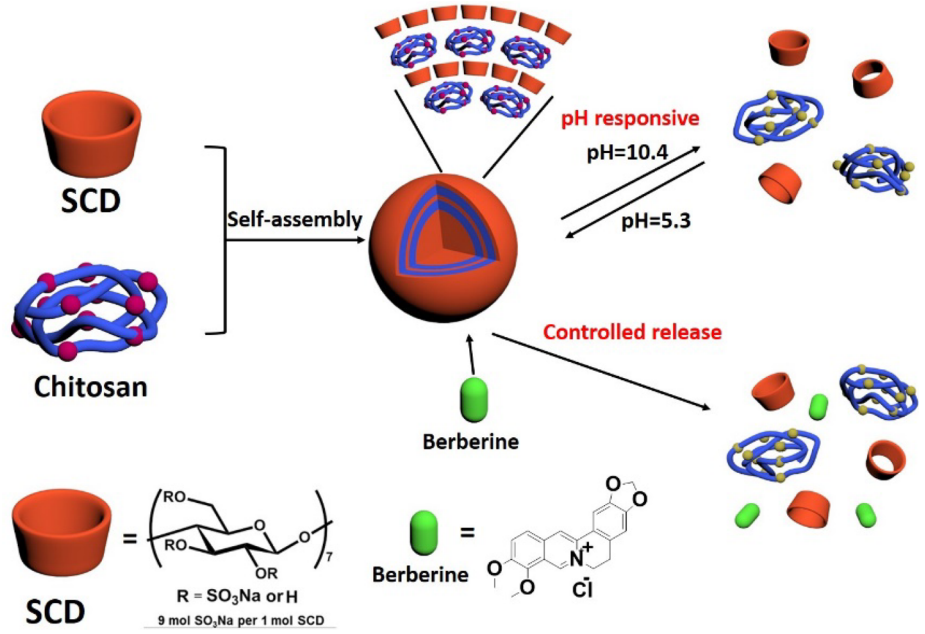
Scheme 1. Construction of SCD/Chitosan Supramolecular Assemblies



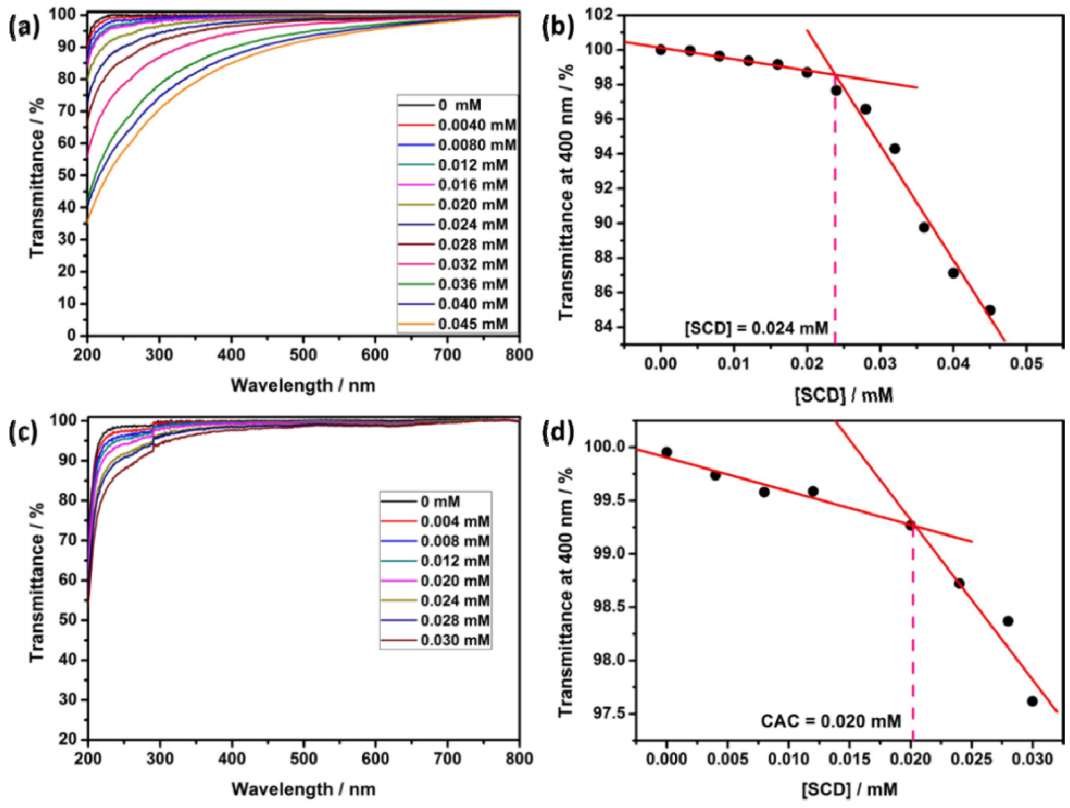


Figure 1. (a) Optical transmittance and (b) dependence of optical transmittance at 400 nm versus the SCD concentration of aqueous solutions containing SCD at different concentrations and chitosan-0.95 (10 μg/mL) at 25 °C. (c) Optical transmittance and (d) dependence of optical transmittance at 400 nm versus the SCD concentration of aqueous solutions containing SCD at different concentrations and chitosan-0.6 (20 μg/mL) at 25 °C.

solution (Figures S1 and S2). In contrast, none of free chitosan (chitosan-0.95 and chitosan-0.6) and free SCD exhibited the Tyndall effect at pH 5.3. By keeping the concentration of one component as a constant and changing the concentrations of the other component, the critical aggregation concentrations (CAC) of SCD/chitosan supramolecular assemblies were measured by investigating the optical transmittance of fixed component. As shown in Figure 1, when increasing the concentrations of SCD from 0 to 0.030 mM, the optical transmittances of chitosan-0.6 (20 μg/mL) at 400 nm versus the concentration of SCD showed the different linear variation with the appearance of an inflection point at 0.020 mM.

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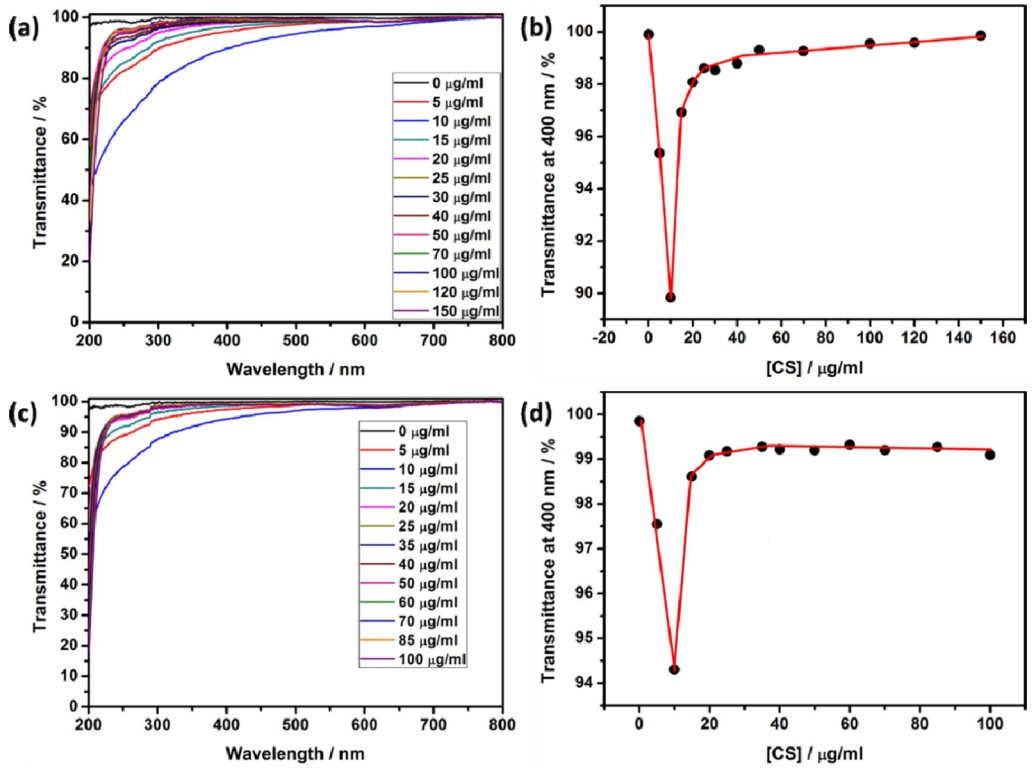

Figure 2. (a) Optical transmittance and (b) dependence of optical transmittance at 400 nm on the chitosan-0.95 concentration of aqueous solutions containing chitosan-0.95 at different concentrations and SCD (0.035 mM) at 25 °C. (c) Optical transmittance and (d) dependence of optical transmittance at 400 nm on the chitosan-0.6 concentration of aqueous solutions containing chitosan-0.6 at different concentrations and SCD (0.030 mM) at 25 °C.

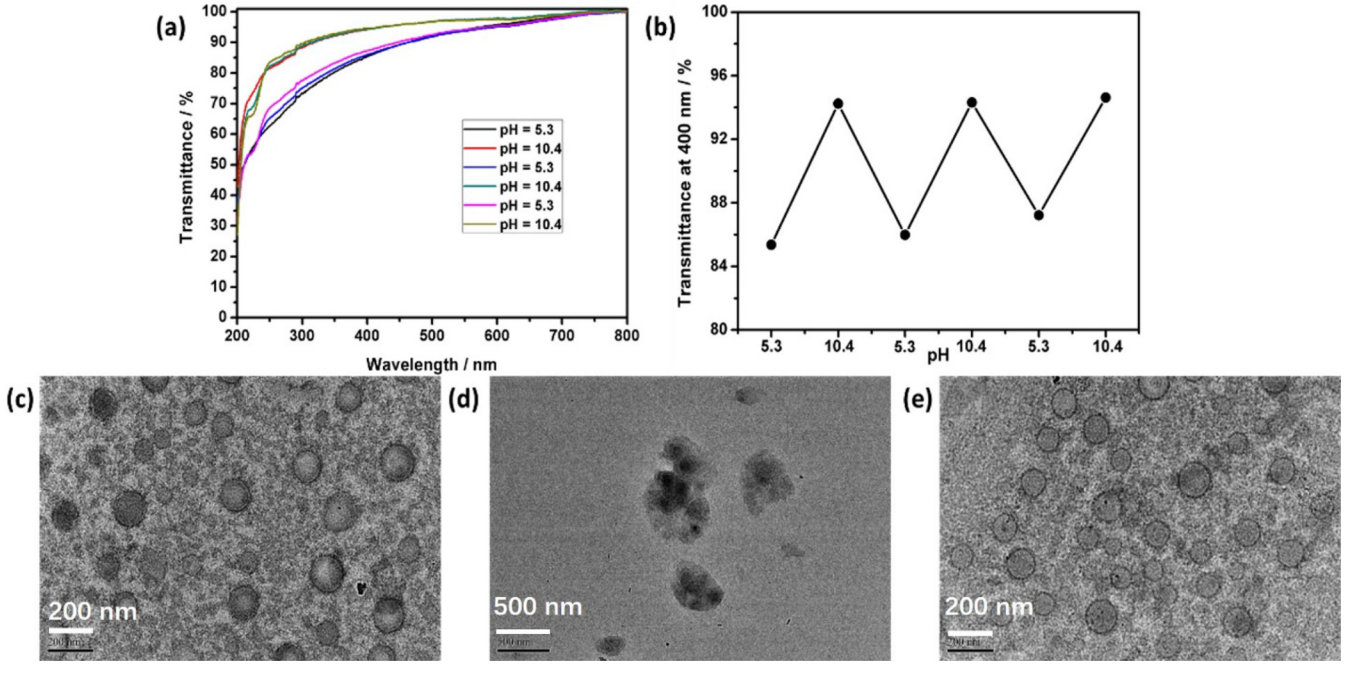

Figure 3. (a) Optical transmittance and (b) dependence of optical transmittance at 400 nm of SCD/chitosan-0.95 nanoparticles with pH varying between 5.3 and 10.4. (c–e) TEM images of (c) original SCD/chitosan-0.95 nanoparticles, (d) disassembled nanoparticles after pH was adjusted to 10.4, (e) reassembled nanoparticles after pH was adjusted back to 5.3, respectively. ([SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL).

Therefore, the complexation-induced CAC value of SCD in the presence of chitosan-0.6 should be 0.020 mM. In the case of chitosan-0.95, the CAC value of SCD was determined as 0.024 mM.

The preferable SCD/chitosan mixing ratio between was also investigated. When changing the concentration of chitosan from 0 to 100 μg/mL in a SCD solution (0.030 mM), the optical transmittances at 400 nm sharply decreased before the

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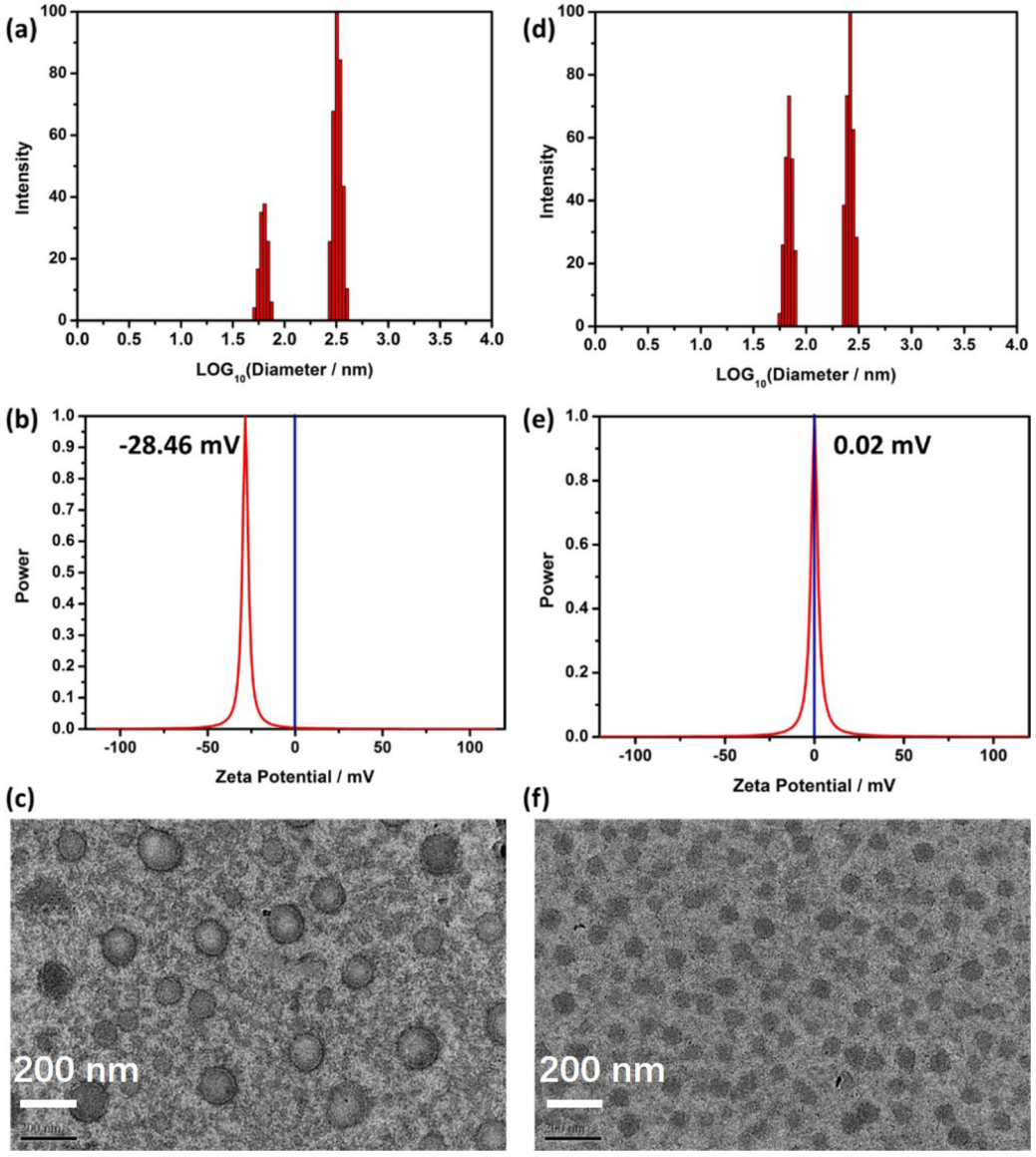

Figure 4. (a) DLS, (b) zeta potential, and (c) TEM image of SCD&chitosan-0.95 nanoparticles ([SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL). (d) DLS data, (e) zeta potential, and (f) TEM image of the SCD&chitosan-0.6 nanoparticles ([SCD] = 0.030 mM, [chitosan-0.95] = 10 μg/mL).

concentration of chitosan-0.6 reached 10 μg/mL, and then gradually recovered after increasing concentration of chitosan-0.6 to 100 μg/mL (Figure 2). Accordingly, the preferable mixing ratios of SCD/chitosan assemblies were measured as 0.030 mM SCD/10 μg/mL chitosan-0.6 and 0.035 mM SCD/10 μg/mL chitosan-0.95 at pH 5.3. In the control optical transmittance experiments, no obvious transmittance changes of free SCD or free chitosan was observed under the same conditions (Figures S3 and S4).

DLS, TEM, and SEM were carried out to investigate the size and morphology of SCD/chitosan supramolecular assembly. As shown in Figure 3, the average hydrodynamic diameters of SCD/chitosan-0.6 and SCD/chitosan-0.95 were measured as 122 and 173 nm, respectively, by DLS. Moreover, the TEM and SEM images of SCD/chitosan assemblies all showed a number of spherical nanoparticles. Their average diameters were measured as ca. 90 nm for SCD/chitosan-0.6 and 150 nm for SCD/chitosan-0.95, which were similar to that of SEM results but a little smaller than the corresponding values of DLS results.

According to the stability requirement of supramolecular nanoparticles for their applications, we detected the optical transmittance of SCD/chitosan supramolecular nanoparticles versus time at room temperature. In the case of SCD/chitosan-0.6 or SCD/chitosan-0.95, there are nearly no changes of optical transmittance for at least 7h (Figures S6 and S7). In addition, the optical transmittance SCD/chitosan nanoparticles also showed no obvious changes with varying the temperature from 10 to 70 °C (Figures S8 and S9). These jointly indicated a good stability of SCD/chitosan nanoparticles.

Considering the ionic structure of building blocks, pH is expected as an important governing factor in the controlled assembly/disassembly of supramolecular assemblies, because the pH changes could affect the protonation degree of SCD and/or chitosan. The optical transmittance of SCD/chitosan-0.95 nanoparticle obviously increased with the pH changing

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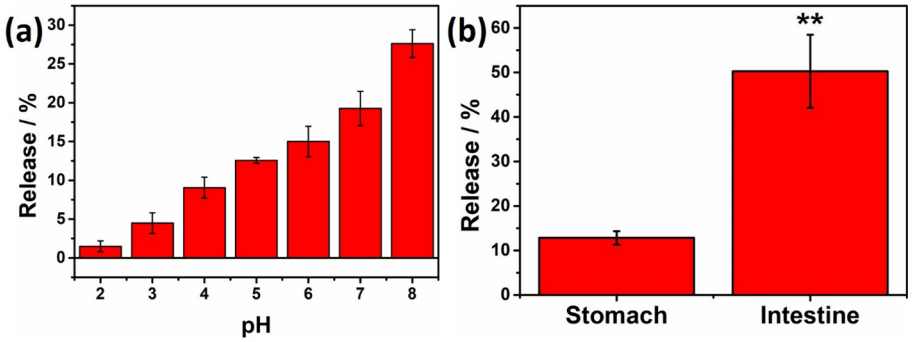

Figure 5. (a) Illustration of pH-responsive release of BE from SCD&chitosan nanoparticle with varying pH ranging from 2 to 8. ([SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL, [berberine] = 0.035 mM, experiment 3 times (n = 3)). (b) In vivo BE release from the supramolecular assemblies. The stomach and the intestine were sampled from the treated mice after intragastric administration of the supramolecular assemblies (containing 35 μM BE) for quantification of the released BE. ** indicates significant difference in released BE contents between the groups (P < 0.01).

from 5.3 to 10.4, referring to the disassembly of nanoparticle. However, when the pH returned to 5.3, the enhanced optical transmittance decreased to the original level indicating the regeneration of nanoparticle (Figure 3a), and this cycle could be repeated several times (Figure 3b). Similar phenomenon was also observed by TEM (Figure 3c–e). When the pH of SCD/chitosan-0.95 was adjusted to 10.4, no nanoparticles could be found in TEM images, but these nanoparticles reappeared when the pH was adjusted back to 5.3. These results demonstrated a good pH-responsive and reversible assembly/disassembly property of SCD/chitosan nanoparticles.

The zeta potentials of SCD&chitosan-0.6 nanoparticle and SCD&chitosan-0.95 nanoparticle were measured as 0.02 and −28.46 mV, respectively (Figure 4), indicating that SCD&chitosan-0.6 nanoparticle was nearly electroneutral but SCD&chitosan-0.95 nanoparticle was highly anionic. Therefore, we deduce that, possessing many negative charges at the outer space, the SCD&chitosan-0.95 nanoparticle may have the good ability of loading cationic substrates. Herein, berberine (BE), a representative bacteriostasis drug extracted from traditional Chinese herbs for treating diseases of the digestive tract,[27,28] was used as the model substrate. Through a comparison on the UV–vis absorbance spectra before and after loading BE as well as the UV–vis absorbance standard curve of BE (Figures S12–S15), the loading efficiency and encapsulation efficiency were calculated as 10 and 64% (Figure S16), respectively. In addition, the BE-loaded SCD&chitosan-0.95 nanoparticle was highly stable at room temperature (Figures S17 and S18), and nearly no changes of absorbance curve could be observed within at least 6 h. Additionally, the stabilities of such nanoparticle at pH 2 and the disassembly at pH 8 were investigated. Optical transmittance and TEM results demonstrated that such nanoparticles kept stable for >6 h at pH 2 but were disassembled at pH 8 (Figure S19). Meanwhile, with increasing pH from 2 (referring to an environmental pH of stomach) to 8 (referring to an environmental pH of intestine), the UV–vis absorbance intensity of loaded BE in SCD&chitosan-0.95 nanoparticle was obviously declined, and the release efficiency was calculated as (27.61 ± 1.78) % according to the standard curve of BE (Figure 5a and Figure S20). For further determination, such supramolecular assemblies uploaded with BE were administered into BALB/C mice (6-week old) for releasing BE from stomach to intestine. As expected, the release efficiency in stomach and intestine were calculated as (12.83 ± 1.51) % and (50.26 ± 8.21) %, respectively, indicating an efficient release from stomach to intestine (Figure 5b). These results indicated the good capability of the nanoparticles on loading and controlled release of berberine, especially from stomach to intestine.

In summary, some reversible supramolecular nanoparticles were constructed by two kinds of heterocharged saccharides, SCD and chitosan, and showed the good loading and pH-responsive release property toward berberine. These obtained nanoparticles could disassemble by increasing pH and recover by decreasing pH back. The interval of the varying pH to release berberine fits well with the difference of pH between stomach and intestine, which thus enables the application potential of supramolecular nanoparticles in precise drug release.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b08651.

Experimental section, tyndall experiments, UV–vis absorbance and transmittance data, SEM images (PDF)

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Notes

The authors declare no competing financial interest.

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