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Supporting Information

Facile and Green fabrication of Multiple Magnetite Nano-cores @Void@Porous Shell Microspheres for Delivery Vehicles

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Experimental Details

Fabrication of rattle type multiple magnetite cores microspheres with porous biopolymer shell (RMMCMs): 5 g *Chlorella pyrenoidosa* cells (purchased from Kang Bao Food Factory) was dispersed in 5 % Ferric ammonium citrate solution (100 mL) and shaken at 150 rpm for 12 h and then isolated by centrifugation, the solid product was washed at least 5 times with deionized water and then homogeneously dispersed in 6 % NaOH (80 mL), the mixture was then transferred to a 100 mL Teflon-sealed autoclave, maintained at 105°C for 1.5 h, the solid product was centrifugal separated, then washed with abundant deionized water until pH neutrality was established. The resulting suspension (80 mL) was placed in a 100 mL Teflon-sealed autoclave and the pH value was adjusted to 10 with 0.5 mol/L HCl or NaOH, heated up to a temperature in the 170-200°C range and maintained at this temperature for 4 h. After the autoclave had cooled down to the room temperature, the black products were magnetic separated, then washed with deionized water and alcohol several times. A part of wet samples were treated by freeze-drying process for the instrument analysis. All the chemical reagents are of analytical grade.

Characterization methods:

Transmission electron microscope (TEM) microphotographs were obtained with H-7500 (Hitachi, Japan) at 80 kV. The samples for TEM observation were prepared by dispersing the RMMCMs in alcohol and dropping a droplet of the suspension on the TEM mesh, which could be analyzed by TEM after air-drying.

Field emission transmission electron microscope (FETEM) images, energy-dispersive X-ray (EDX) spectrum, elemental mapping and selected-area electron diffraction (SAED) were analyzed using a JEM-2100F (JEOL, Japan) with acceleration voltage of 200 kV.

Cold field-emission scanning electron microscopy (FESEM) microphotograph was obtained with SU8020 (Hitachi, Japan) at 2.0 kV.

N2 adsorption/desorption isotherms were measured using a Micromeritics ASAP 2020 instrument. The pore distribution of shells was obtained from the desorption branch of the isotherms by the BJH (Barrett-Joyner-Halenda) method.

RMMCMs powder pattern were recorded on an X'Pert Pro MPD X-ray diffractometer (Philips, the Netherlands) with a Cu-K radiation at 40 kV and 40 mA with a scanning rate of 5 °min^{-1}.

The zeta potential analysis of magnetite cores inside the RMMCMs and the FITC-BSA were done with Zetasizer 2000 zeta potential analyzer (Malvern, United Kingdom). The magnetite cores inside the RMMCMs were obtained by sonic disruption of the microspheres and followed by magnetic separation of these nanoparticles. The magnetite nanoparticles and FITC-BSA were respectively dispersed in different pH buffer solutions (pH 5, 7 and 9). Each sample was measured by three times.

SFM images were carried out in air at room temperature using a Nanoscope IIIa multimode scanning force microscope (Digital Instruments, Santa Barbara, CA) in contacting model. The samples were prepared by adequate mixing equal amounts of 1.6 mg mL^{-1} BSA and 1.2 mg mL^{-1} RMMCMs solutions (0.01 M acetate buffer, pH 5.0) for 24 hours at 4°C. Then the RMMCMs with BSA inside was magnetic separated and washed with adequate cold pH 5 buffer solution (4°C) to remove the excess BSA. Meanwhile, the sample of RMMCMs without loading BSA was prepared too. The BSA loading efficiency was examined by the difference between the initial concentration and the equilibrium concentration of the BSA solution, and the protein contents were determined by the Bradford method. The samples for SFM investigation were carried out by dropping a droplet of the RMMCMs suspensions (with or without loading BSA at pH 5) on the freshly cleaved mica at 4°C, which could be analyzed by SFM after air-drying.

CLSM images were collected on a Leica TCS SP5 laser scanning confocal microscope (Leica, Germany) equipped with a 100 × oil immersion objective. The images of the RMMCMs in encapsulation and release of FITC-BSA were obtained immediately by using 488 nm excitation laser line. The encapsulation of FITC-BSA into RMMCMs was employed by mixing equal amounts of 1.6 mg mL^{-1} FITC-BSA and 1.2 mg mL^{-1} RMMCMs solutions (0.01 M acetate buffer, pH 5) for 24 hours at

4°C and 200 rpm. The release of FITC-BSA from RMMCMs was carried out by re-dispersing the RMMCMs with FITC-BSA inside in pH 7 (0.01 M phosphate buffer) and pH 9 (0.01 M carbonate buffer) solution respectively.

The tests in the existence of Fe (Ⅱ): 50.0 mg sample was extracted with 4.75 ml of 0.5 M hydrochloric acid and 0.25 ml ammonium fluoride with the adding of ultrasonic at 40°C for 1 h. The mixture was centrifugal separated and pipette 0.2 ml supernatant into a polyethylene beaker. Add 1 ml 1% 1, 10-phenanthroline solution, 1.5 ml 3 M hexamethylenetetramine buffer, and 1.3 ml deionized water in that order, swirling the solution after addition of each regent and UV-Vis spectra was used to record the absorbance of the samples. Added Fe (II) ($FeCl_2$) was not oxidized and added Fe (III) ($FeCl_3$) was not reduced by this procedure[1].

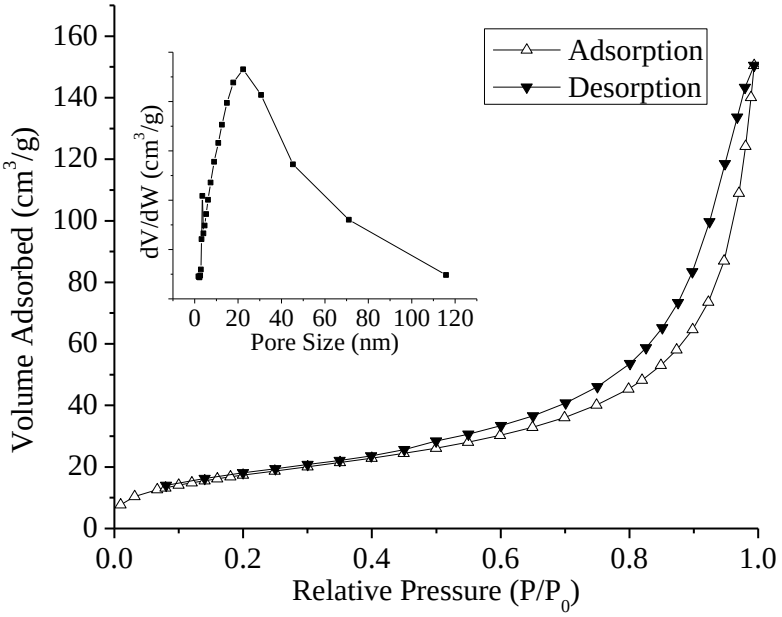

Figure S1. N_2 adsorption-desorption isotherm and pore size distribution curve of RMMCMs

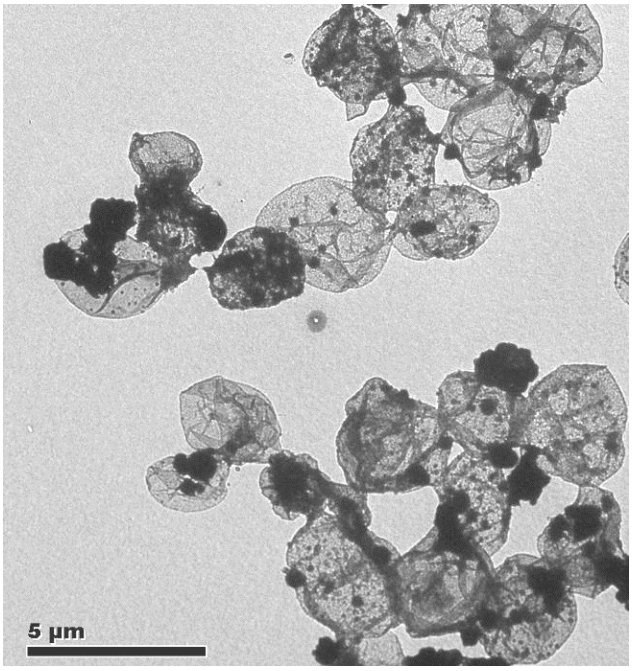

Figure S2. Wide range of TEM image of RMMCMs

Table S1. The lattice spacing, d (Å), deduced from the analysis of SAED and XRD patterns and the values corresponding to standard bulk magnetite (JCPDS no.99-0073)

diffraction ring	SAED	XRD	XRD(standard bulk Fe_3O_4)	hkl
1	4.712	4.845	4.848	111
2	2.876	2.972	2.969	220
3	2.425	2.534	2.532	311
4	2.047	2.094	2.099	400
6	1.547	1.613	1.616	511
7	1.439	1.483	1.484	440

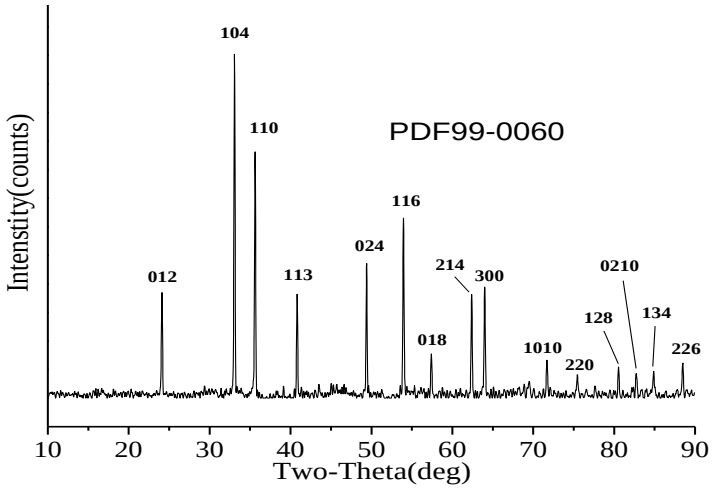

Figure S3 the XRD patterns of the Fe_2O_3 powder obtained under 200°C, 4h without adding Chlorella pyrenoidosa.

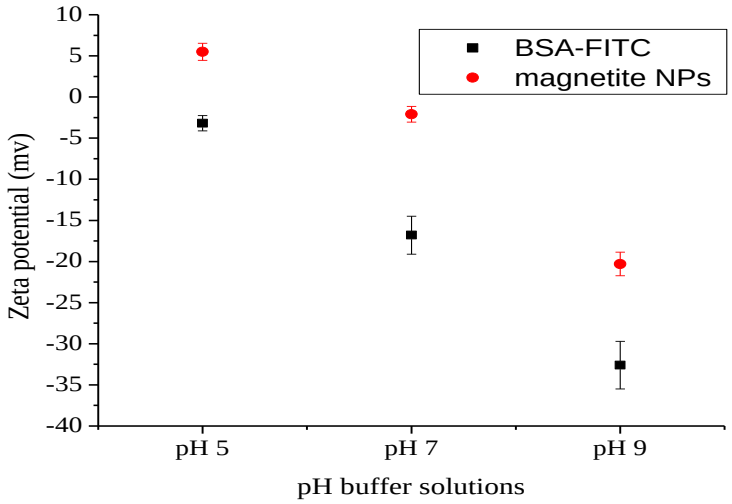

Figure S4 zeta potentials of BSA-FITC and magnetite nanoparticles dispersed in pH 5, 7 and 9 buffer solvents.

At pH 5, the FITC-BSA (isoelectric point 4.7) was negatively charged, however, the magnetite nanoparticles (isoelectric point 6.5) were positively charged, therefore, FITC-BSA was then found to spontaneously accumulate inside the RMMCMs, which was dominated by electrostatic attraction. While at pH 7, the magnetite cores inside the RMMCMs were negatively charged (the zeta potential is -2.1 ± 0.4 mV), and FITC-BSA was still negatively charged (the zeta potential is -16.8 ± 2.3 mV), therefore, electrostatic repulsive force resulted in FITC-BSA and magnetite cores repelling each other, and the FITC-BSA was released out of the RMMCMs. when the pH value was further increased to 9, the higher electrostatic repulsive force could cause the more complete release of FITC-BSA. Theoretically, so long as the pH value is chosen between the isoelectric points of the target molecules and the magnetite cores within the RMMCMs, many other target materials could be auto-encapsulation inside the RMMCMs.

[1] a) D. R. L. a. E. J. P. Phillips, *Appl. Environ. Microbiol* **1986**, *52*, 751-757; b) H. Tamura, K. Goto, T. Yotsuyanagi, M. Nagayama, *Talanta* **1974**, *21*, 314-318.