Supporting information

Self-assembly amphiphilic polysaccharide-based co-delivery system for egg white derived peptides and curcumin with oral bioavailability enhancement

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Methods

Transmission Electron Microscopy (TEM). TEM images were conducted with a Tecnai Spirit electron microscope (FEI, Netherlands). Each of sample solution was dropped onto a copper wire mesh and negatively stained by phosphotungstic acid in advance[1].

Atomic Force Microscopy (AFM). The AFM observation for samples was performed using a Veeco Nanoscope V Multimode 8 scanning probe microscope under the ambient conditions (25 ℃, relative humidity of 25%). Prior to AFM scanning, 10 μL of each sample was dropped on the smooth mica sheets carefully and air-dried for 4 h.

X-ray Photoelectron Spectroscopy (XPS). The surface elemental composition of samples was detected by a Thermo ESCALAB 250Xi spectrometer (Waltham, MA) using an Al Kα X-ray excitation source. The high-resolution spectra were obtained at a 30.00 eV pass energy with a step size of 0.050 eV[2].

Fourier Transform Infrared Spectroscopy (FTIR). FTIR was performed to explore the interaction mechanism within the NPs. Different samples were freeze-dried in advance and then made into tablets at a specific mass ratio (sample:potassium bromide = 1:100). The corresponding spectra were recorded at a resolution of 4 cm^{-1} over the 4000-400 cm^{-1} range.

^{1}H NMR. Lyophilized samples were fully dissolved in deuterated dimethyl sulfoxide (DMSOd6) to reach a concentration of 5 mg/mL. Then, the ^{1}H NMR spectra was determined by a Bruker Advance III 500 MHz spectrometer (Billerica, MA). The chemical shifts were reported in ppm, respectively[3].

X-ray Diffraction (XRD). To better illustrate the co-encapsulation mechanism of EWDP and curcumin in NPs, freeze-dried samples were detected by an X-ray diffractometer (Bruker, Germany) with 40 kV accelerating voltage and 40 mA tube current. The 2θ angel range was set as 5-50° with a scanning rate of 0.24°/min[4].

Differential Scanning Calorimetry (DSC). DSC was performed to investigate the thermal properties and crystallinity of NPs. Samples were heated from 30 to 230 ℃ (10 ℃/min) in the hermetically sealed aluminum pans with a nitrogen flow of 20 mL/min[5].

Cytotoxicity Assay Caco-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% nonessential amino acid and 1% penicillin-

streptomycin. The cells (90 μL) were seeded in the 96-well plates with a density of 8000 cells per well, respectively. After overnight incubation, the cells were exposed to the samples (10 μL) diluted in phosphate buffered saline (PBS) with different EWDP/curcumin concentrations for 24 h. Afterwards, 20 μL MTS solution was loaded to each well for 2 h (37 ℃, 5% CO_2), and then the UV absorbance at 490 nm was recorded by a microplate reader (BioTek, Winooski, VT) to calculate the corresponding cell viability[6].

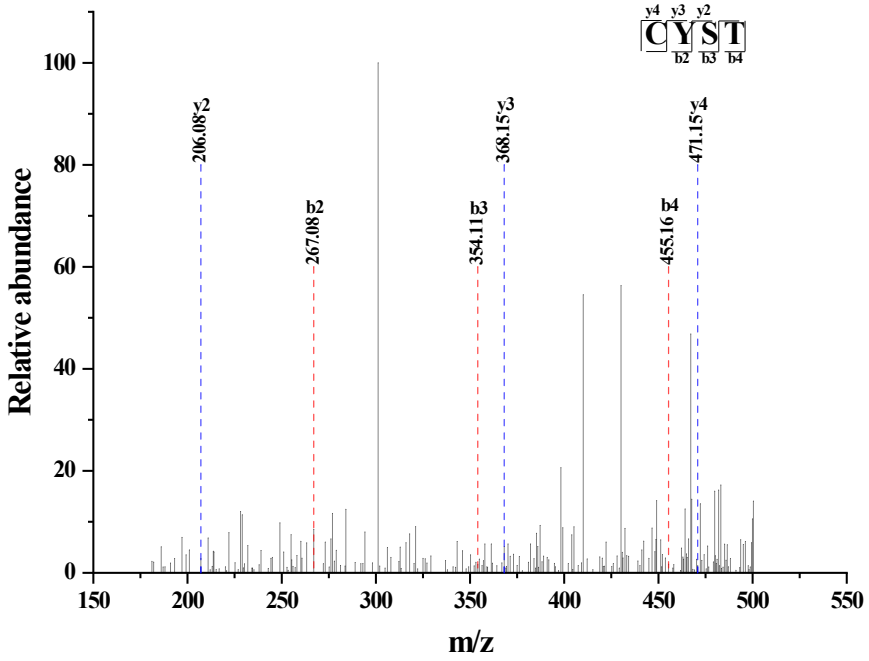

Fig. S1 The mass spectrum of EWDP (CYST). The corresponding b and y ions were shown in the spectrum.

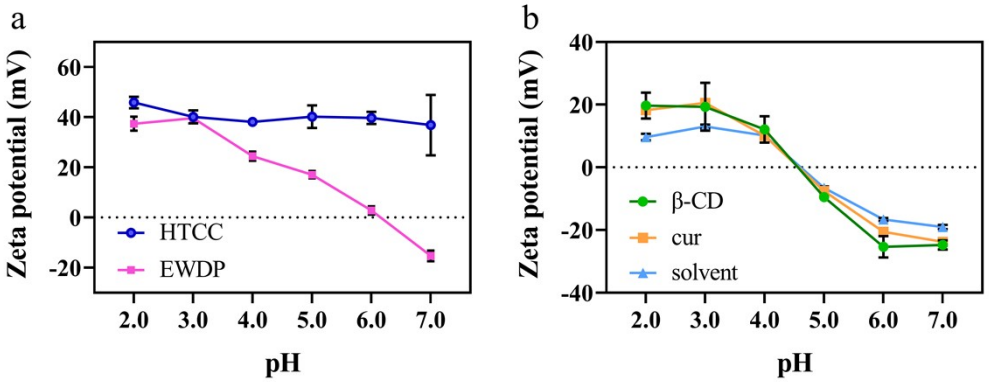

Fig. S2 Zeta potential of native core-shell materials and solvent under various pH values (2.0-7.0).

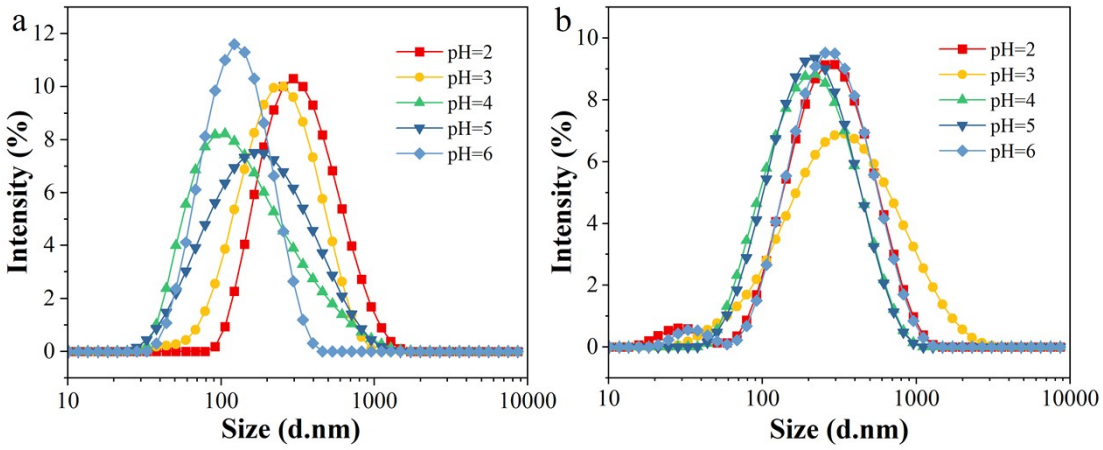

Fig. S3 The size distribution of samples under different pH values (2.0-6.0). (a) HTCC-β-CD NPs. (b) HTCC-EWDP-β-CD-cur NPs.

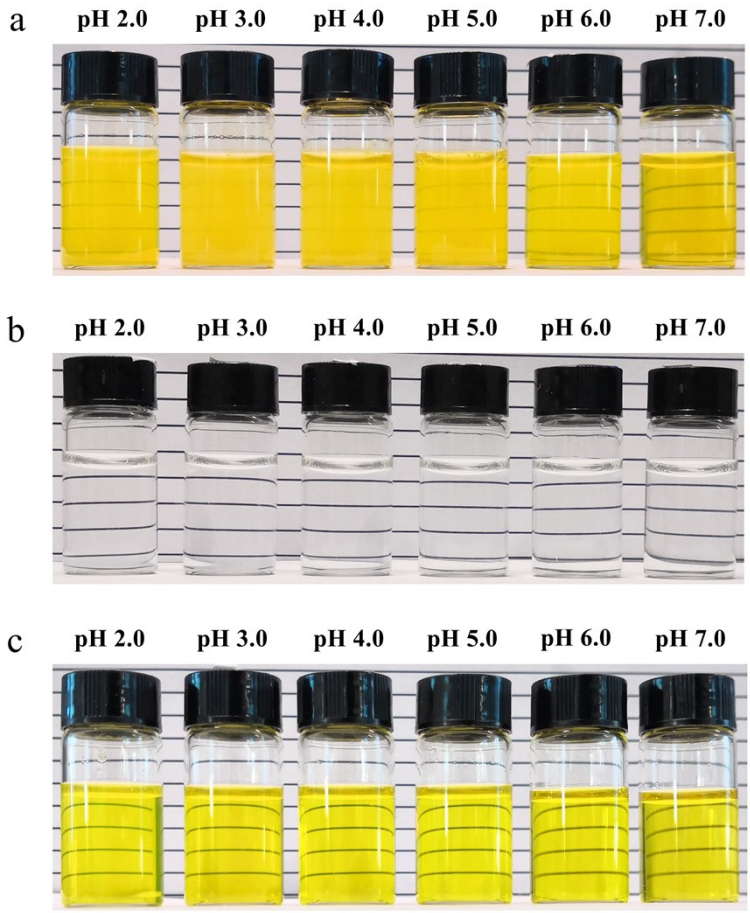

Fig. S4 Images of samples under different pH values (2.0-7.0). (a) The simple mixture of EWDP and curcumin. (b) HTCC-β-CD NPs. (c) HTCC-EWDP-β-CD-cur NPs. The concentration of EWDP and curcumin for different samples was 0.5 mg/mL and 0.05 mg/mL.

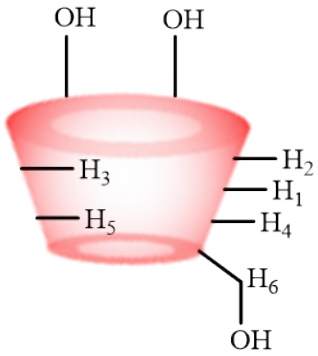

Fig. S5 Schematic illustration of different protons distribution within the β-CD molecule.

Table S1 ^{1}H NMR analysis of β-CD protons' chemical shifts within the composite NPs after encapsulation

Protons	$\Delta\delta_{HTCC\text{-}\beta\text{-}CD}$	$\Delta\delta_{\beta\text{-}CD\text{-}cur}$	$\Delta\delta_{HTCC\text{-}\beta\text{-}CD\text{-}cur}$	$\Delta\delta_{HTCC\text{-}EWDP\text{-}\beta\text{-}CD\text{-}cur}$
H1	-0.001	-0.003	-0.004	-0.020
H2	-0.001	-0.004	-0.004	-0.021
H3	0.000	-0.009	-0.009	-0.314
H4	0.003	0.000	-0.001	-0.002
H5	0.000	-0.006	-0.007	-0.024
H6	0.002	-0.005	-0.007	-0.020

$\Delta\delta = \delta_{\text{the corresponding NPs}} - \delta_{\beta\text{-CD}}$.

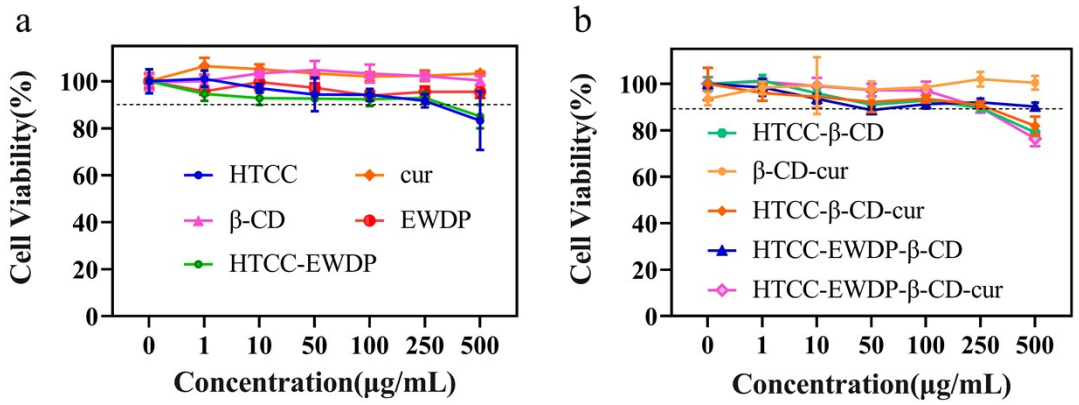

Fig. S6 Cytotoxicity evaluation for different samples. All the samples showed no significant cytotoxicity on Caco-2 cell after incubation for 24 h (cell viability ≥ 90%).

References

1 M. Ma, T. Sun, P. Xing, Z. Li, S. Li, J. Su, X. Chu and A. Hao, A supramolecular curcumin vesicle and its application in controlling curcumin release, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2014, **459**, 157-165.

2 X. Hu, Y. Wang, L. Zhang and M. Xu, Construction of self-assembled polyelectrolyte complex hydrogel based on oppositely charged polysaccharides for sustained delivery of green tea polyphenols, *Food Chem.*, 2020, **306**, 125632.

3 S. Goswami and M. Sarkar, Fluorescence, FTIR and 1H NMR studies of the inclusion complexes of the painkiller lornoxicam with β-, γ-cyclodextrins and their hydroxy propyl derivatives in aqueous solutions at different pHs and in the solid state, *New J. Chem.*, 2018, **42**, 15146-15156.

4 S. Feng, Y. Sun, D. Wang, P. Sun and P. Shao, Effect of adjusting pH and chondroitin sulfate on the formation of curcumin-zein nanoparticles: Synthesis, characterization and morphology, *Carbohydr. Polym.*, 2020, **250**, 116970.

5 Y. Ren, L. Huang, Y. Wang, L. Mei, R. Fan, M. He, C. Wang, A. Tong, H. Chen and G. Guo, Stereocomplexed electrospun nanofibers containing poly (lactic acid) modified quaternized chitosan for wound healing, *Carbohydr. Polym.*, 2020, **247**, 116754.

6 J. Xiao, S. Nian and Q. Huang, Assembly of kafirin/carboxymethyl chitosan nanoparticles to enhance the cellular uptake of curcumin, *Food Hydrocolloids*, 2015, **51**, 166-175.