Supporting information

for

Superparamagnetic submicro-megranates: Fe_3O_4 nanoparticles coated with highly cross-linked organic/inorganic hybrids

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***Chemicals**:* 1-octadecene (ODE, 90%, technical grade), oleic acid (OL, Chemical grade), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Chemical grade), hexachlorocyclotriphosphazene (HCCP, 98%, Aldrich), 4,4'-sulfonyldiphenol (BPS, 98%, Aldrich), triethylamine (TEA), aqueous ammonia solution($NH_4 \cdot OH$, 25wt %), polyoxyethylene (5) nonylphenyl ether (Igepal CO-520), tetraethyl orthosilicate (TEOS), bovine serum albumin (BSA), pH Buffer Solutions and all other organic solvents such as acetone, hexane, chloroform, tetrahydrofuran (THF) and ethanol are commercially available products. All chemicals were used as received without any further purification. Water was purified with a Milli-Q-system (Milipore, Bedford).

Synthesis of Fe_3O_4 nanoparticles: The synthesis of iron oxide nanoparticles was carried out using a modified procedure published elsewhere.[1] In a typical experiment, 2.78 g (3 mmol) of iron oleate complex which treated at 30 °C in a vacuum oven for 24 h before using, 0.48 mL of OL (1.5 mmol) and 10 mL of ODE were mixed in a three-neck round-bottom reaction flask. The reaction mixture was then heated to about 320 °C with a heating rate of 3.3 °C/min under stirring and the reaction mixture was kept refluxing for a further 1h. The initial reddish-brown color of the reaction solution turned brownish-black. The resulting solution was then cooled down to room temperature and a mixture of 10 mL of hexane and 40 mL of acetone was added to the reaction flask to precipitate the nanoparticles. The products were separated by centrifugation and washed 3 times with a mixture of hexane and acetone (1:1 v/v). After washing, the resulting nanoparticles were separated by centrifugation and dissolved in cylcohexane for further use.

Synthesis of Fe_3O_4@SiO_2 nanoparticles: In a typical experiment,[2] 8.75 g of Igepal CO-520 was added to 120 mL of cyclohexane and subjected to ultrasonic treatment for 15 min. Then, 1 mL of Fe_3O_4 solution in cyclohexane (5 mg/mL) was added to the Igepal solution. After the mixture had been stirred for 3 h, 0.88 mL of ammonia solution (25%) was added. Finally, 0.6 mL of TEOS was added, and the mixture was allowed to age for 48 h for hydrolysis and condensation of the silica precursor. When methanol was added into the reaction solution, Fe_3O_4@SiO_2 nanoparticles were precipitated. They were collected by both magnetic separation and centrifugation, washed with methanol, and re-dispersed in ethanol.

Synthesis of Fe_3O_4@SiO_2@PZS: To a 100-mL round-bottom flask, 20 mg of Fe_3O_4@SiO_2 , 20 mg of HCCP, and 45mg of BPS (at a mass ratio of 4: 4: 9) were added. Subsequently, 60 mL of the mixture of tetrahydrofuran (THF) and anhydrous alcohol (1:1 by volume) were added. After ultrasonic irradiation for 10 min, 2 mL of TEA was injected into the above reaction mixture. The solution was then maintained at room temperature for 8h under ultrasonic irradiation (50 W, 40 kHz). The resulting solid were collected by a magnet, washed with THF and distilled water several times.

BSA Adsorption on the Fe_3O_4@SiO_2@PZS: The adsorption of BSA on the Fe_3O_4@SiO_2@PZS was investigated at room temperature. In general, 10 mL of BSA solution of certain concentration (0.5 - 2.5 mg/mL) and 1 mg of Fe_3O_4@SiO_2@PZS were mixed together and left for stirring varying pH value, time and BSA initial concentration. Then the BSA-mediated aggregates of magnetic nano-adsorbents were separated magnetically from the BSA solution under an external magnetic field. BSA concentration in the supernatant was measured by UV-vis spectrometer at 278 nm. Therefore, the adsorbed mass of protein was calculated by mass balance.

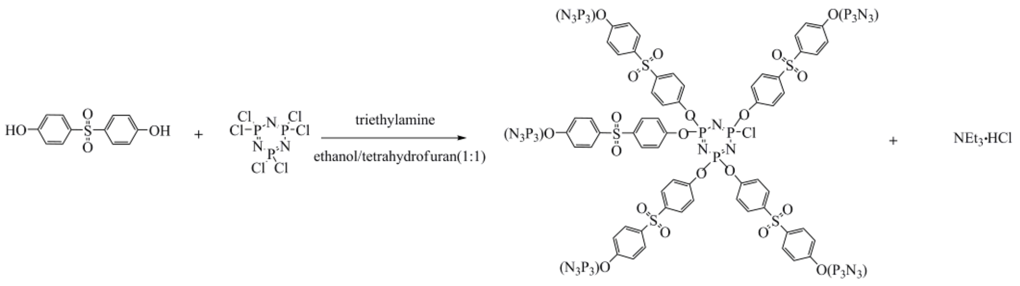
Characterizations: The size and morphologies of the as-prepared samples were characterized by a transmission electron microscope (TEM, JEM-2010, JEOL, Japan), and a high-resolution transmission electron microscope (HR-TEM, JEM-2100F, JEOL, Japan) operating at an acceleration voltage of 200 kV. The selected area electron diffraction (SAED) patterns of the samples were collected on TEM. The elemental composition of samples was determined by energy dispersive X-ray spectrometry (EDS) (Link-Inca, model 622, U.K.) on the TEM. The size and distributions of all as-prepared nanomaterials were determined from the TEM or SEM micrographs using ImageJ (V1.41, NIH, USA) for image analysis.[3] X-ray diffraction (XRD) patterns were collected on the powder diffractometer (D/max-2200/PC, Rigaku, Japan) using Cu Kα radiation (40 kV, 20 mA). Diffraction patterns were collected from 20° to 70° at a speed of 3 °/min. Fourier transform infrared (FTIR) spectra were recorded on a Paragon 1000 (Perkin Elmer) spectrometer. The samples were dried overnight at 45 °C in a vacuum and thoroughly mixed and crushed with KBr to fabricate KBr-pellet. Thermogravimetric analysis (TGA) data were collected using a Universal V3.6 TA Thermal Analysis Q5000 system. The sample was placed in a platinum crucible and heated under a flow of N_2 from 100 ^oC to 700 ^oC at a heating rate of 10 ^oC/min. The magnetization curve was measured at 300 K under a varying magnetic field with physical properties measurement system (PPMS-9, Quantum Design, USA). Photography was taken with digital camera (IXUS 800IS, Cannon, Japan). BSA concentration in supernatant was measured by UV-vis spectrometer (Shimadzu UV 1601 PC) at 278 nm. The Zeta potential of submicron was measured on a NanoSizer (Nano Series, Malvern, UK).

Notes and references

1. J. Park, K. J. An, Y. S. Hwang, J. G. Park, H. J. Noh, J. Y. Kim, J. H. Park, N. M. Hwang and T. Hyeon, *Nat. Mater.*, 2004, **3**, 891.

2. N. R. Jana, C. Earhart and J. Y. Ying, *Chem. Mater.*, 2007, **19**, 5074.

3. W. S. Rasband, *ImageJ*; National Institutes of Health: Bethesda, MD, 1997-2009; http://rsbweb.nih.gov/ij/



BPS HCCP PZS TEACl

Fig. S1 The polycondensation of monomers BPS and HCCP and the organic-inorganic hybrid cross-linked structure of the PZS product as shell of superparamagnetic colloids.

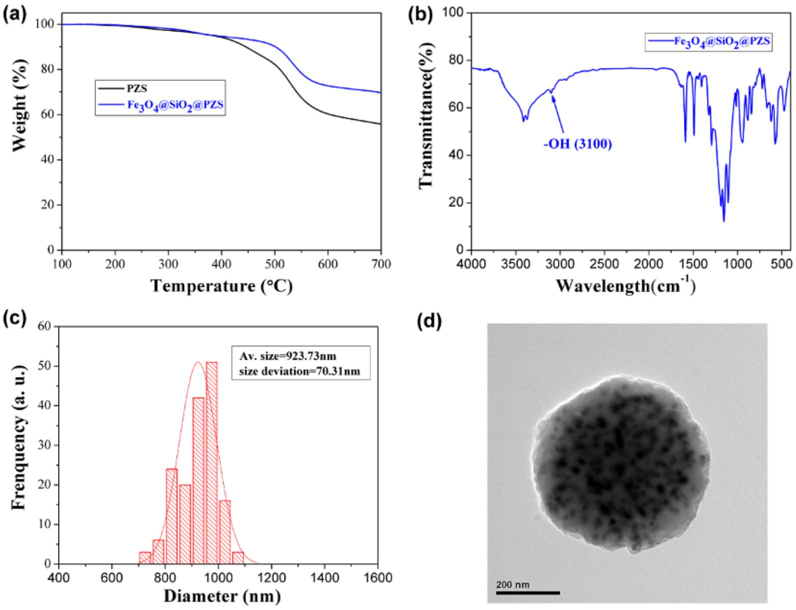


Fig. S2 Characterization of as-prepared Fe_3O_4@SiO_2@PZS: (a) TGA curve, the amount of organic phase is about 68.70% (at a mass ratio of Fe_3O_4@SiO_2/HCCP/BPS= 4:4:9), (b) FT-IR spectrum, and (c) Size distribution based on Fig. 2e (d) TEM image of Fe_3O_4@SiO_2@PZS (size: 552 nm).

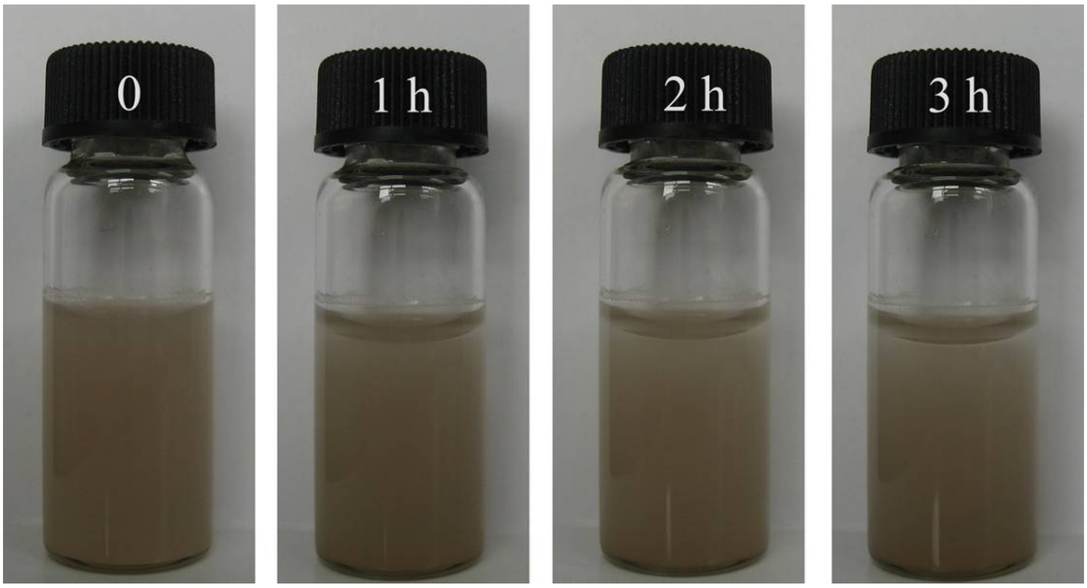


Fig. S3 Photographic images of Fe_3O_4@SiO_2@PZS in water (concentration: 1 mg/mL)

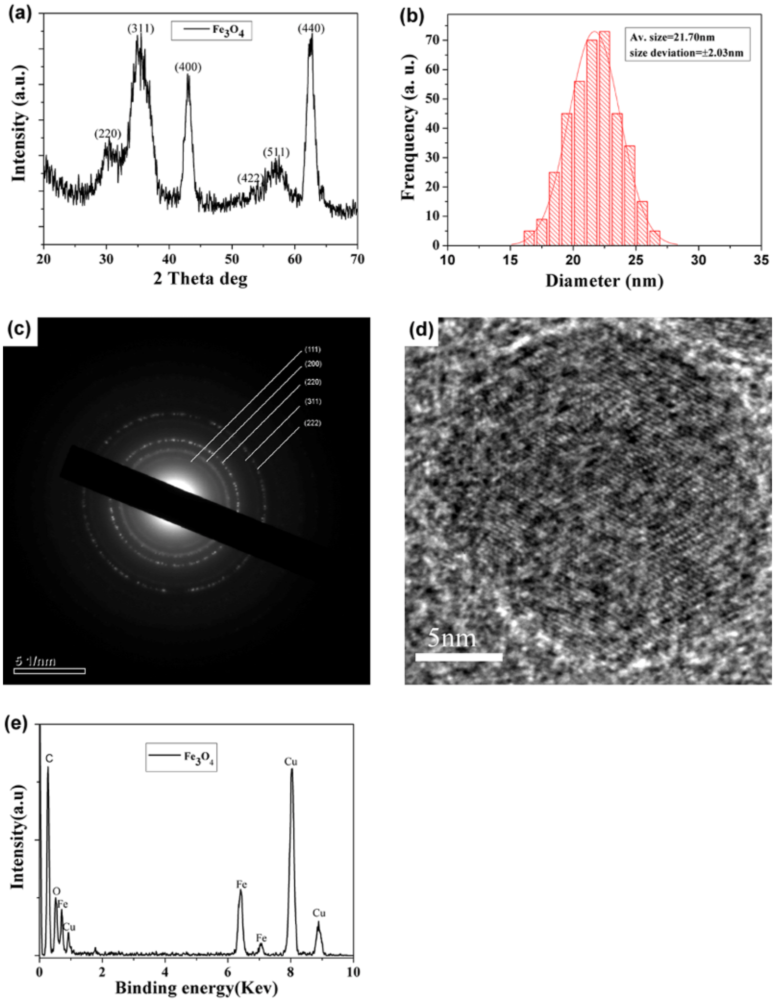


Fig. S4 Characterization of as-prepared Fe_3O_4 nanoparticles: (a) XRD spectrum, (b) Size distribution based on Fig 2a, (c) SAED pattern, (d) HR-TEM image and (e) EDS spectrum.

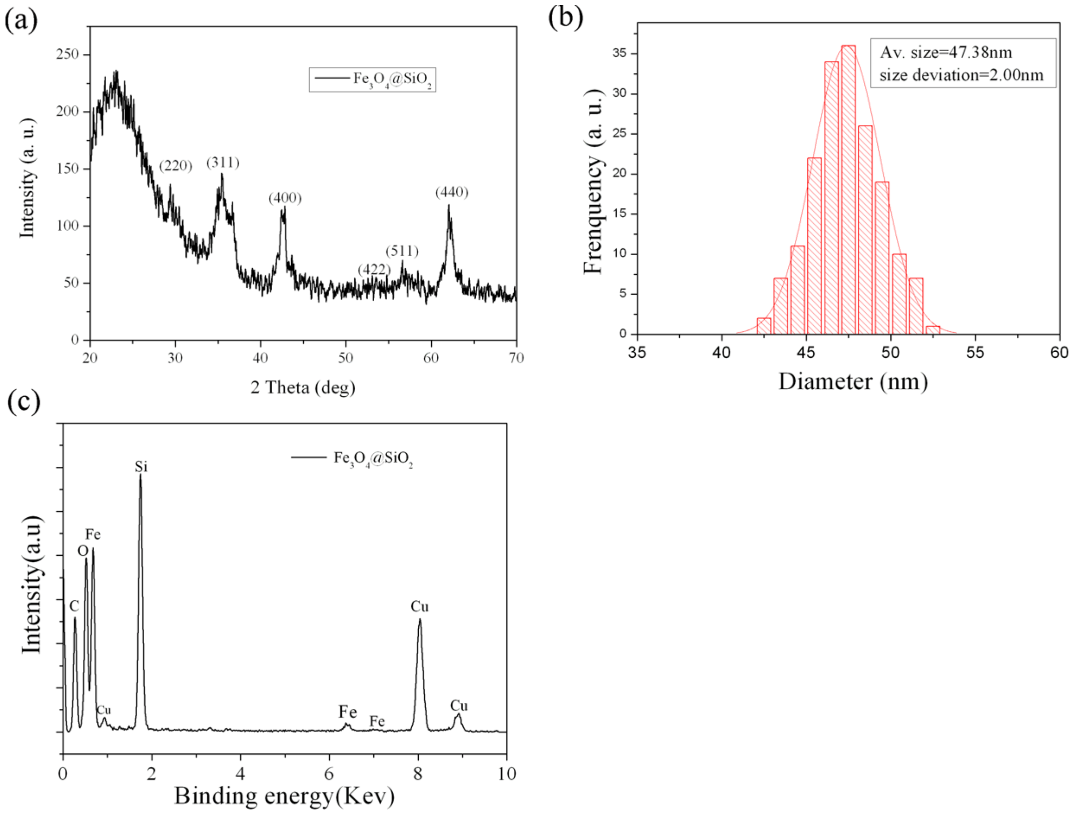


Fig. S5 Characterization of as-prepared $Fe_3O_4@SiO_2$: (a) XRD spectrum, (b) Size distribution based on Fig 2b, and (c) EDS spectrum.

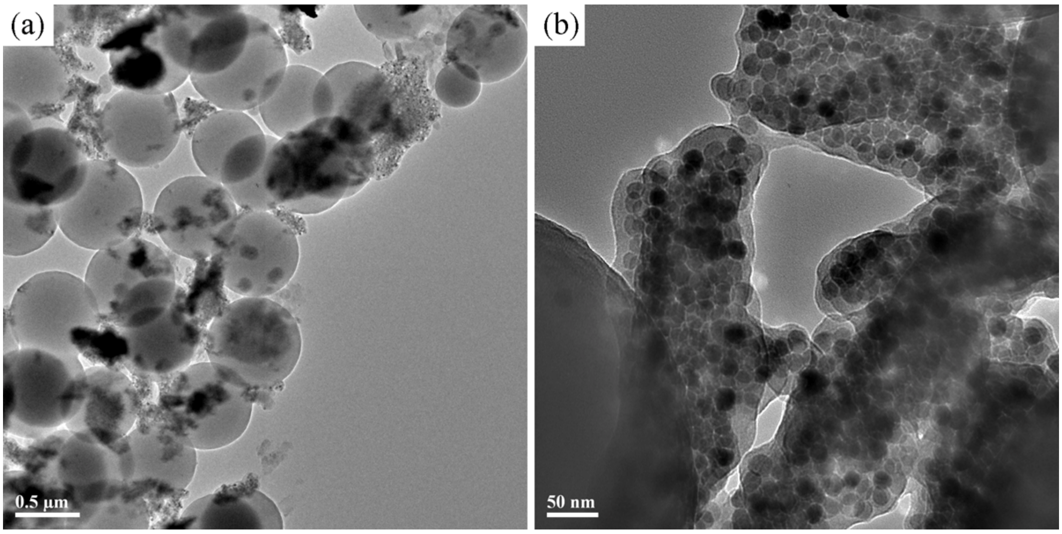


Fig. S6 (a, b) Typical TEM images of ill-defined Fe_3O_4/PZS mixture

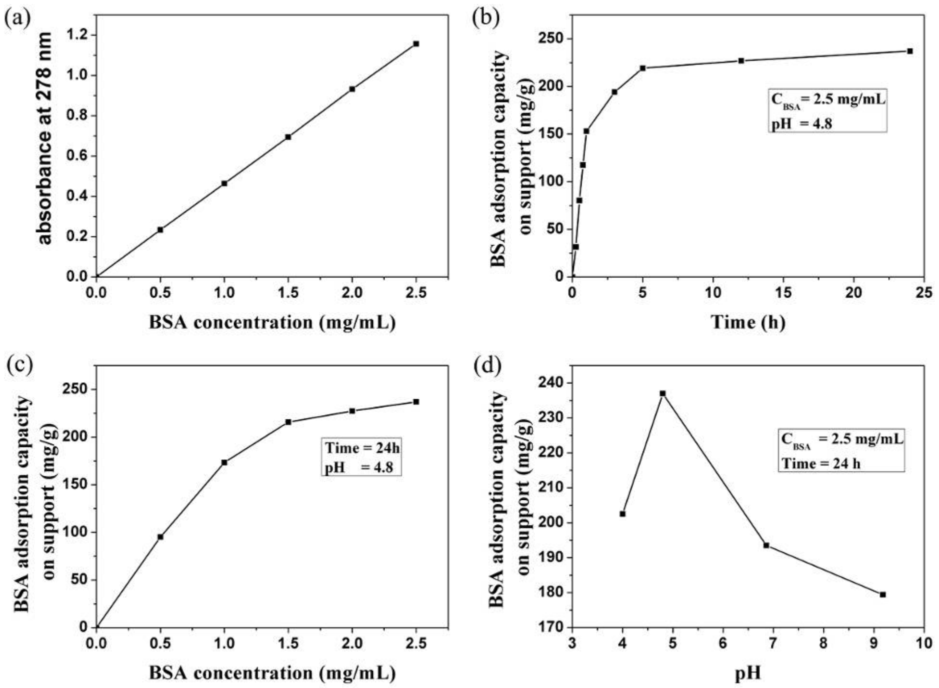


Fig. S7 (a) The standard calibration curve of BSA concentration *vs* absorbance at 278 nm. The solid line is a linear regression fit. (b) Influence of BSA initial concentration on BSA adsorption. Fe_3O_4@SiO_2@PZS content: 1mg; the total volume of protein solution: 10 mL; adsorption time: 24 h; pH=4.8. (c) BSA adsorption rate. Fe_3O_4@SiO_2@PZS content: 1mg; BSA initial concentration: 2.5 mg/mL; the total volume of protein solution: 10 mL; pH=4.8. (d) Influence of pH value on BSA adsorption. Fe_3O_4@SiO_2@PZS content: 1mg; BSA initial concentration: 2.5 mg/mL; the total volume of protein solution: 10 mL; adsorption time: 24 h.