A Green and Facile Strategy for Fabrication of All-Natural Porous Proteinaceous Microspheres

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Materials

Zein (grade Z3625) and Nile Red were purchased from Sigma-Aldrich (USA). Soybean lecithin and *Spirulina* phycocyanin were purchased from Tokyo Chemical Industry Co., Ltd (Japan). Fluorescein isothiocyanate (FITC) isomer and Span 80 were purchased from Macklin Biochemical Co., Ltd (China). A mixture of caprylic/capric triglycerides (GTCC) was obtained from Guangzhou Chou Qin Biotechnology Co., Ltd (China). Ethanol (AR, >99.7%), acetone, *n*-hexane, *n*-hexadecane and Congo Red (CR) were supplied by Sinopharm Chemical Reagent Co., Ltd (China). Deionized water (Milli-Q grade, 18.2 MΩ•cm) was used in all experiments. All chemicals were used as received.

Dye absorption

The equilibrium adsorption capacity, q_e ($mg \cdot g^{-1}$), and the adsorption capacity at time t, q_t ($mg \cdot g^{-1}$), were calculated by:

$$q_e = \frac{V(C_0 - C_e)}{m}$$

$$q_t = \frac{V(C_0 - C_t)}{m}$$

Where V(L) is the volume of the solution used; and m is the mass of the adsorbents (g); C_0 is the initial concentration of CR solution; C_e and C_t are the concentration of CR at equilibrium and time t ($mg \cdot L^{-1}$).

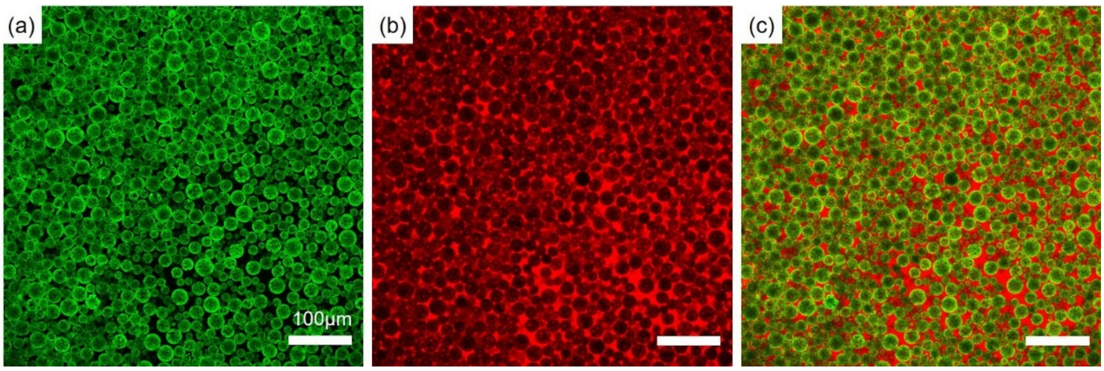

Fig. S1 CLSM images of porous zein protein microspheres in green channel (a), red channel (b), and merged channel (c), respectively.

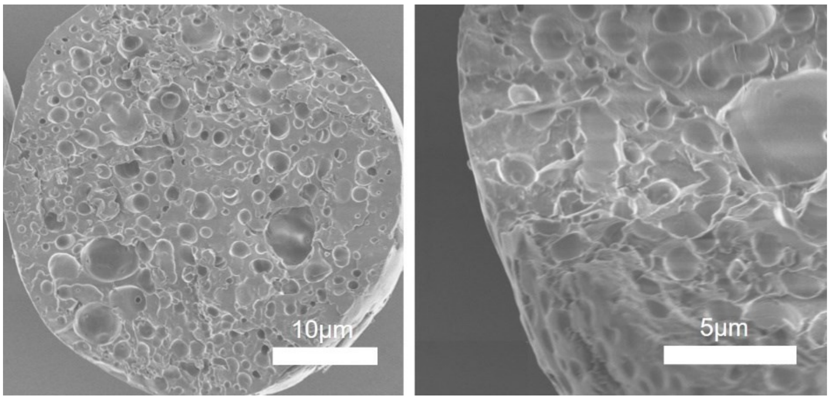

Fig. S2 SEM images of cross-section of the porous zein microspheres.

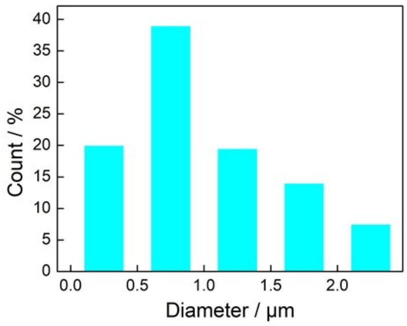

Fig. S3 Distribution of the internal pore diameter, measured by image J.

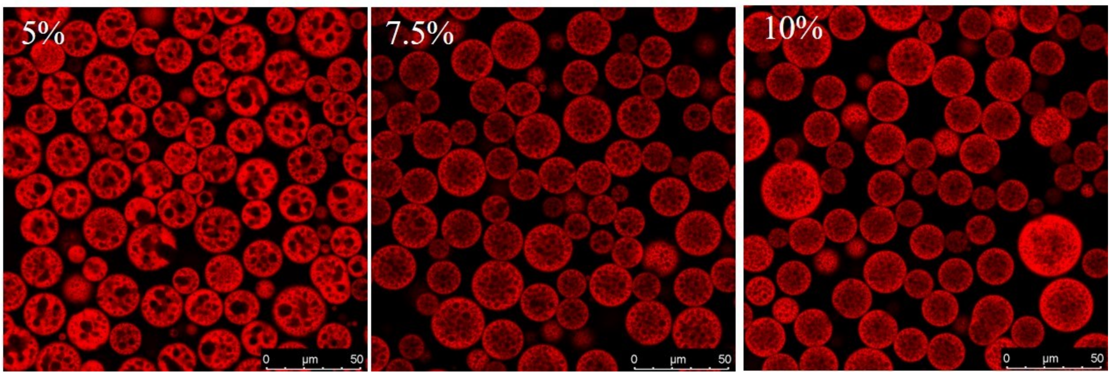

Fig. S4 CLSM images of porous protein microspheres with different lecithin concention.

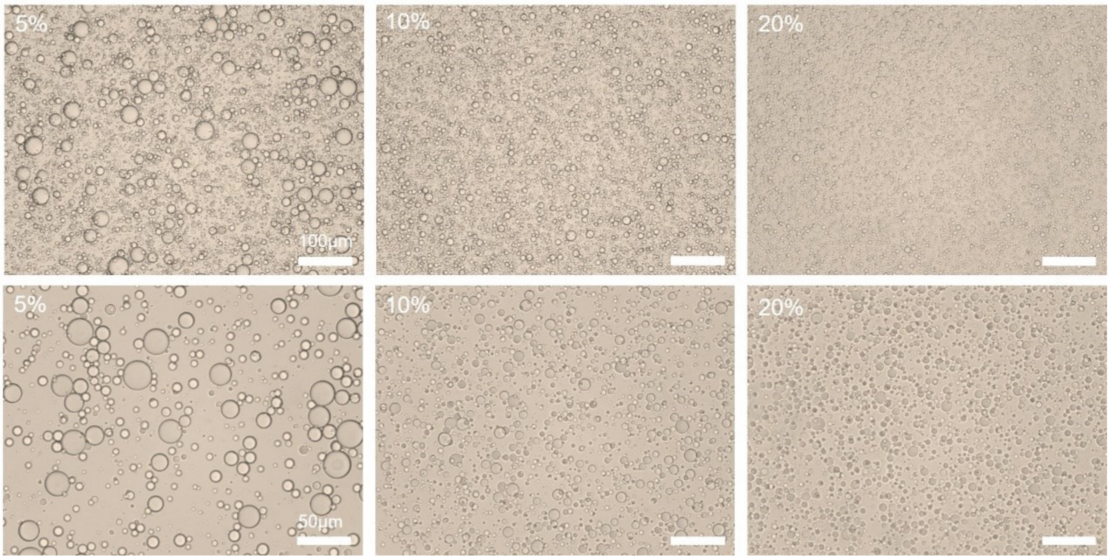

Fig. S5 Optical microscope images of GTCC-in-(ethanol/water) emulsions stabilized by different concentration of zein.

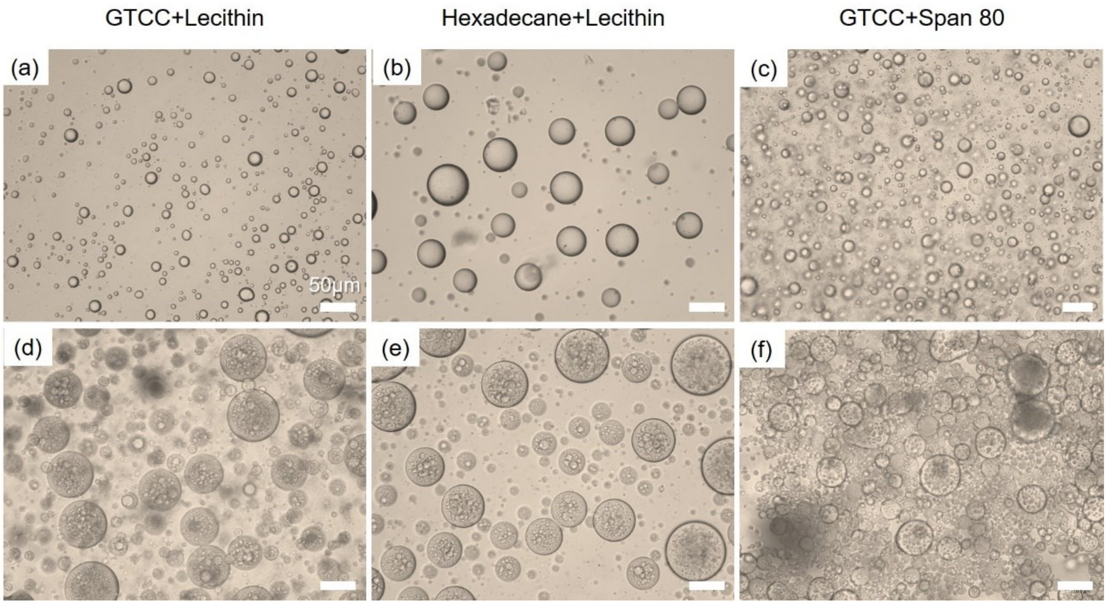

Fig. S6 Optical microscope images of emulsions prepared with different oils and emulsifiers. (a-c) Without addition of zein; (d-f) With 20% zein.

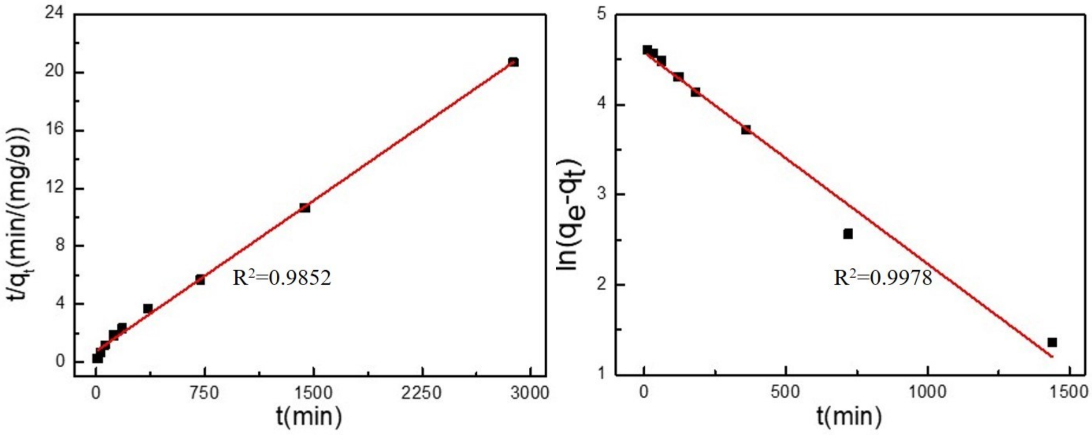

Fig. S7 Pseudo-first-order kinetic (a) and pseudo-second-order kinetic models (b) for CR adsorption on the prepared samples (adsorbent dose=20 mg; and initial CR concentration = 70 mg/L).

Kinetic models fittings: The pseudo-first-order and pseudo-second-order kinetic models can be transformed to expressions as follows:

$$\ln\left(q_e - q_t\right) = lnq_e - k_1 t$$

$$\frac{t}{q_t} = \frac{1}{q_e^2 k_2} + \frac{t}{q_e}$$

where q_e and q_t (mg/g) are the amounts of CR adsorbed at equilibrium and at any time t (min), respectively; and k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) are the rate constants of the pseudo-first-order and pseudo-second-order kinetic model, respectively.

Figure S7 shows the results of the two model fittings and the correlation coefficients and parameters are also summarized in Table S1, it is obvious that both fittings show a linearity, but the correlation coefficient R^2 of the pseudo-second-order kinetic model (0.9978) is higher than that of the pseudo-first-order kinetic model (0.9852), indicating that the pseudo-second-order kinetic model are more adapted to for illustrating CR adsorption of the samples.

Table S1. Parameters of two kinds of kinetic models for CR adsorption on prepared sample microspheres

		Pseudo-first-order model			Pseudo-second-order model		
Concentration (mg/L)	$q_{e,exp}$(mg/g)	R^2	k_1(min^{-1})	q_e(mg/g)	R^2	k_2(g mg^{-1} min^{-1})	q_e(mg/g)
70	139.26	0.9852	0.0023	97.12	0.9978	6.2039×10^{-5}	144.92

Langmuir isotherm model fitting: Langmuir model can be mathematically expressed as follows:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m K_L}$$

Where C_e ($mg\cdot L^{-1}$) is the equilibrium concentration, q_e ($mg\cdot g^{-1}$) is the equilibrium adsorption amount, q_m ($mg\cdot g^{-1}$) is the adsorption amount at equilibrium concentration, and K_L is the equilibrium adsorption constant.

Table S2. Parameters of Langmuir isotherm model for CR adsorption on prepared sample microspheres

Langmuir isotherm model	
q_{max}/$mg\cdot g^{-1}$	217.39
K_L/$L\cdot mg^{-1}$	1.2105
R^2	0.9997

	Mean diameter	Standard deviation	Polydispersity index (PDI)
Porous zein microspheres	19.5μm	5.05	0.067
Pore sizes	1.07 μm	0.833	0.6017

Table S3. The mean diameter、standard deviation and polydispersity index (PDI) of porous zein microspheres and pore size from statistical results, respectively.