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Supporting Information

A simple route to renewable high internal phase emulsions (HIPEs) strengthened by successive cross-linking and electrostatics of polysaccharides

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1. Materials and methods

1.1 Materials

Chitosan of medium molecular weight (Mw = 190–310 kDa, 75–85% degree of deacetylation, viscosity 200–800 cP) and pectin powder, from citrus peel, were purchased from Sigma-Aldrich (St. Louis, MO, US). 100% pure corn oil (Mazola, ACH Food Companies, Inc., Cordova, TN) was purchased from a local supermarket. Glutathione (GSH) and β-carotene (type I, synthetic, >93% purity) were purchased from Sigma-Aldrich (St. Louis, MO, US). All other reagents used were analytical grade.

1.2 Preparation of oil-filled polysaccharide microspheres by ultrasonication

Polysaccharide microspheres were synthesized based on a previous method with a slight modification.[1] Briefly, chitosan was dissolved in acetic acid solution under magnetic stirring. The solution was filtered (filter pore size 50 μm) to remove insoluble components and the pH was adjusted to 2.0 using hydrochloric acid. Pectin was dissolved separately in distilled water and the pH adjusted to 2.0. The concentration of each single polysaccharide ranged from 0.5 to 1.5 wt% (w/w). 4 mL of chitosan and 4 mL of pectin solution were mixed and over-layered with 4 mL of corn oil, followed by exposure to high-intensity ultrasound using a 750 Watt ultrasonic processor with a high powered sonic tip operated at 20 kHz frequency (VC 750, Sonics vibra-cell, Sonics & Materials, Newtown, CT, USA). The bottom of a 13 mm diameter ultrasonic horn was immersed at the oil/water interface and the systems were sonicated in an ice bath for 10 min at different amplitudes (20%, 30%, 40% and 50%) corresponding to acoustic powers of 150, 225, 300, and 375 W cm^{-2} (5 s on, 2 s off).

To utilize non-cross-linked polysaccharides, we switched the pH of the sonicated microsphere suspension to high values (3-5) before further centrifugation.

1.3 Preparation of HIPEs

The freshly prepared polysaccharide microspheres were then transferred into a 50 mL Falcon tube and centrifuged at 10000 *g* for 5 min. A rigid cream layer, i.e. the HIPE, would form on top of the aqueous phase after centrifugation. The aqueous phase was also collected for calculating the internal phase volume fraction of the HIPEs. For comparison, conventional HIPEs were prepared by a standard homogenization emulsification method.[2] The required volume of polysaccharides and corn oil was homogenized with a T25 digital Ultra Turrax (IKA-Werke, Wilmington, NC) at 13000 rpm. The corn oil was then added dropwise, continuously, during homogenization until the desired internal oil fraction was achieved. To produce porous materials, cyclohexane was used instead of corn oil to prepare the HIPE templates.

1.4 Morphological characterization

To perform scanning electron microscopy (SEM), HIPEs were placed onto a silicon wafer, freeze-dried, and sputtered with gold. The porous structure of the freeze-dried HIPEs was observed on a LEO 1550 SEM equipped with a GEMINI field emission column. Confocal imaging of fresh HIPEs was carried out on a Zeiss confocal microscope (LSM 710, Carl Zeiss, Göttingen, Germany). The structural morphology was studied by directly adding Nile red into the obtained HIPEs. Excitation/emission wavelengths for Nile red was 488/566 nm, respectively.

1.5 FTIR spectra

Fourier-transform infrared (FTIR) spectra of pure chitosan, pectin and chiitosan/pectin freeze-dired foam were recorded on an IRAffinity-1S spectrometer equipped with a single-reflection ATR accessory (Shimadzu Corp., Kyoto, Japan). The FTIR spectra were scanned between 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

1.6 Thermogravimetric analysis (TGA)

The TGA curves were obtained from a thermogravimetric analyzer (TGA Q500, TA Instruments, New Castle, DE, USA). The measurements were conducted under nitrogen gas at a flow rate of 60 mL min^{-1}. Approximately 5 mg of the sample was loaded onto the platinum pan and heated from 20 to 600 °C, at a rate of 10 °C min^{-1}. The thermogravimetric curve was plotted as the first derivative of mass loss percent (%) over temperature (°C) *vs*. heating temperature (°C). The first derivative curve was plotted using the Universal Analysis 2000 software (Version 4.5A, TA instruments, New Castle, DE, USA.

1.7 X-ray photoelectron spectroscopy (XPS)

XPS was carried out to investigate the chemical state of the polysaccharides using a Surface Science Instruments SSX-100 XPS system at an operating pressure of ~2× 10^{-9} Torr.

1.8 Rheology measurements

Dynamic rheological measurements were performed on an AR1000-N controlled stress rheometer (TA Instruments, Inc., Ghent) using the plate geometry. A range of experiments, including amplitude (stress = 0.1–1000 Pa, frequency = 1 Hz), and frequency sweeps (0.1-10 Hz, strain=1%) were carried out at 25 °C. The elastic modulus (G') and loss modulus (G'') were recorded using the RheoWin 3 Data Manager. Viscosity was measured at a range of shear rates of 0–10 s^{-1} at 25 °C.

1.9 Determination of adsorbed polysaccharides on the microspheres.

The amount of non-crosslinked polysaccharides after sonication was determined according to the phenol-sulfuric acid method, as described previously.[3] The microsphere suspension was centrifuged at 15,000 *g* for 20 min. A 30 µL aliquot of the supernatant was taken and placed in a tube. Then, 0.16 mL of freshly prepared 5% (v/v) phenol aqueous solution and 0.8 mL of concentrated sulfuric acid was added. After 20 min incubation at room temperature, the absorbance was recorded at 490 nm with a UV-2600 spectrophotometer (Shimadzu, Japan). Distilled water

was used as a blank. The standard curve of polysaccharide was obstained through poysaccharide in a concentration gradient (y = 1.7762 x + 0.064, R^2=0.9877), where y and x correspond to absorbance and polysachcaride concentration (%, w/w), respectively.

1.10 Dynamic light scattering

Average particle size and zeta potential were carried out based on dynamic light scattering techinque using a commercial zeta-sizer (Nano-ZS90, Malvern Instruments Ltd., UK) with a He/Ne laser (λ = 633 nm) and scattering angle of 90º. Before measurements, aliquots of samples were diluted with the same buffer solution to avoid multiple scattering phenomena due to particle interaction.[4] The measurements were repeated in triplicate and reported as averages.

1.11 In Vitro Release of β -carotenoid from HIPEs

To evaluate the potential of our HIPEs as a nutraceutical/drug delivery system, we prepared β-carotenoid-loaded HIPEs and evaluated the release of β -carotenoid at pH 6.2 with and without the presence of GSH at 10 mM. Initially, 0.06 g β-carotenoid was dissolved in 70 mL corn oil under magnetic stirring. The HIPEs loaded with β-carotenoid were prepared at pH 2 and 5 respectively.

The release of β-carotenoid from HIPEs was conducted based on the membrane-free model with a light modification of a previously described method.[5] Briefly, 0.75 g HIPEs were placed on the bottom of glass vials, followed by the careful addition of 20 mL pH 6.2 buffer with or without GSH (10 mM), and then shaken slowly in a water bath at 37 ± 0.2 ˚C. Because the HIPEs were less dense than water, they floated to the surface of the fluid. At specific time intervals, an aliquot of fluid below the HIPEs was withdrawn and replaced by the same volume of fresh medium. The amount of carotenoid was measured at 450 nm by a UV-2600 spectrophotometer (Shimadzu, Japan). The release rate was calculated as the carotenoid in the collected medium, at a given time,

divided by the initial carotenoid in the HIPEs, and multiplied by 100. Each experiment was run in triplicate, and the data was reported as average values.

Table

Table S1. Average particle size, zeta potential and adsorbed polysaccharides from microspheres prepared at different pHs.

Microspheres	Average particle size (nm)	Zeta potential (mV)	Adsorbed polysaccharides (%)
pH 2	994.3 ± 12.0	39.7 ± 0.7	41 ± 2.4
pH 3	1038.3 ± 47.4	37.3 ± 0.7	49 ± 1.7
pH 4	1156.7 ± 61.6	31.1 ± 1.2	56 ± 2.2
pH 5	1279.7 ± 73.2	25.4 ± 1.4	68 ± 2.7

Figures

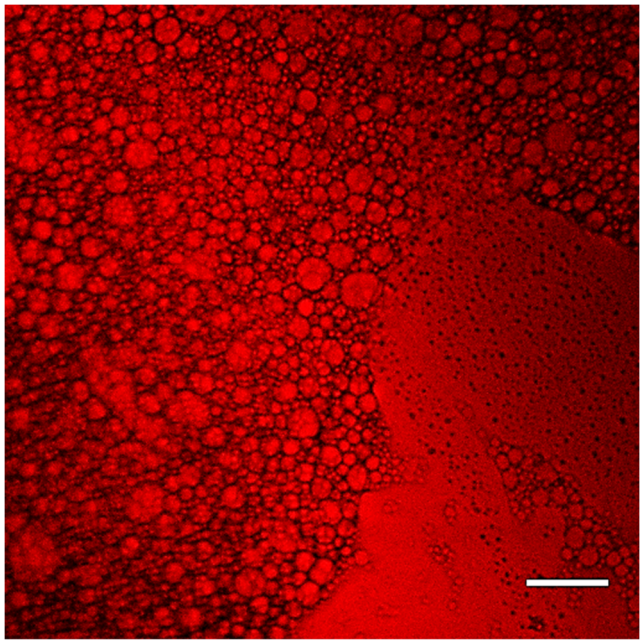

Fig. S1 CLSM image from the cream layer of polysaccharide/corn oil mixture prepared by homogenizing chitosan + pectin + corn oil after centrifugation at 10000 *g* for 5 min. The scale bar is 5 μm.

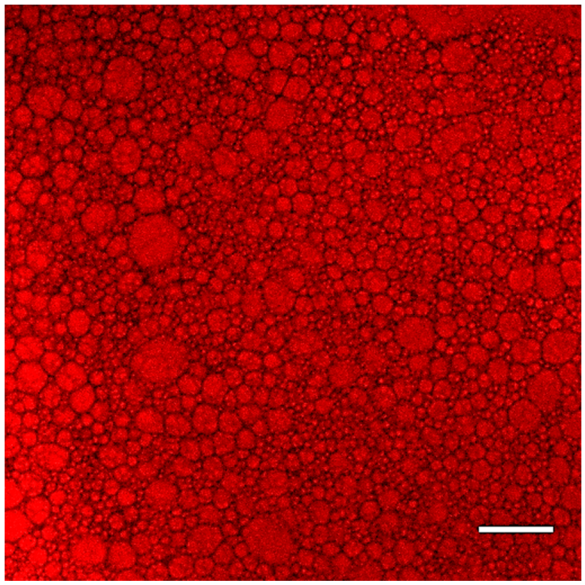

Fig. S2 CLSM image from the cream layer of polysaccharide (chitosan + pectin)/corn oil mixture prepared by ultrasonication (300 W cm^{-2}) after centrifugation at 15000 *g* for 5 min. The scale bar is 5 μm.

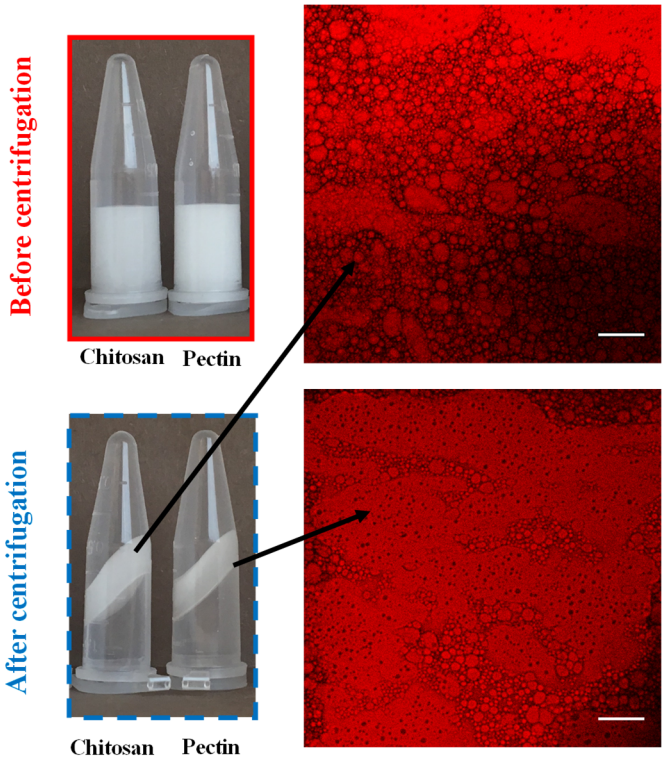

Fig. S3 Visual appearance of sonicated mixture of oil and single polysaccharide prepared by ultrasonication (300 W cm^{-2}) before and after 10000 g for 5 min. CLSM images from the cream layer of sonicated mixture of chitosan + corn oil, and pectin + corn oil. All the scale bars are 5 μm.

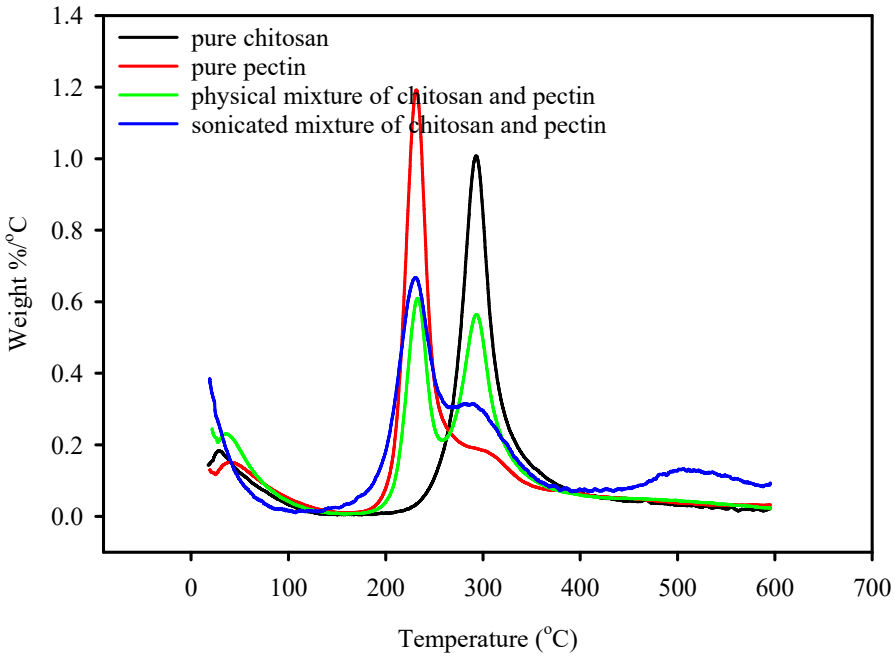

Fig. S4 First derivative TGA curves of pure chitosan, pure pectin, physical mixture of chitosan and pectin, and sonicated mixture of chitosan and pectin.

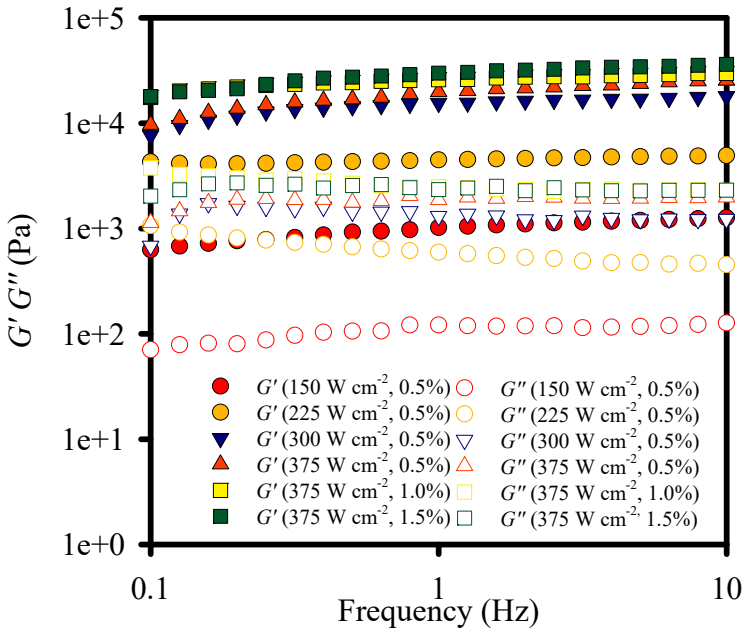

Fig. S5 Frequency sweep of the storage (*G'*) and loss (G'') moduli of the HIPEs synthesized at different ultrasonic intensities (150-375 W cm^{-2}) and polysaccharide concentrations (0.5-1.5 wt%).

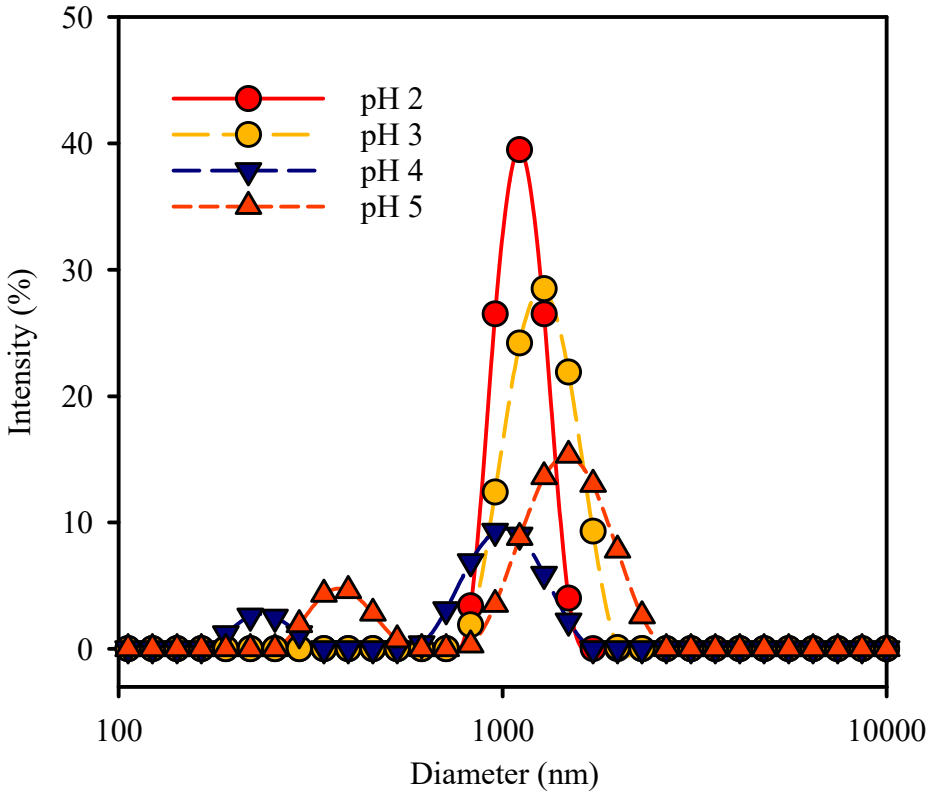

Fig. S6 Diameter distribution of microspheres prepared at different pHs.

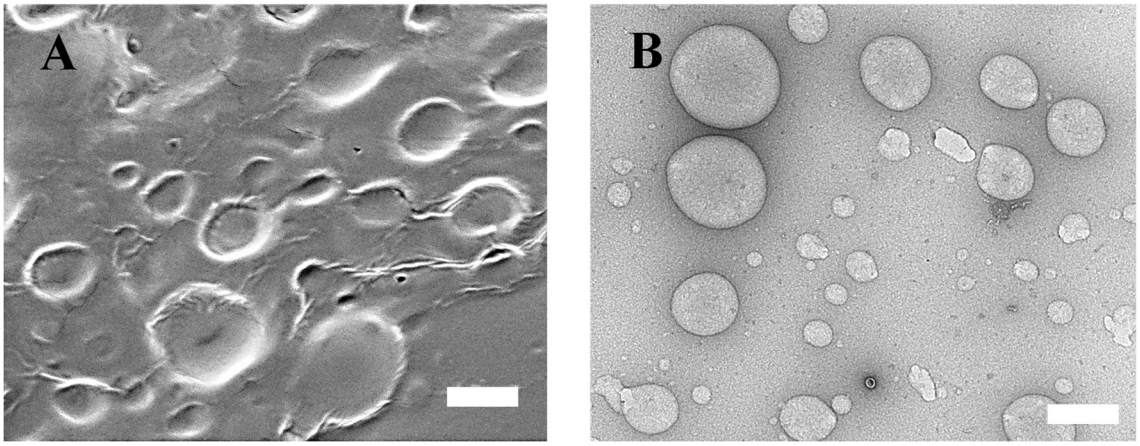

Fig. S7 (A) SEM and (B) TEM images of chitosan/pectin microspheres prepared at pH 2. The scale bars are 1 μm.

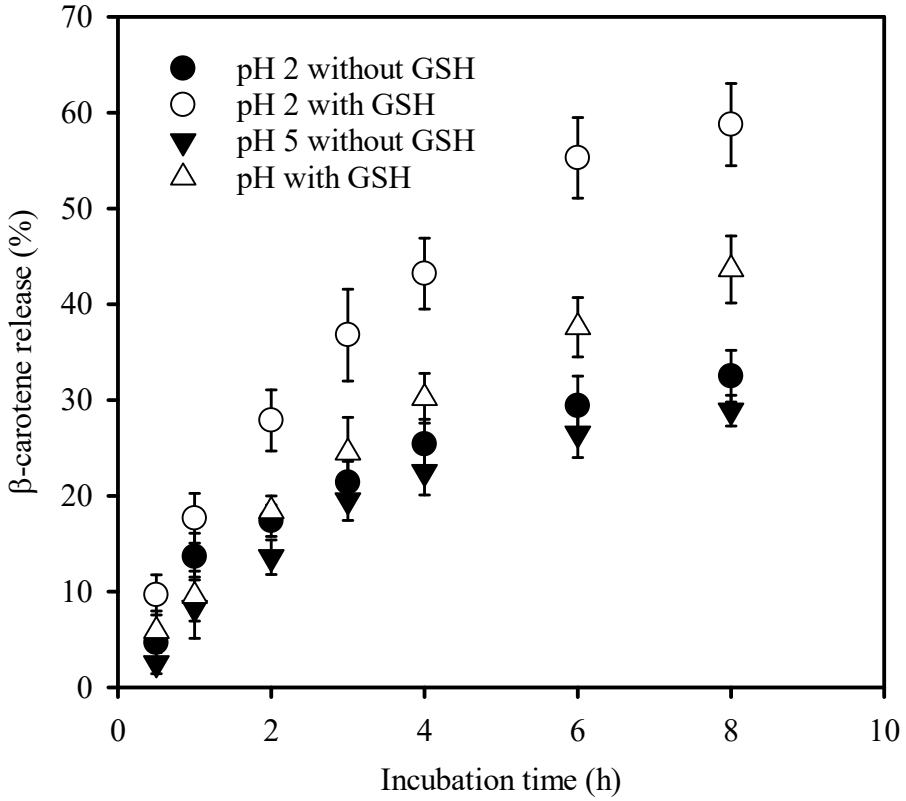

Fig. S8 Release behaviors of β-carotene from the HIPEs prepared at pH 2 and 5 during incubation in a phosphate buffer solution (pH 6.2) without and with the GSH of 10 mM.

References

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