Supporting Information

for

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

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Experimental section

Materials Preparation. Chitosan with a deacetylation degree of 60% (MV 14000-220000) was purchased from Sigma-Aldrich. Chitosan with a deacetylation degree of 95% (MV 106) was purchased from Aladdin. Sulfato-â-cyclodextrin (SCD) was purchased from Shandong Binzhou Zhiyuan Bio-Technology Co. HCl, NaOH were all analytical agents and were purchased from the Tianjin Chemical Reagents company. Berberine chloride was purchased from Sigma-Aldrich. All of these were used without further purification.

Preparation of SCD/chitosan nanoparticles. Chitosan was dissolved in 1% (*v*/*v*) acetic acid, and a certain amount of concentrated sodium hydroxide solution was added until the pH of the solution reached 5.3. The pH was verified with a pH meter calibrated with two standard buffer solutions. Then a SCD solution was mixed in dropwise with the chitosan solution, and the mixture was kept for 1h to obtain the SCD/chitosan nanoparticle.

BE-loaded nanoparticles. BE-loaded nanoparticles were prepared as follows: A certain amount of BE was added to a solution containing chitosan, and then some SCD and water were added until the volume of the solution reached 25 mL. The ultimate concentration of BE, chitosan, and SCD for BE-loaded nanoparticles in the controlled release experiments were 0.035 mM, 10 ìg/mL, and 0.035 mM, respectively. Subsequently, the prepared BE-loaded nanoparticles were purified by dialysis

(molecular weight cut off 3500) in distilled water several times until the water outside the dialysis tube exhibited negligible BE fluorescence.

UV-Vis Spectroscopy. UV-Vis spectra and the optical transmittance of the aqueous solution (pH = 5.3) were measured in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

Fluorescence Spectroscopy. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell Peltier accessory to control temperature.

TEM experiments. High-resolution TEM images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

SEM Experiments. SEM images were recorded on a Hitachi S-3500N scanning electron microscope. The sample for SEM measurements was prepared by dropping the solution onto a coverslip, followed by evaporating the liquid in air.

DLS Measurements. The sample solution for DLS measurements was prepared by filtering solution through a 450 nm Millipore filter into a clean scintillation vial. The samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90°.

Zeta Potential Measurement. Zeta potential of the SC4A−protamine vesicle was measured by Zeta PALS + BI-90 instrument (Brookhaven Co. USA).

***In vivo* BE Release Assay.** To determine BE release from the supramolecular assemblies in the stomach and in the intestine, 2 mL of the supramolecular assemblies (containing 35 μM BE) were intragastrically administered into BALB/C mice (6-week old). After 20 min of administration, the mice were executed, and the stomach and the intestine were sampled. The sampled organs were cut and suspended in 5 mL of the distilled water, and then dialyzed using dialysis bags (molecular weight cutoff 3500 D) in 100 mL of water for 1 h at 37 °C with gentle shaking. OD_{345} of the solution outside the dialysis bags were determined using a UV/Vis spectrophotometer (SmartSpec Plus, Bio-Rad). The released BE in the stomach and in the intestine was quantified based on the standard curve.

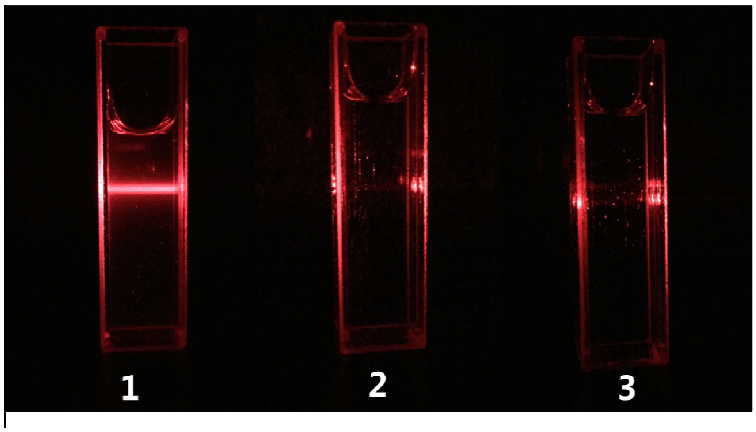

Figure S1. Tyndall effect of SCD/chitosan-0.95(1), free SCD(2), free chitosan-0.95(3) in aqueous solutions at pH 5.3. [SCD] = 0.035 mM, [chitosan-0.95] = 10μg/mL,.

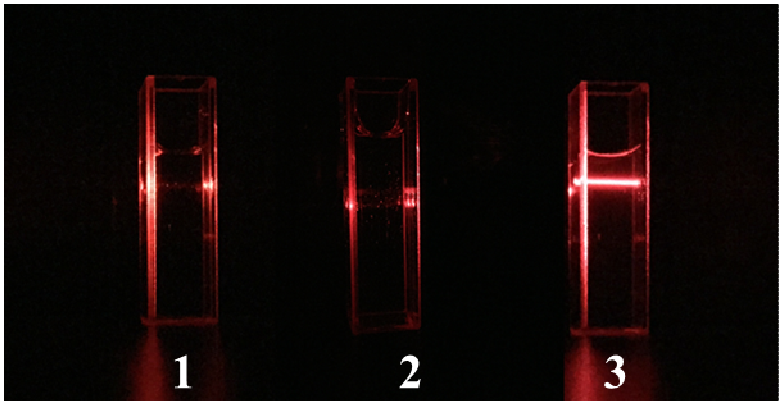

Figure S2. Tyndall effect of free SCD (1), free chitosan-0.6 (2), SCD/chitosan-0.6 (3) in aqueous solutions at pH 5.3. [SCD] = 0.030 mM, [chitosan-0.6] = 10μg/mL.

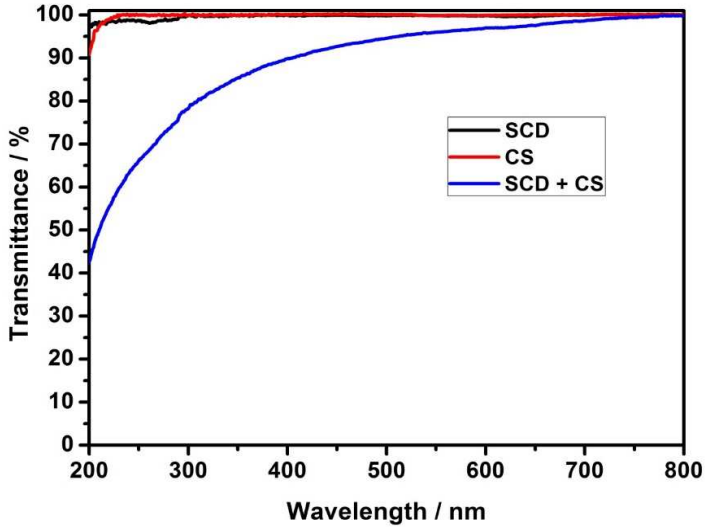

Figure S3. Optical transmmitance of SCD, chitosan-0.95 (CS), and SCD/chitosan-0.95 (SCD+CS) in aqueous solutions at 25°C. [SCD] = 0.035 mM, [chitosan-0.95] = 10μg/mL.

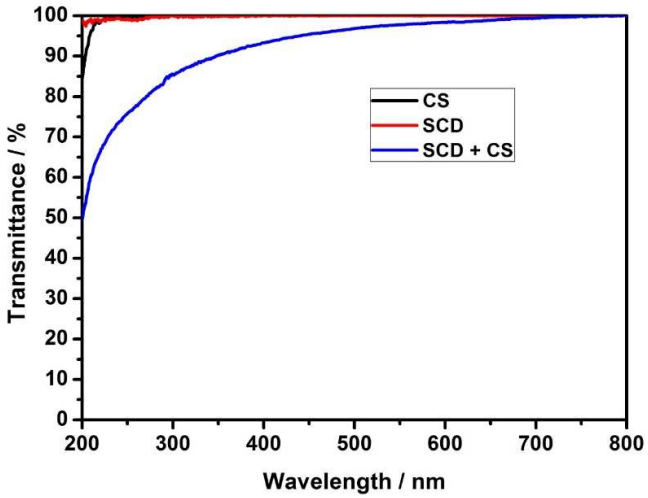

Figure S4. Optical transmmitance of SCD, chitosan-0.6 (CS), SCD/chitosan-0.6 (SCD+CS) in aqueous solutions at 25°C. [SCD] = 0.030 mM, [chitosan-0.6] = 10μg/mL.

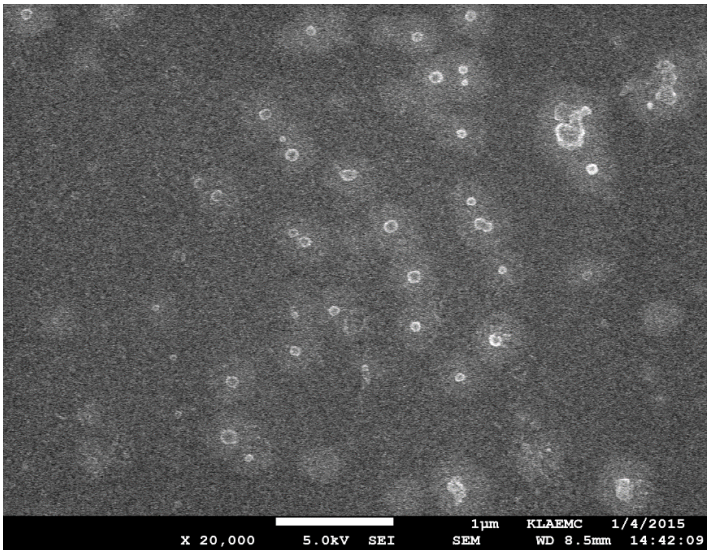

Figure S5. SEM image of SCD/chitosan-0.95 nanoparticles.

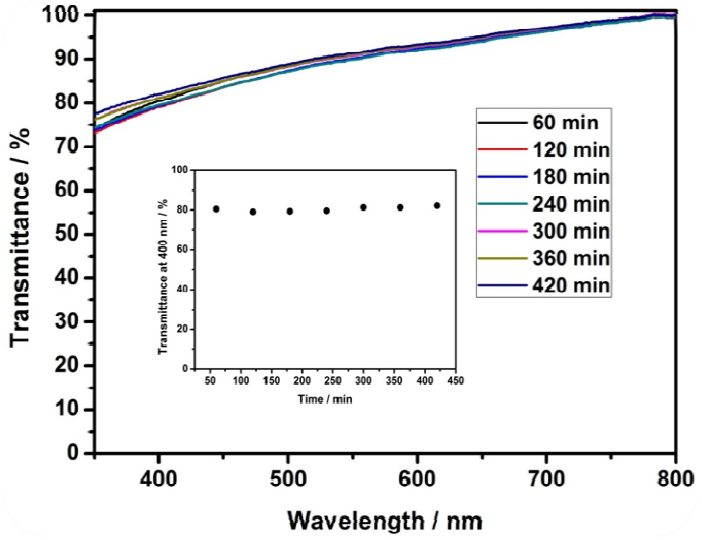

Figure S6. Optical transmittance of SCD/chitosan-0.95 nanoparticles at different time within 6 h at 25 °C in water. Inset: dependence of the optical transmittance at 400 nm on time. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL, pH 5.3.

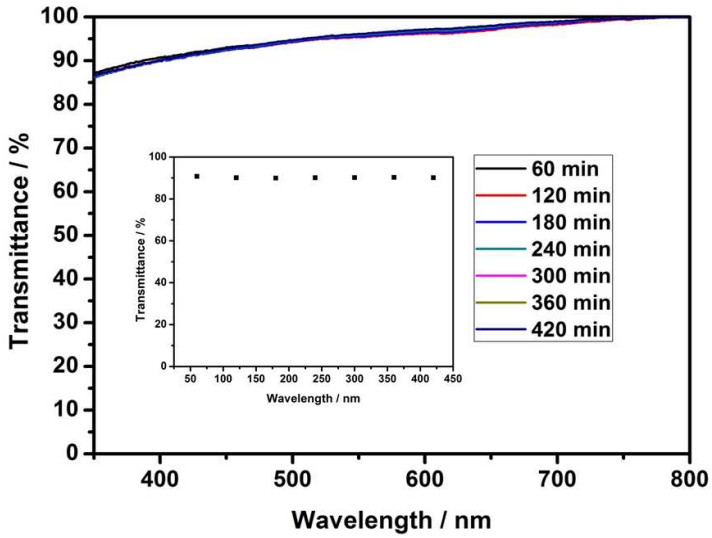

Figure S7. Optical transmittance of SCD/chitosan-0.6 nanoparticles at different time within 6 h at 25 °C in water. Inset: dependence of the optical transmittance at 400 nm on time. [SCD] = 0.030 mM, [chitosan-0.6] = 10 μg/mL, pH 5.3.

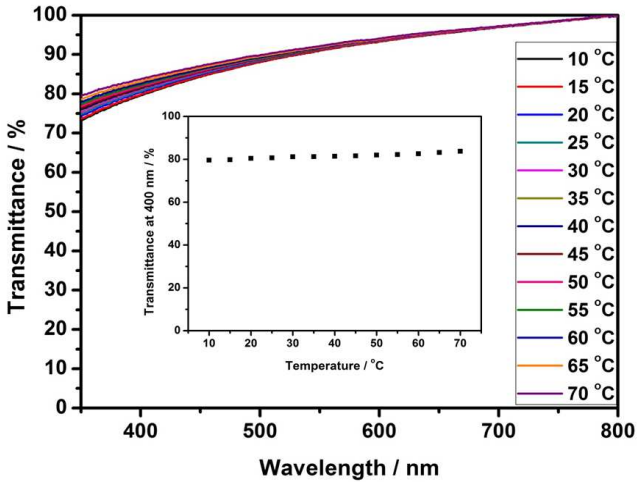

Figure S8. Optical transmittance of SCD/chitosan-0.95 nanoparticles at different temperatures from 10°C to 70°C in water. Inset: dependence of the optical transmittance at 400 nm on temperature. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL, pH 5.3.

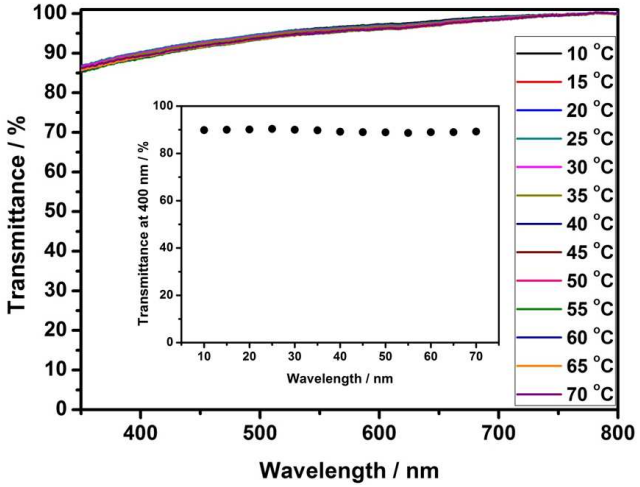

Figure S9. Optical transmittance of SCD/chitosan-0.6 nanoparticles at different temperatures from 10°C to 70°C in water. Inset: dependence of the optical transmittance at 400 nm on temperature. [SCD] = 0.030 mM, [chitosan-0.6] = 10 μg/mL, pH 5.3.

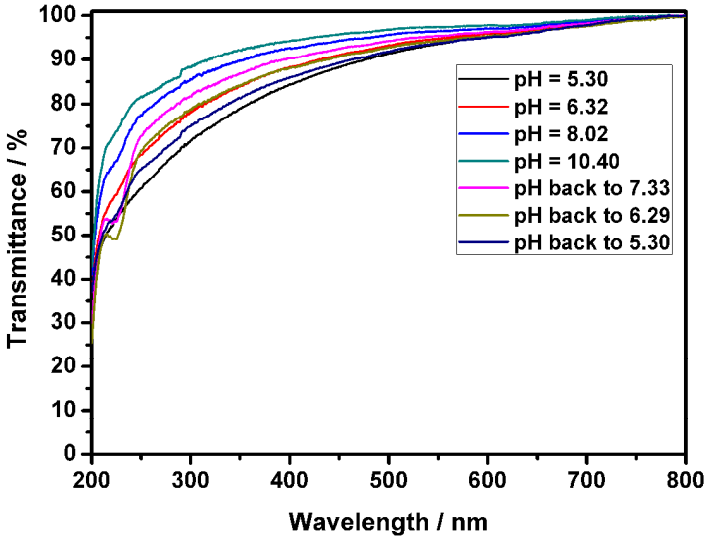

Figure S10. Optical transmittance of SCD/chitosan-0.95 nanoparticles at a cyclic variational pH between 5.3 and 10.4 at 25°C in water. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL.

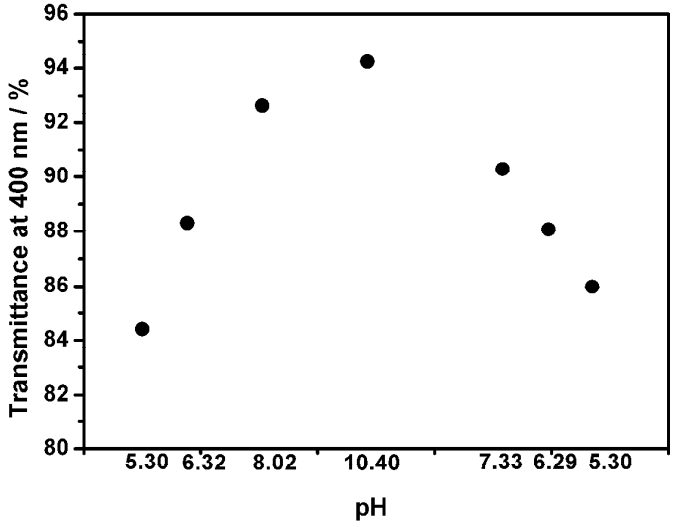

Figure S11. Dependence of the optical transmittance at 400 nm on a cyclic variational pH between 5.3 and 10.4 at 25°C in water. [SCD] =0.035 mM, [chitosan-0.95] = 10 μg/mL.

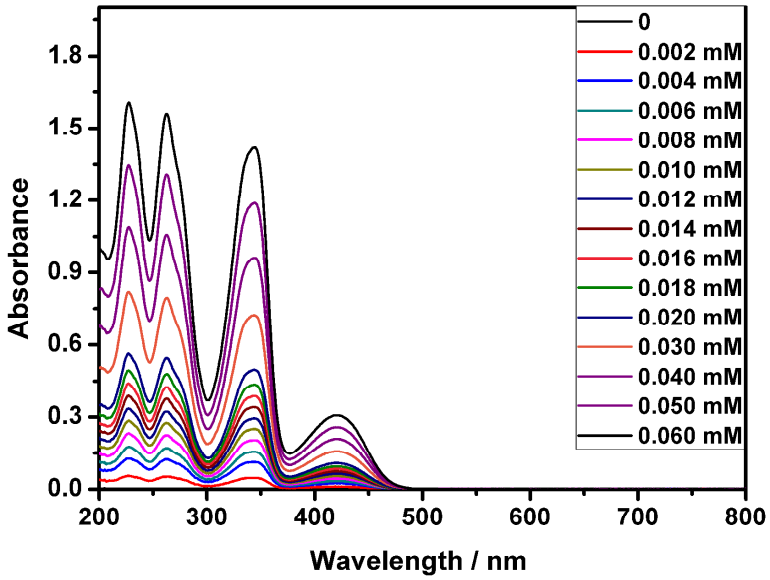

Figure S12. UV-Vis spectra of berberine chloride (BE) of different concentrations at 25°C in water, pH 5.3.

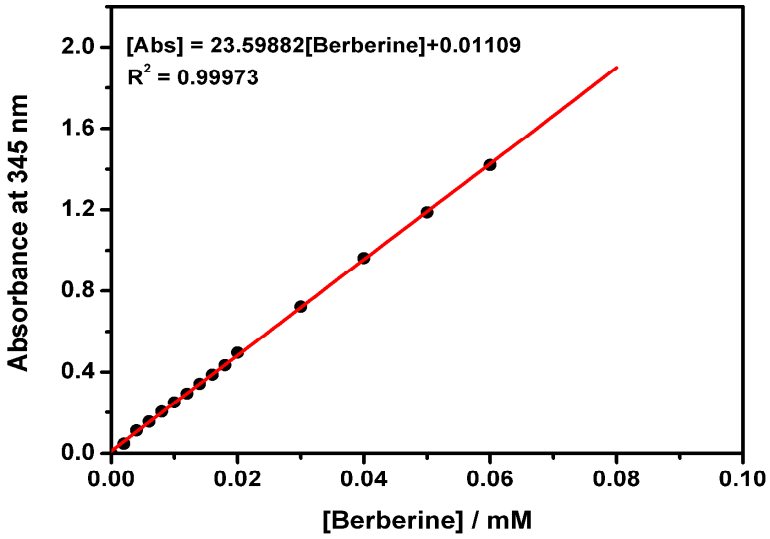

Figure S13. Standard curve of BE depending on absorbance at 345 nm of BE of different concentrations at 25°C in water, pH 5.3.

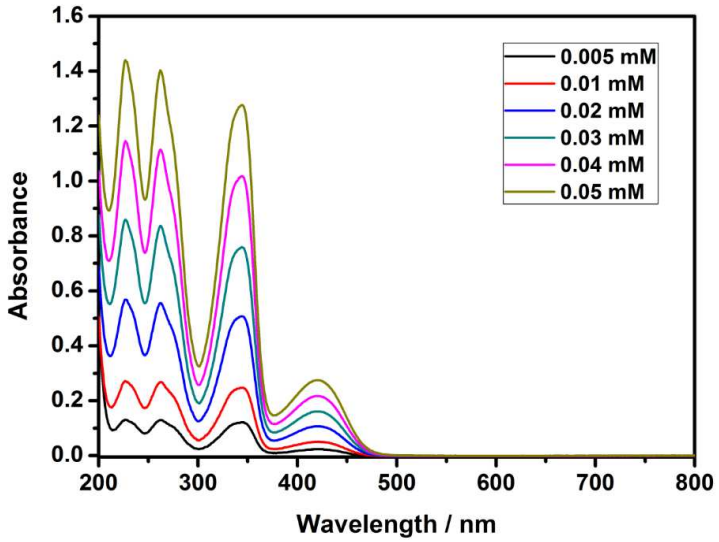

Figure S14. UV-Vis spectra of berberine chloride (BE) of different concentrations at 25°C in water, pH 10.4.

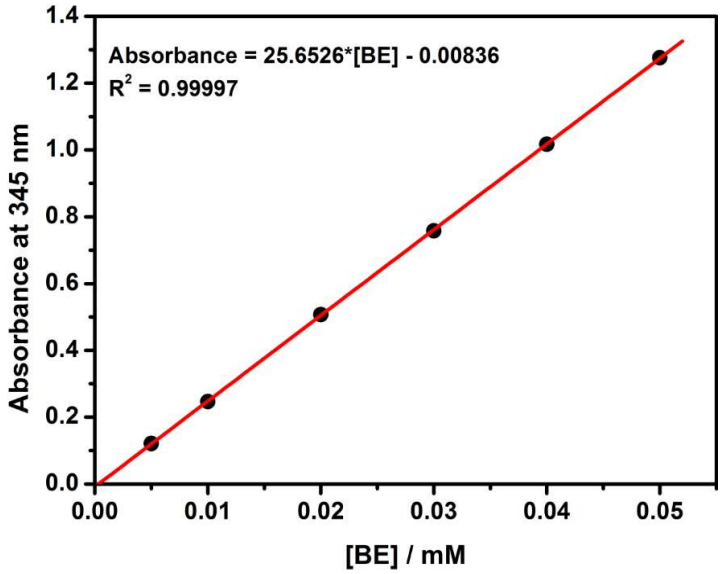

Figure S15. Standard curve of BE depending on absorbance at 345 nm of BE of different concentrations at 25°C in water, pH 10.4.

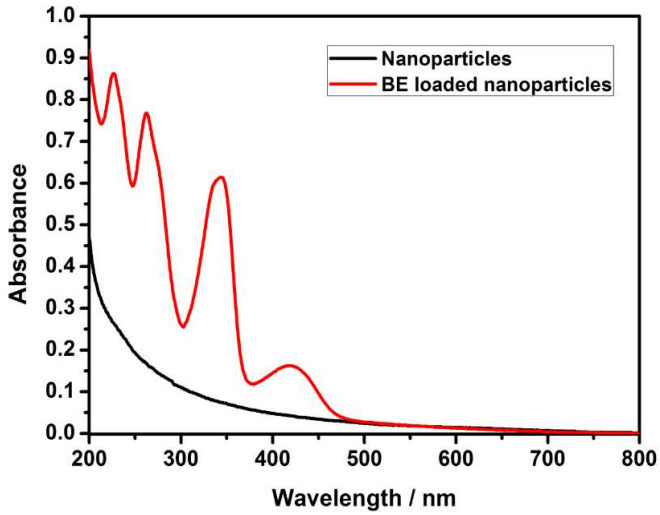

Figure S16. UV-Vis spectra of SCD/chitosan-0.95 nanoparticles and berberine chloride (BE) loaded nanoparticles at 25°C in water, pH 5.3. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL.

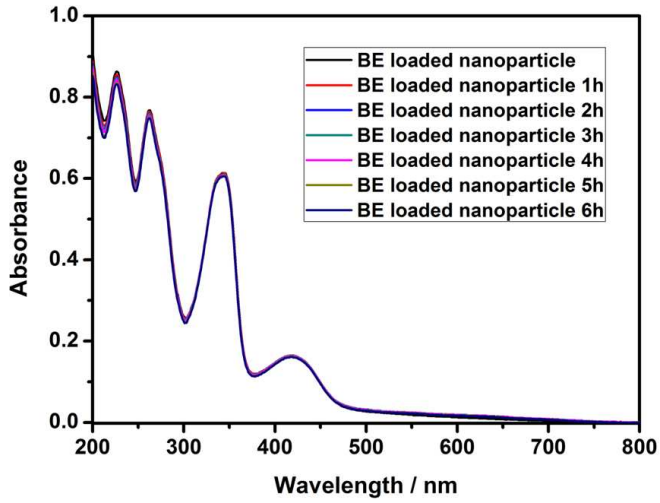

Figure S17. UV-Vis spectra of BE loaded SCD/chitosan-0.95 nanoparticles at different time within 6 h at 25 °C in water. Inset: dependence of the optical transmittance at 400 nm on time. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL, pH 5.3.

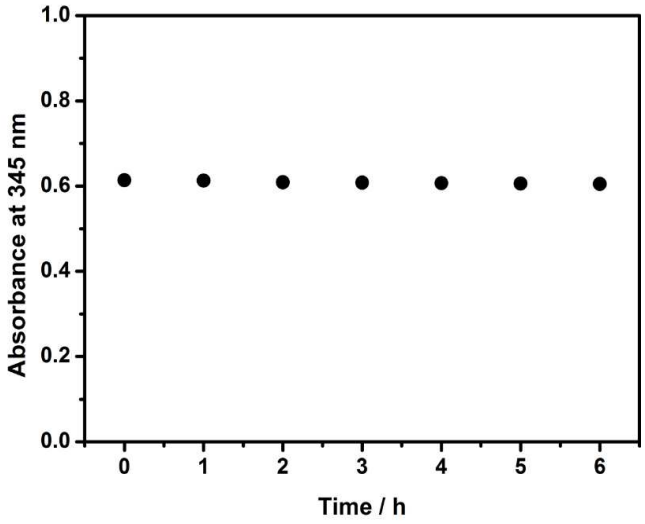

Figure S18. Dependence of UV-Vis spectra at 345 nm on time at 25°C in water, pH 5.3. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL.

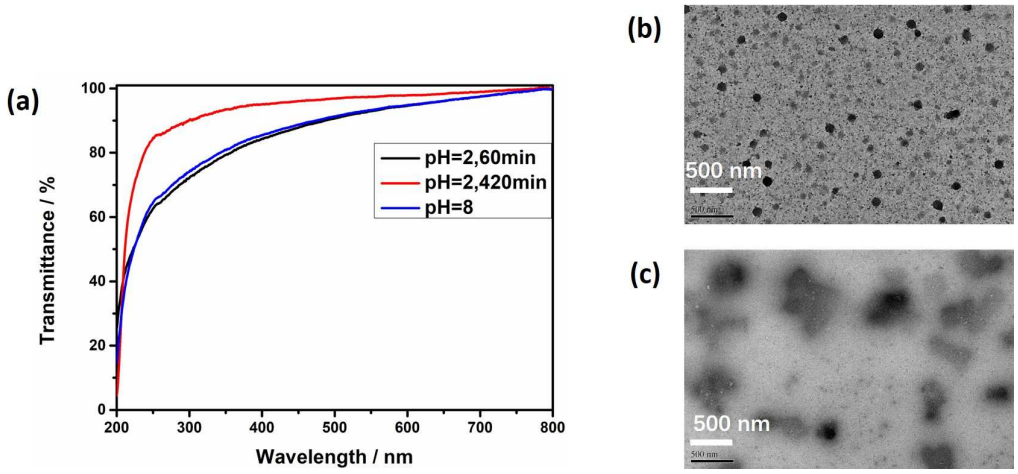

Figure S19. (a) Optical transmittance of SCD/chitosan-0.95 nanoparticles in pH = 2 for 1 h and 7 h and disassembled in pH = 8; (b, c) TEM images of SCD/chitosan-0.95 nanoparticles in pH = 2 (b) and disassembled in pH = 8 (c). [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL.

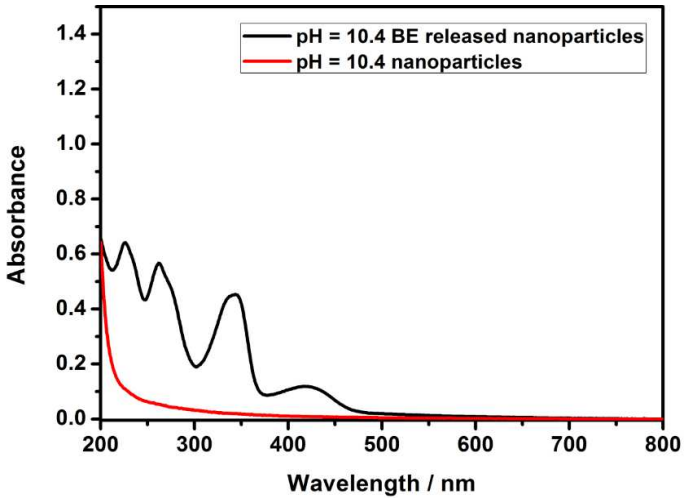

Figure S20. UV-Vis spectra of disassembled SCD/chitosan-0.95 nanoparticles and berberine chloride (BE) released nanoparticles at 25°C in water, pH 10.4. [SCD] = 0.035 mM, [chitosan-0.95] = 10 μg/mL.