Electronic Supplementary Material (ESI) for RSC Advances


# Supporting Information

## Microwave-assisted hydrothermal crystallization: an ultrafast route to MSP@mTiO$_2$ composite microspheres with uniform mesoporous shell

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## Experimental Section

**Materials.** Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ammonium acetate ($NH_4OAc$), ethylene glycol (EG), anhydrous ethanol, trisodium citrate dehydrate and aqueous ammonia solution (25%) were purchased from Shanghai Chemical Reagents Company and used as received. β-Casein, bovine serum albumin, fetuin, 2,5-dihydroxybenzoic acid (2,5-DHB, 98%), ammonium bicarbonate (ABC, 99.5%) and 1-1-(tosylamido)-2-phenyl-ethyl chloromethyl ketone (TPCK)-treated trypsin (E.G 2.4.21.4) were purchased from Sigma (St.Louis, MO). Acetonitrile (ACN, 99.9%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck (Darmstadt, Germany). Phosphoric acid (85%) was purchased from Shanghai Feida Chemical Reagents Ltd. (Shanghai, China). Matrix DHB was dissolved in acetonitrile (ACN)/water (50/50, v/v) solution containing 1% $H_3PO_4$ by keeping DHB at 10 $mg \cdot mL^{-1}$. Deionized water (18.4 MΩ cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA). Field-emission transmission electron microscopy (FE-TEM) images were taken on a JEM-2100F transmission electron microscope at an accelerating voltage of 200 kV. Samples dispersed at an appropriate concentration were cast onto a carbon-coated copper grid. Magnetic characterization was carried out with a vibrating sample magnetometer (VSM) on a Model 6000 physical property measurement system (Quantum, USA) at 300K. XRD patterns were collected on a X'Pert Pro (Panalytical, The Netherlands) diffraction meter with Cu KR radiation at λ =0.154 nm operating at 40 kV and 40 mA. Nitrogen adsorption-desorption measurements were performed on an ASAP2020 (Micromeritics, USA) accelerated surface area analyzer at 77 K. Before measuring, the samples were degassed in a vacuum at 120 $^oC$ for at least 6 h.

***Preparation of MSPs stabilized by citrate***

The magnetic colloidal supraparticles (MSPs) stablibized by citrate were prepared through a modified solvothermal reaction. Typically, 1.350 g of $FeCl_3 \cdot 6H_2O$, 3.854 g of $NH_4Ac$ and

0.400 g of sodium citrate were dissolved in 70 ml of ethylene glycol. The mixture were stirred vigorously for 1 h at 170 $^{o}$C to form a homogeneous black red solution, and then transferred into a Teflon-lined stainless-steel autoclave (100 ml capacity). The autoclave was heated at 200 $^{o}$C and maintained for 16 h, then it was cooled to room temperature. The black product was washed with ethanol and collected with the help of a magnet. The cycle of washing and magnetic separation was repeated for several times. The final product was dispersed in ethanol for further use.

***Preparation of MSP@$TiO_2$ core/shell microspheres***

The MSP@$TiO_2$ core/shell microspheres were synthesized by directly coating $TiO_2$ layer on the surface of MSPs in the mixed solvent of ethanol and acetonitrile at room temperature by hydrolyzing TBOT in the presence of ammonia. Briefly, about 50 mg of the as-prepared MSPs were dispersed in a mixed solvent containing 75 mL of ethanol and 25 mL of acetonitrile with the aid of ultrasonic and then mixed with 0.5 mL of $NH_3 \cdot H_2O$ at room temperature. Finally, a solution of 1 mL of TBOT in 15 mL of ethanol and 5 mL of acetonitrile was added to above suspension under stirring. After reacting for 1.5 h, the products were collected by magnetic separation and washed with ethanol and acetonitrile for several times.

***Preparation of MSP@m$TiO_2$ core/shell microspheres by using microwave-assisted hydrothermal crystallization***

The mesoporous $TiO_2$ shell were achieved by treating the obtained $Fe_3O_4$@$TiO_2$ microspheres by a microwave-assisted hydrothermal process. The microwave-assisted hydrothermal process was carried out at in a commercial-available microwave synthesizer CEM Discovery (CEM Inc. NC, US) with single mode and continuous power of 2.45 GHz. The synthesizer was attached with a automation system to provide autamated reation handling capabilities. The 35 mL quartz moded vessel was sealed by a crimp cap. In a typical reaction, 50 mg of the as-synthesized MSP@$TiO_2$ microspheres was dispersed in 18 mL mixed solvent containing 12 mL of ethanol and 6 mL of deionized water. The mixture was then transferred into a microwave reaction vessel (35 mL capacity) and placed in the vessel holder. The reaction condition was programmed by a intelligent software and could be facilely altered. The maximum power was set as 180 W and the pressure cut-off limit was 150 psi. The temprature was set as 180$^{o}$C and the reaction time is determined as needed. The temprature was quickly ramped from room temprarure to the desired temperature and once the reaction is complete,

the air flow was increase to rapidly cool the reation system. The as-prepared MSP@m$TiO_2$ products were collected by a magnet and washed with ethanol for 3 times.

***Preparation of MSP@m$TiO_2$ core/shell microspheres by using conventional hydrothermal crystallization***

The experimental process for preparing MSP@m$TiO_2$ core/shell microspheres by using conventional hydrothermal crystallization is as follows. Typically, the as-synthesized MSP@$TiO_2$ microspheres were dispersed in 60 mL mixed solvent containing ethanol (40 mL) and deionized water (20 mL). The mixture was then transferred to a Teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated at a certain temperature and maintained for 20 h. Then it was cooled to room temperature, the as-prepared MSP@m$TiO_2$ products were collected by a magnet and washed with ethanol for 3 times.

***Preparation of Tryptic Digest of Standard Proteins***

β-casein, BSA were each dissolved in 25 mM ABC at pH 8.0 (1mg/mL for each protein) and denatured by boiling for 10 min. Protein solutions were then incubated with trypsin at an enzyme/substrate ratio of 1:40 (w/w) for 12 h at 37 °C to produce proteolytic digests, respectively. The tryptic peptide mixtures were stored at -20 °C until further use.

***Selective enrichment of phosphopeptides with MSP@m$TiO_2$ microspheres***

The obtained MSP@m$TiO_2$ was first washed with ethanol for three times and then suspended in deionized water at 10 mg/mL. Tryptic digests of β-casein and BSA was dissolved in 200 μL loading buffer (50% ACN containing 7.5% TFA), then 2 μL MSP@m$TiO_2$ was added and incubated at room temperature for 60 min, respectively. After that, MSP@m$TiO_2$ with captured phosphopeptides was separated from the mixed solutions by applying an external magnet. After washing with 200 μL loading buffer to remove the nonspecifically adsorbed peptides, the trapped phosphopeptides were eluted with 10 μL 5% $NH_3$•$H_2O$ for the further MS analysis.

***Evaluation of the enrichment capacity of MSP@m$TiO_2$ towards phosphopeptides***

In order to evaluate of the enrichment capacity of MSP@m$TiO_2$ towards phosphopeptides, equivalent amounts of MSP@m$TiO_2$ were used to selectively enrich phosphopeptides from β-casein; a series of samples with varying amounts of phosphopeptides were prepared. After the samples were loaded, the flow-through fractions were analyzed using MALDI-TOF MS.

When the total amount of β-casein was lower than the capacity of the materials, the phosphopeptides could not be detected. Once the phosphopeptide's signal was detected by the MALDI-TOF MS—meaning that the material could not capture all of the phosphopeptides at the concentration—the enrichment capacity of the material could be estimated.

***MALDI Mass Spectrometry***

1 μL of the eluate was deposited on the MALDI probe, and then 1 μL of matrix solution DHB was deposited for MS analysis. MALDI-TOF mass spectrometry analysis was performed in positive reflection mode on a 5800 Proteomic Analyzer (Applied Biosystems, Framingham, MA, USA) with a Nd: YAG laser at 355 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV. The range of laser energy was optimized to obtain good resolution and signal-to-noise ratio (S/N) and kept constant for further analysis. External mass calibration was performed by using standard peptides from myoglobin digests.

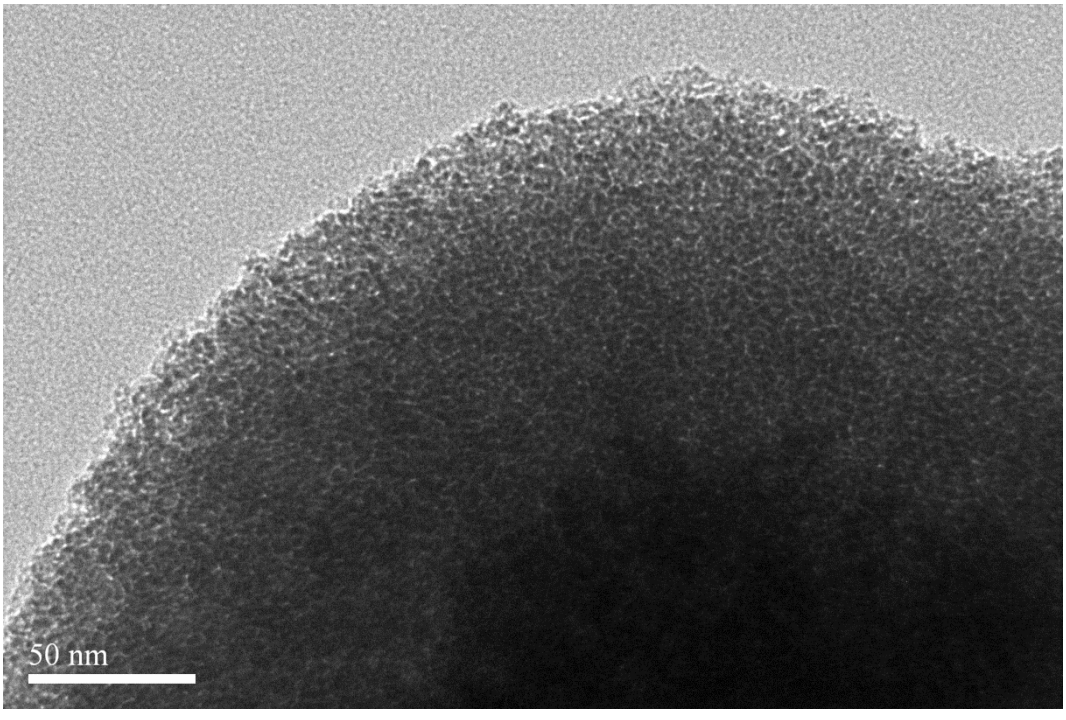


**Fig. S1** HRTEM image of the $TiO_2$ shell of MSP@m$TiO_2$ prepared the reaction time of 0 min.

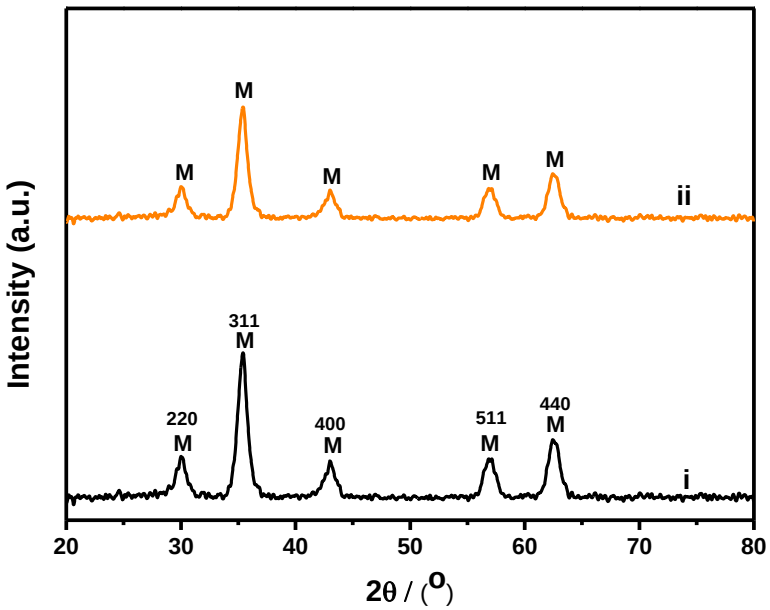


**Fig. S2** PXRD patterns of (i) MSPs, (ii) MSP@$TiO_2$

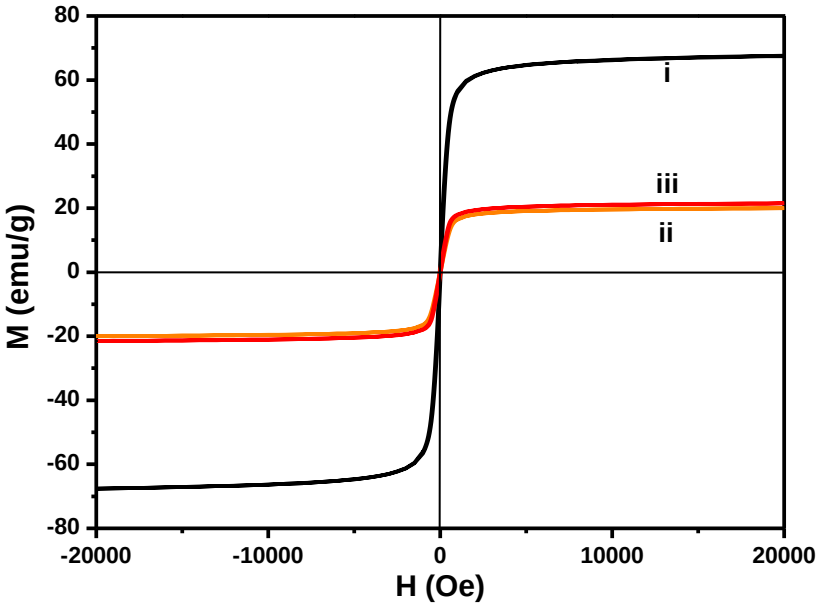


**Fig. S3** Magnetic hysteresis curves of (i) MSPs, (ii) MSP@$TiO_2$ and MSP@m$TiO_2$ prepared under the reaction time of 10 min.