Supplementary Information for

Synthesis of superparamagnetic Fe_3O_4/PMMA/SiO_2 nanorattle with periodic mesoporous shell for lysozyme adsorption

1. Materials

Iron (Ⅲ) acetylacetonate, 1, 2-hexadecanediol (90%), oleic acid (90%), oleylamine (>70%) and benzyl ether (99%) were purchased from Sigma Aldrich. Methyl methacrylate (MMA), polyvinyl alcohol (PVA, M_w=2.2KD), ammonium persulfate (APS) and sodium dodecylbenzene sulfonate (SDBS) were obtained from Kelong Chemical Company (Chengdu, China). Tetraethylorthosilicate (TEOS) was obtained from Kemiou Chemical Company (Tianjin, China). Lysozyme was purchased from Solarbio Company. And other reagents were all analytical grade. Water used in the experiments was made from Milli-Q (Milli-Q Academic, Millipore, France).

2. Synthesis of Fe_3O_4 nanoparticles

Superparamagnetic Fe_3O_4 nanoparticles were prepared via thermal decomposition method. $Fe(acac)_3$ (2 mmol), 1,2-hexadecanediol (10mmol), oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20 mL) were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 200 ℃for 2 h, and then heated and refluxed at 300℃for another 1 h. The product was then precipitated with ethanol, centrifuged, and redispersed into hexane.

3. Synthesis of Fe_3O_4/PMMA composite nanospheres

Fe_3O_4/PMMA composite nanospheres were prepared by modified miniemulsion polymerization method according to our previous research [1]. 24 mg of SDBS and 881 μL PVA solutions were dissolved in 10 mL water. 400 μL Fe_3O_4 ferrofluids and 293 μL MMA with the weight ratio of 1:6 were added. The mixture was ultrasonicated at 50 % power level for a period of time (SONICS & MATERIALS INC. VCX 130PB, 130W) to form miniemulsion. The miniemulsion was transferred to a three-neck flask and purged with nitrogen gas throughout the procedure. To initiate the polymerization, 0.8 ‰ APS was added at 80 ℃with stirring at a speed of 300 rpm. The reaction lasted for 5 h. The as-received magnetic nanospheres were separated by the external magnetic field, and then washed with distilled water for several times. Finally, the product was centrifuged for 5 min at 3500 rpm and re-dispersed in distilled water.

4. The synthetic protocol of Fe_3O_4/PMMA/SiO_2 nanorattles with periodic mesoporous shells

1 mL Fe_3O_4/PMMA ferrofluids with the concentration of 20 mg/mL were dispersed in 9 mL CTAB solution with the concentration of 12mg/mL. The mixture was ultrasonicated for a period of time to form microemulsion. The microemulsion and 0.2 mL TEOS were added into a solution composed of 40 mL CTAB solution of the concentration of 5 mg/mL and 1.2 mL ammonia. The reaction lasted at ambient temperature for 24 h under vigorous stirring. The as-received magnetic nanorattles were separated and dialyzed with alcohol for two days. The nanorattles were re-dispersed in distilled water.

5. Adsorption of lysozyme

10 mg of Fe_3O_4/PMMA/SiO_2 nanorattles were added into 5 mL lysozyme solution (1mg/mL, 0.01 M, PH=11, $NaHCO_3$-Na_2CO_3 buffer). After stirred at 30 ℃ for 24 h, the nanorattles were removed from the lysozyme solution by outer magnetic field (about 200 mT) for less 10 s. The lysozyme concentration was measured by UV-vis spectroscopy at 297 nm. The calibration curve was constructed with lysozyme solution (0.05-1.00 mg/mL). The adsorption amount of lysozyme was calculated according to the following equation:

$$q = V_o (C_o - C)/W$$

Where q is the equilibrium adsorbed amount in the nanorattles (mg/g); C_o and C are the protein concentrations (mg/mL) at initial and equilibrium solution, respectively; V_o is the volume of the aqueous phase (mL); and W is the weight of the nanorattles (g). Desorption of the lysozyme from the nanorattles were performed in 1 M NaCl solution. The experiment was repeated three times for quality control.

6. Characterization

Hydrodynamic diameters and the size distribution of Fe_3O_4/PMMA/SiO_2 nanorattles were determined by dynamic light scattering (DLS, Malvern Nano-ZS, wavelength of 632.8 nm). The morphology and internal detailed structure were observed by scanning electron microscopy (SEM, HITACHI S4800) and transmission electron microscopy (TEM, JEM-100CX, JEM-2010, Japan electronic). Fourier-transform infrared (FTIR, Perkin-Elmer spectrometer) spectra were recorded from the wave number of 500 to 4000 cm^{-1}. The crystals structures were characterized by powder X-ray diffraction (XRD, X' Pert Pro MPD, Philips, Netherlands) at 40 Kv and 25 mA with Cu/Kα (λ = 0.154 nm) radiation source. The mesoporous structure was characterized by Small angle X-ray diffraction pattern (SAXRD, X'Pert PRO, Philips, Netherlands) using Cu/Kα radiation source (λ = 0.154 nm). Thermogravimetric analysis (TGA) measurements were performed on simultaneous thermal analysis (STA 449 C Jupiter, NETZSCH). The mass losses of the dried samples were carried out under N_2 with a heating rate of 10 ℃ min^{-1} from 35 to 700 ℃.The magnetization measurements of samples were performed by a vibrating sample magnetometer (VSM, Model BHV-525, Riken Japanese Electronics Company) with the field from 0 to 20,000 Oe at 300 K. Nitrogen adsorption and BJH pore size distribution were obtained at 77K on a surface area and pore size analyzer (QuadraSorb SI, America). The concentration of lysozyme was measured at 297 nm by UV-vis spectroscopy (Perkin-Elmer Lambda 650S).

7.

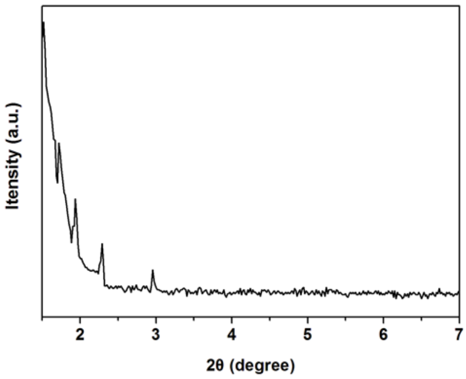

Fig. S1 SAXRD pattern of the Fe_3O_4/PMMA/SiO_2 nanorattles with periodic mesoporous shells

Reference

F. Lan, K.-X. Liu, W. Jiang, X.-B. Zeng, Y. Wu and Z.-W. Gu, Nanotechnology, 2011, 22, 225604.