Supporting information

Facile synthesis of superparamagnetic mesoporous zeolite microspheres for the capacious enrichments of enzyme and protein

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Experimental Section

I. Chemicals

Tetrapropylammonium hydroxide (TPAOH, 25\% in water), tetraethyl orthosilicate (TEOS), aluminium isopropoxide [Al(\textsuperscript{i}Pro)\textsubscript{3}], methyl methacrylate (MMA, 99\%), potassium peroxodisulfate (KPS, +99\%), sodium dodecyl sulfate (SDS, 98.5\%), NaOH, iron nitrate (Fe(NO\textsubscript{3})\textsubscript{3}, 9H\textsubscript{2}O) and ethanol (99.9\%) were purchased from Aldrich.

II. Direct synthesis of the mesoporous zeolite microspheres

2.1 Mesoporous zeolite microspheres (MZMs) were synthesized by our method described previously\textsuperscript{1}

2.2 Preparation of superparamagnetic mesoporous zeolite microspheres (SmMZMs): A certain amount of MZMs was taken in vessel and subjected to vacuum. After thirty minutes, the vacuum pump was turned off and an ethanol solution of iron nitrate (0.5
M concentration were used in the synthesis procedure) was allowed to enter the system till normal atmospheric pressure was achieved, and the system status was held for ten minutes. After that, the iron nitrate solution was removed by centrifugation and the precipitate was dried under flowing air at room temperature. After drying, the powder was washed twice with absolute ethanol. Then, the powder was dried under flowing air again. After that, the powder was calcined at 573 K for 3 h to yield the mesoporous zeolite microspheres with hematite cores. The reduction was carried out by the thermal treatment in mixed H₂ (5% volume percentage) and Ar (95% volume percentage) gases at 683 K for 5 h, and the final SmMZMs were obtained.

2.3 Enzyme and Protein Immobilization:

2.3.1 Experiment of Adsorbing Protein and Enzyme:

(A) A total of 2.0 mg of SmMZMs was dispersed in 1.0 mL of a 20 mM phosphate buffer solution (PBS) containing 1.0 mg mL⁻¹ of protein at different pH values (from pH 2 to 12) and kept in a water bath at 37 °C for 1 h. The adsorption amount of the proteins was obtained through measuring the UV absorbance of the protein solution at λ=280 nm before and after adsorption. For comparison, (B) the adsorption behavior of SmMZMs for lipase was also determined as follows. A total of 1.0 mg of SmMZMs was dispersed in 1.0 mL of a 20 mM phosphate buffer solution (PBS) containing 0.05 mg mL⁻¹ of lipase from Candida rugosa at different pH values (from pH 2 to 12) and kept in a water bath at 37 °C for 1 h. The adsorption amount of the proteins was obtained through measuring the UV absorbance of the protein solution at λ=250 nm before and after adsorption. To accurately calculate the adsorption amount, a calibration curve was created by using a series of protein and enzyme solutions with different concentrations in 20 mM PBS at pH 7.0 before measurement.

2.4 Experiment of Adsorbing Dynamics of Protein and Enzyme:

A total of 1.0 mg of SmMZMs was dispersed in 1.0 mL of a 0.05 mg mL⁻¹ enzyme solution buffered at pH 5 for lipase from Candida rugosa adsorption and 2.0 mg of SmMZMs was dispersed in 1.0 mL of a 1.0 mg mL⁻¹ protein solution buffered at pH 6 for ovalbumin adsorption, respectively, and kept in a water bath at 37 °C for 0 to 130 min. The adsorption amount of the protein and enzyme at different adsorption
times was obtained through measuring the UV absorbance of the protein and enzyme solution at $\lambda=280$ nm and 250 nm, respectively, as mentioned above.

**III. Characterization**

Powder XRD patterns were recorded on a Rigaku D/Max 2200PC diffractometer using Cu Kα radiation (40 kV and 40 mA) with the scanning rate of 0.4° min$^{-1}$ for small angle tests and 4° min$^{-1}$ for wide angle tests, respectively. The N$_2$ sorption measurements were performed using Micromeritics Tristar 3000 and Micrometerics ASAP 2020 porosimeters at 77 K for mesoporosity and microporosity, respectively, and the mesoporous specific surface area and the pore size distribution were calculated using the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively. The micropore size distribution and pore volume were calculated by the Horvath-Kawazoe method. FE-SEM (Field Emission Scanning Electron Microscopy) analysis was performed on a JEOL JSM6700F electron microscope. Transmission electron microscopy (TEM) images were obtained on a JEOL-2010F electron microscope operated at 200 kV. $^{27}$Al and $^{29}$Si MAR NMR measurements were performed on a Bruker DMX500 spectrometer and a Bruker DSX300 spectrometer, respectively. A vibrating magnetometer (PPMS Model 6000 Quantum Design) was used to study the magnetic properties. The UV/Vis absorbance spectra were measured using a Shimadzu UV-3101PC spectrophotometer.
Scheme S1. The schematic process of superparamagnetic mesoporous zeolite microspheres (SmMZMs) in three steps: (A) adding Mesoporous zeolite microspheres (MZMs) sample into vessel and vacuumizing; (B) pump being switched off and iron nitrate solution filled in; (C) decomposition iron nitrate and formation of magnetic nanoparticles in mesopore channels by calcination and reduction.
Fig. S1 SEM images (a, b) of the prepared sample SmMZMs

Fig. S2 TEM image (a) of sample SmMZMs and the HRTEM image (b) of Fe₃O₄
Fig. S3 Separation of SmMZMs nanoparticles from suspension without the magnetic field (a) and under an external magnetic field (b)
Fig. S4 Adsorption amount of lipase from Candida rugosa in SmMZhMs at different PH values (a) and adsorption amounts of lipase from Candida rugosa at pH 5 in SmMZHMs as a function of time (b).
Fig. S5 XRD pattern of the prepared sample MZMs (a), MZMs-Fe$_2$O$_3$ (hematite, b) and SmMZMs (c).

Table S1 Structural parameters of samples MZMs, SmMZMs and Conventional ZSM-5

<table>
<thead>
<tr>
<th></th>
<th>BET (m$^2$/g)</th>
<th>Micropore Volume (cm$^3$/g)</th>
<th>Mesopore Volume (cm$^3$/g)</th>
<th>Mesopore Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZMs</td>
<td>394</td>
<td>0.17</td>
<td>0.70</td>
<td>13</td>
</tr>
<tr>
<td>SMMZMs</td>
<td>382</td>
<td>0.17</td>
<td>0.67</td>
<td>13</td>
</tr>
<tr>
<td>Conventional ZSM-5</td>
<td>124</td>
<td>0.06</td>
<td>/</td>
<td>/</td>
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Reference: