Electronic Supplementary Material (ESI) for Soft Matter.

pH-Driven encapsulation of curcumin in self-assembled casein nanoparticles for enhanced dispersibility and bioactivity

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Supplementary information

Methods

Differential Scanning Calorimetry (DSC)

The crystallinity of lyophilized samples, sodium caseinate (NaCas) and prestine curcumin powder was characterized using a model Q2000 calorimeter (TA Instrument, New Castle, DE, USA). Ten mg of powder was sealed in hermetic aluminum pans and heated from 30 to 250 °C at a rate of 5 °C/min. Nitrogen was used as the transfer gas at a flow rate of 50 mL/min. The instrument was calibrated using Indium under the same conditions as the samples.

UV-vis Spectroscopy

Curcumin was extracted from a fresh dispersion using chloroform as previously described and diluted to 4.5 μg/mL in chloroform. Free curcumin sample was prepared by directly dissolving the curcumin powder in chloroform at 4.5 μg/mL. The curcumin samples were scanned from 250 to 600 nm at 21 °C using a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA).

Particle size stability at physiological conditions

The dispersion prepared with an initial curcumin concentration of 1.0 mg/mL was diluted 10 times in 10 mM phosphate-buffered-saline (PBS, pH 7.4) and incubated at 37 °C on a temperature controlled magnetic stir plate (Fisher Scientific, Pittsburgh, PA, USA) operating at a stirring speed of 100 rpm. After incubation for 0, 24, and 48 h, the sample was diluted 2 times using deionized water, and the hydrodynamic diameter was measured (Delsa Nano analyzer, Beckman Coulter, Atlanta, GA, USA).

In vitro *release profile*

The *in vitro* release profile of curcumin from NaCas nanoparticles was determined in PBS using a dialysis bag method[1]. Briefly, free curcumin pre-dissolved in dimethyl sulfoxide

(DMSO) or the curcumin dispersions prepared with various initial curcumin concentrations were diluted to the same concentration (100 μg/mL) using deionized water. Five mL of the diluted sample was placed in a dialysis tubing with a molecular-weight–cut-off of 14 kDa (Sigma-Aldrich Corp., St. Louis, MO, USA). The dialysis bag was then placed in a beaker containing 100 mL of 10 mM PBS at pH 7.4 with 0.5% v/v Tween® 20. The entire beaker was maintained at 37 ºC on a temperature controlled magnetic stir plate with mild stirring for 10 h. Two mL of a sample was collected periodically and 2 mL of the PBS with Tween® 20 was replenished to the beaker to maintain a total volume of 100 mL. The sampled PBS with released curcumin was measured for absorbance at 419 nm and was determined for curcumin concentration based on a standard curve with various concentrations of curcumin dissolved in the same medium.

Results and discussion

The DSC thermograms are presented in Figure S1. Pristine curcumin showed a sharp peak at 177 °C, indicating its highly crystalline structure. The thermograms of NaCas and encapsulated curcumin were similar. The absence of the endothermic peak after encapsulation indicates the amorphous structure of curcumin in casein capsules.

When the UV-vis spectrum of free curcumin was compared to that extracted from dispersion (Figure S2), there was no detectable difference. The results support the observations from NMR, indicating the pH treatments at the encapsulation conditions did not cause chemical structural changes of curcumin.

The curcumin dispersion was stable during 48-h incubation in PBS at 37 ˚C (Table S1). During dialysis against PBS with 0.5% Tween® 20, the release of curcumin followed mostly the first-order kinetics (Figure S3). The release rate was smaller at a lower content of curcumin, and the sample prepared with an initial curcumin concentration of 2 mg/mL showed the similar release profile as free curcumin pre-dissolved in DMSO. Tween® 20 is well-established for its ability to replace caseins encapsulating lipids (emulsion droplets).[2] When a higher amount of curcumin is encapsulated in the same mass of caseins, the amount of casein is lower after diluting to the same curcumin concentration, and therefore, the replacement of caseins by Tween® 20 may be faster. The end result is the faster dissolution of curcumin in Tween® 20 micelles that enabled the diffusion through the dialysis tubing, as indicated by the faster release rate in Figure S3.

Table S1. The hydrodynamic diameter of curcumin dispersion after incubation at pH 7.4 and 37 °C for 0-48 h.

Time (h)	Hydrodynamic diameter (nm)*
0	163.75 ± 8.15[a]
24	171.75 ± 13.08[a]
48	177.21 ± 16.11[a]

*Numbers are mean ± standard deviation (n = 3). Different superscript letters indicate significant differences ($P < 0.05$).

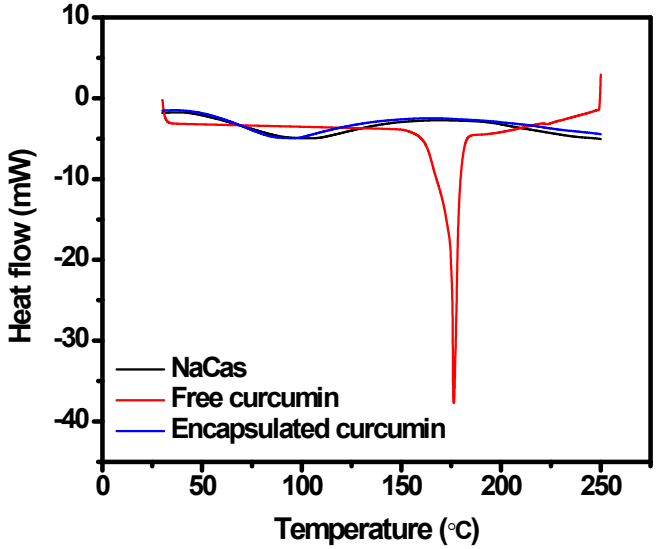

Figure S1. DSC thermograms of the sodium caseinate (NaCas, black), curcumin crystals (red), and curcumin encapsulated in NaCas (blue) during heating from 30 to 250 °C at a rate of 5 °C/min. The NaCas sample was prepared at conditions identical to encapsulation and lyophilized for DSC.

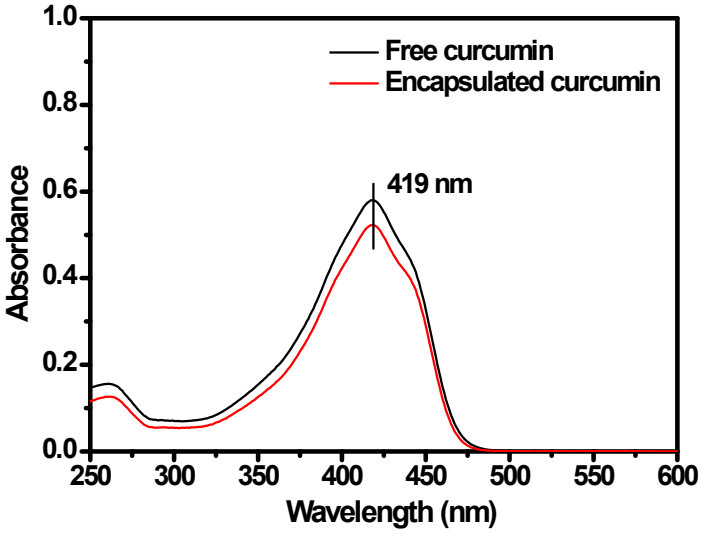

Figure S2. UV-Vis absorption spectra of 4.5 μg/mL free curcumin (directly dissolved in chloroform, black) and that extracted from casein nanocapsules using chloroform (red).

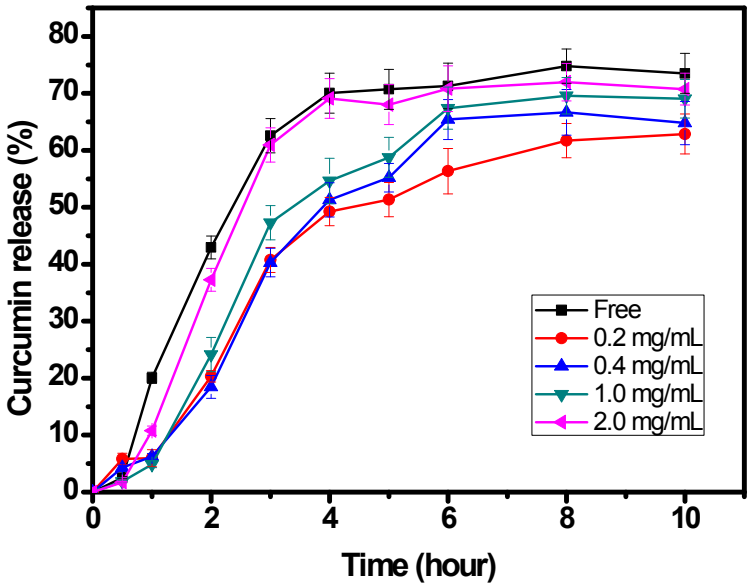

Figure S3. Release profile of free curcumin pre-dissolved in DMSO or curcumin encapsulated in caseins at different initial concentrations at pH 7.4 and 37 °C in PBS with 0.5% v/v Tween® 20. Error bars are standard deviations (n = 3).

References

1. Y. C. Luo, B. C. Zhang, M. Whent, L. L. Yu and Q. Wang, *Colloid Surf. B-Biointerfaces*, 2011, **85**, 145.

2. E. Dickinson, C. Ritzoulis, and M.J. Povey (1999). *J. Colloid Interface Sci,* 1999, **212**, 466.