

Properties of submicron sized vaterite particles for incorporation of proteins

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Materials and methods

Materials. Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich, ethylene glycol was obtained from ABCR. Mucin from porcine stomach, ≈ 600 kDa, Type III (M-1778), bovine liver catalase, porcine pepsin 4500 U/mg, hydrogen peroxide (Sigma–Aldrich, USA, China), trypsin 40 U mg^{-1} , chymotrypsin from bovine pancreas 61 U mg^{-1} (Fluka, Germany), albumin from human serum (Fraction V), recombinant human lactoferrin from the milk of transgenic goats (preparation ‘Caprabel’, Belarus) was employed.

All chemicals used for the preparation of buffer solutions were at least laboratory grade and purchased from Sigma–Aldrich. All other chemicals were used without further purification. Double distilled water was used in all experiments.

Synthesis of micron vaterite particles. 3 ml of 1 M CaCl_2 , 9 ml 0.05 M Tris buffer (pH 7.0), was stirred for 10 min (200 rpm) followed by the addition of 3 ml of 1 M Na_2CO_3 . Stirring was continued for 45 s (200 rpm), then the suspension was left for 15 min without stirring for precipitation. After precipitation of the crystals, the supernatant solutions were separated by centrifugation for 1 min at 1000 rpm; the precipitates were washed three times with double distilled water and lyophilized.

Synthesis of submicron vaterite particles. 5 ml of ethylene glycol and 0.5 ml 0.5M CaCl_2 were stirred for 1 min 500 rpm in 15 ml beaker followed by the addition of 0.5 ml 0.5M Na_2CO_3 . Stirring was continued for 1 h 500 rpm, then the suspension was centrifuged for 15 min 14500 rpm, the precipitate was ablated twice with double distilled water using diffusion on an ultrasonic stirrer between ablutions. The particles were dried at 90 °C.

Coprecipitation into submicron vaterite particles. 5 ml of ethylene glycol, 0.3 ml 10 mg ml^{-1} protein preparation solution (mucin, albumin, catalase, lactoferrin or chymotrypsin) in 0.05M Tris buffer (pH 7.0) and 0.35 ml 0.5M CaCl_2 were stirred for 1 min 500 rpm in 15 ml beaker followed by the addition of 0.35 ml 0.5M Na_2CO_3 . Stirring was continued for 1 h 500 rpm, then the suspension was centrifuged for 15 min 14500 rpm, the precipitate was ablated twice with double distilled water using diffusion on an ultrasonic stirrer between ablutions and lyophilized. The encapsulation of protein preparation into particles [loading capacity (LC)] was assessed by analysis of supernatant and wash solutions.

$\text{LC} = (\text{total protein added} - \text{free non-entrapped protein})/\text{weight of particle}$

Adsorption on submicron and micron vaterite particles. 10 mg of dried particles (nCC or mCC) were weighed and albumin, lactoferrin, or mucin were added to a final concentration of 1.5 mg ml^{-1} , catalase to a concentration of 0.3 mg ml^{-1} , and chymotrypsin to a concentration of 0.5 mg ml^{-1} , bringing the reaction mixture volume to 2 ml using 0.05 M Tris buffer (pH 7.0). The suspension was incubated for 30 min at 300 rpm. After incubation, the particles were centrifuged and washed with Tris buffer. The encapsulation of protein preparation into particles [loading capacity (LC)] was assessed by analysis of supernatant and wash solutions.

Catalase activity. Catalase solution or suspension of the particles loaded with catalase was diluted with 0.05 M Tris buffer to the same protein concentration. An aliquot of 820–870 μl of 0.05 M Tris buffer (pH 7.0) was mixed with 30–80 μl of diluted catalase solution and 100 μl of 176 mM H_2O_2 solutions reaching the total volume of the reaction mixture of 1000 μl . For the measurement of the activity of immobilized catalase, a control experiment was always set up with the same concentration of nCC or mCC, which did not contain catalase. Optical density at a wavelength of 240 nm was recorded for 60 s.

Chymotrypsin activity. Chymotrypsin or suspension of nCC or mCC loaded with chymotrypsin were diluted to the same protein concentration. 0.1–0.2 mg ml^{-1} . Then 950 μl of 0.05 M Tris buffer (pH 7.8) and 20 μl of chymotrypsin solution (pH 3.0 or 7.4) diluted to a concentration of 0.1–0.2 mg ml^{-1} or a suspension of microcrystals in 0.05 M Tris buffer (pH 7.8) were mixed with 30 μl of a 0.8 mg ml^{-1} solution of N-benzoyl-L-tyrosine p-nitroanilide in acetonitrile. In control samples, instead of chymotrypsin solutions, 0.05 M Tris buffer or suspensions of pristine nCC or mCC were added. The obtained samples were incubated for 20 min at 25 °C and stirring at 550 rpm. The suspensions were centrifuged for 1 min at 3000 rpm, the supernatant solution was separated, and the optical density was measured at a wavelength of 410 nm. The relative activity of chymotrypsin in solutions and in suspensions of nCC or mCC, equalized by protein content, was calculated as the ratio of the optical density in the samples (minus the optical density in control experiments) to the optical density of the samples of the chymotrypsin solution with pH 3.0.

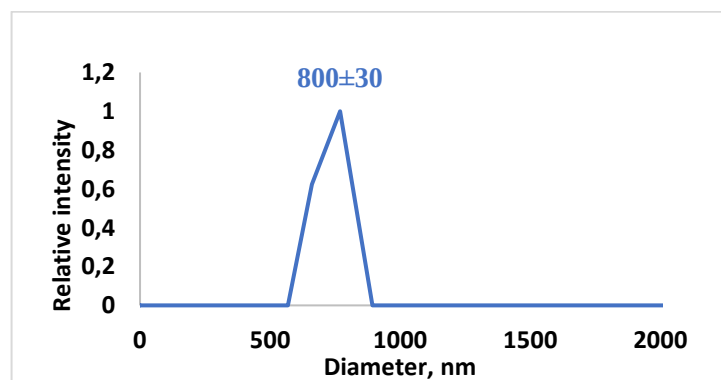


Figure S1 nCC particle size distribution according to dynamic light scattering data.

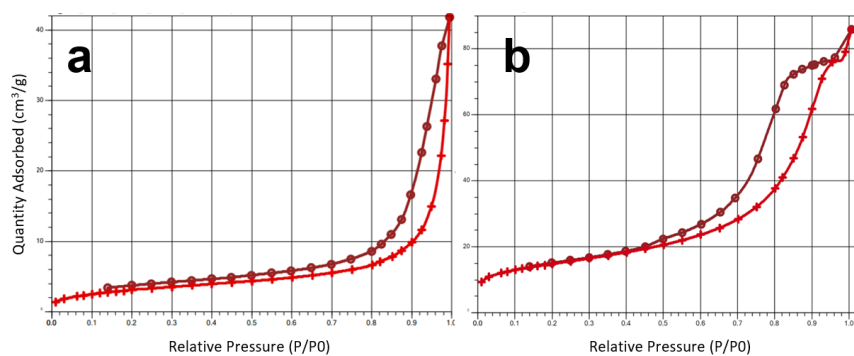


Figure S2 Nitrogen adsorption–desorption isotherms of (a) nCC and (b) mCC according to Brunauer–Emmett–Teller method.

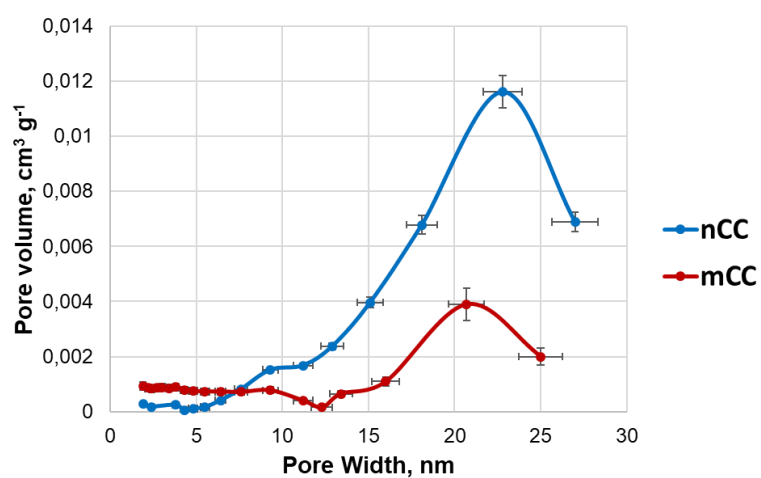


Figure S3 Pore size distribution of nCC and mCC according to Barrett–Joyner–Halenda method. Cumulative pore volume is $0.037099 \text{ cm}^3 \text{ g}^{-1}$ for nCC and $0.016829 \text{ cm}^3 \text{ g}^{-1}$ for mCC.

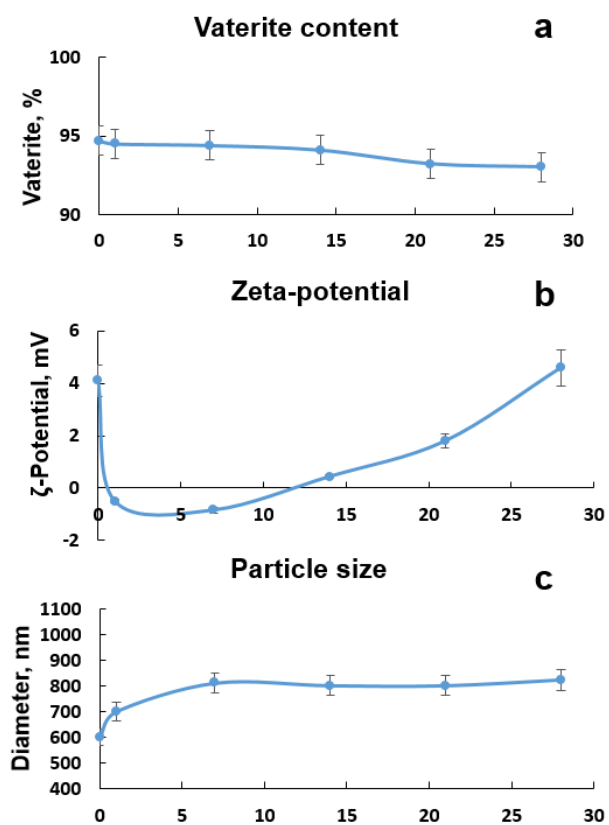


Figure S4 Changes in (a) vaterite content, (b) ζ -potential and (c) particle size during incubation of nCC in PBS buffer.