Supporting Information

Thermo-sensitive Metal Chelation Dual-template Epitope Imprinting Polymer using Distillation-precipitation Polymerization for Simultaneous Recognition of Human Serum Albumin and Transferrin

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EXPERIMENTAL SECTION

Reagents.

Multi-walled carbon nanotubes (CNTs) and acrylic acid (AA) were provided by TCI (Tokyo, Japan). Iron nitrate nonahydrate ($Fe(NO_3)_3•9H_2O$), polyethylene glycol 2000 (PEG), anhydrous sodium acetate (NaAc) and ethylene glycol ($C_2H_6O_2$) were purchased from Feng Chuan Chemicals (Tianjin, China). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4•12H_2O$), sodium dihydrogen phosphate hydroxide ($NaH_2PO_4•2H_2O$), sodium dodecyl sulfate (SDS), polyvinylpyrrolidone K-30 (PVP) and ammonium hydroxide ($NH_3•H_2O$, 25%) were obtained by GuangFu Fine Chemicals (Tianjin, China). Tetraethoxysilicane (TEOS), 3-methacryloyloxypropyltrimethoxysilane (MPS), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN) were bought from J&K Scientific Ltd. (Beijing, China). Zinc acrylate ($C_6H_6O_4Zn$) was provided by Aladdin Bio-Chem Technology (Shanghai, China). Acetonitrile (ACN), methanol (MeOH, 99.5 %), acetic acid (HAc, 100%) and ethanol (EtOH, 100%) were purchased from Concord Technology Co., Ltd (Tianjin, China). N-isopropylacrylamide (NIPAM), chromatographic grade acetonitrile (ACN) and analytical grade trifluoroacetic acid (TFA) were provided by Sigma–Aldrich Co. (Silicon Valley, USA). Phosphate buffer solution (PBS) was prepared via dissolving $Na_2HPO_4•12H_2O$ and $NaH_2PO_4•2H_2O$ into distilled water.

Cytochrome c (Cyt C), bovine serum albumin (BSA), and the human blood sample were purchased from Solarbio (Beijing, China). Human serum albumin (HSA), transferrin (Trf) and myoglobin (Mb) were provided by Sigma–Aldrich Co. (Silicon Valley, USA). The peptide chains including C-terminal fragment (AASQAALGL) of HSA and C-terminal fragment (LEACTFRRP) of Trf were synthesized by GL Biochem Co. Ltd (Shanghai, China).

Characterization.

The morphology of MCNTs, MCNTs@SiO_2, MCNTs@MPS and MCNTs@D-EMIP

were scanned using a JEM100CXII transmission electron microscope (TEM) (JEOL, Japan). Fourier-transform infrared (FT-IR) spectra were recorded using a Vector 22 FT-IR spectrophotometer (Bruker, Germany). Magnetic properties were detected by a LDJ 9600-1 vibrating sample magnetometer (VSM) (LDJ, USA).

Instrument Conditions of HPLC for Detection of Proteins.

HPLC analysis was performed using a high performance liquid chromatograph (LC-20AD, Shimadzu, Japan). Gradient elution was adopted with mobile phase A (ACN with TFA [0.1%, v/v]) and mobile phase B (filtered water with TFA [0.1%, v/v]) with a flow rate of 0.7 ml/min. Protein mixture (HSA, Trf, Cyt C and Mb) and human blood sample were detected with HPLC using a reversed-phase column (Agilent C8, New York, USA) at the wavelength of 214 nm with gradient elution. Within 77 min, the content of mobile phase B (filtered water with 0.1% TFA) changed from 80% to 40%.

RESULTS AND DISCUSSION

The utilization of epitopes were calculated by the following formula:

$$UE = \sum C_e V_e / m_0 \qquad \text{Equation S1}$$

where C_e (mg mL^{-1}) is the concentration of epitope in eluent, V_e (mL) is the volume of the eluent, m_0 (mg) is the mass of the epitope added originally.

Cross-link degree (CLD) also influenced the final imprinting efficiency, which was calculated by the equation as follows:

$$CLD = n_{(\text{cross-linker})} / n_{(\text{cross-linker and functional monomer})} \qquad \text{Equation S2}$$

where $n_{(\text{cross-linker})}$ is the mole amount of cross-linker, $n_{(\text{cross-linker and functional monomer})}$ is the the total mole amounts of cross-linker and functional monomer.

The amount of protein adsorbed by MCNTs@D-EMIP was calculated according to the following formula:

$$Q = (C_0 - C_e)\, V/m \qquad \text{Equation S3}$$

where Q (mg g^{-1}) is the adsorption capacity, C_0 (mg mL^{-1}) is the initial protein

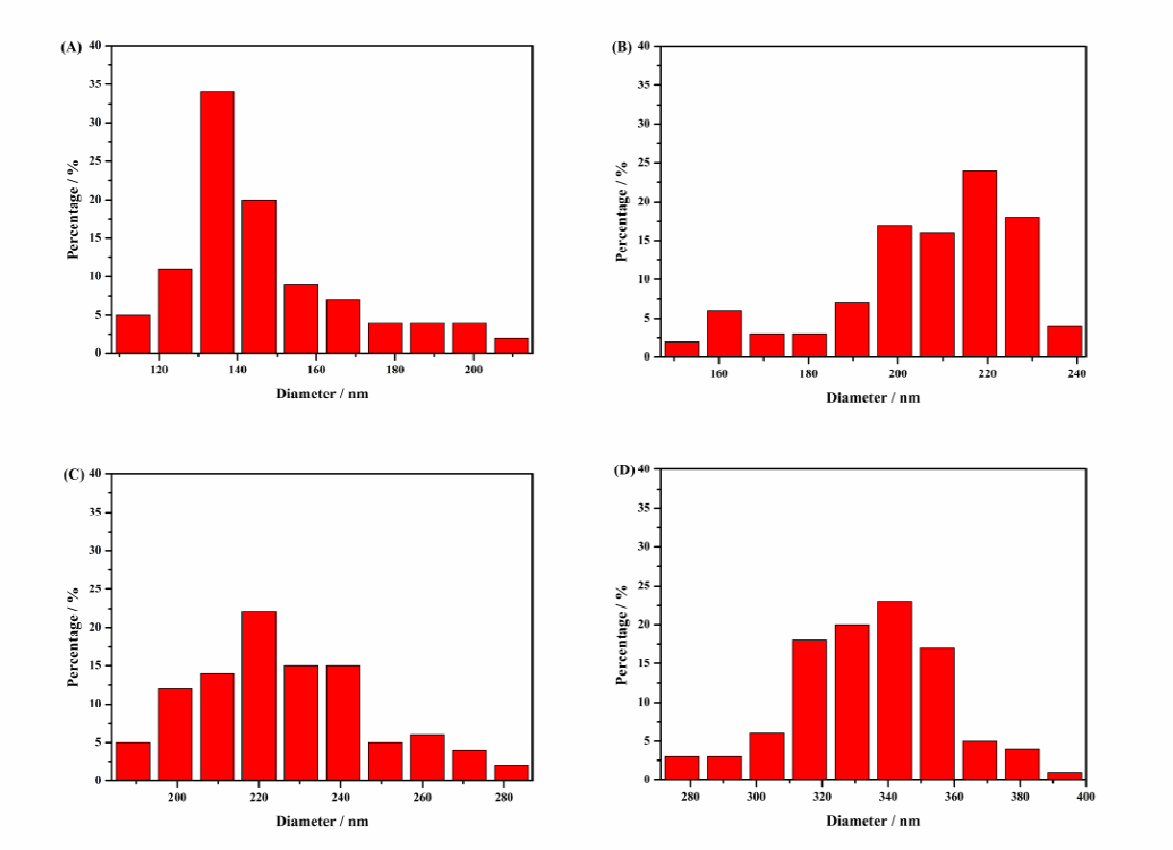
concentration, C_e (mg mL^{-1}) is the supernatant protein concentration after incubation, V (mL) is the volume of the protein solution and m (g) is the mass of MCNTs@D-EMIP used.

The imprinting factor (IF) was used to judge the imprinting effect of the epitope imprinting polymer for the target protein and the selectivity coefficient (K) represents the selectivity property. The IF and K were calculated using the formula as follows:

$$IF = Q_{MIP} / Q_{NIP} \qquad \text{Equation S4}$$

$$K = IF_{tar} / IF_{com} \qquad \text{Equation S5}$$

where Q_{MIP} and Q_{NIP} (mg g^{-1}) are the adsorption capacities of proteins for MCNTs@D-EMIP and MCNTs@D-ENIP, respectively; IF_{tar} and IF_{com} are the imprinting factors for target protein and competitive protein, respectively.



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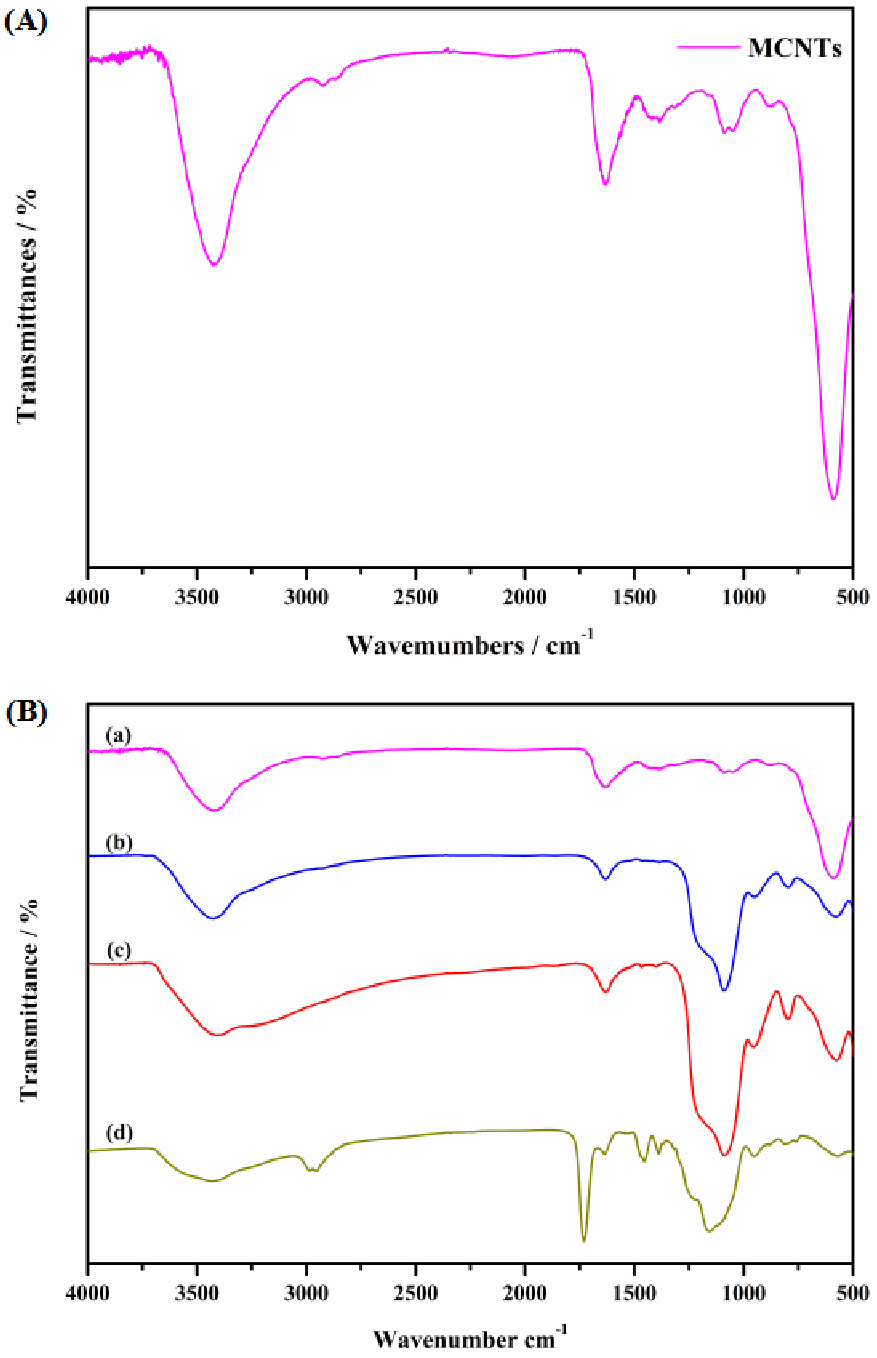

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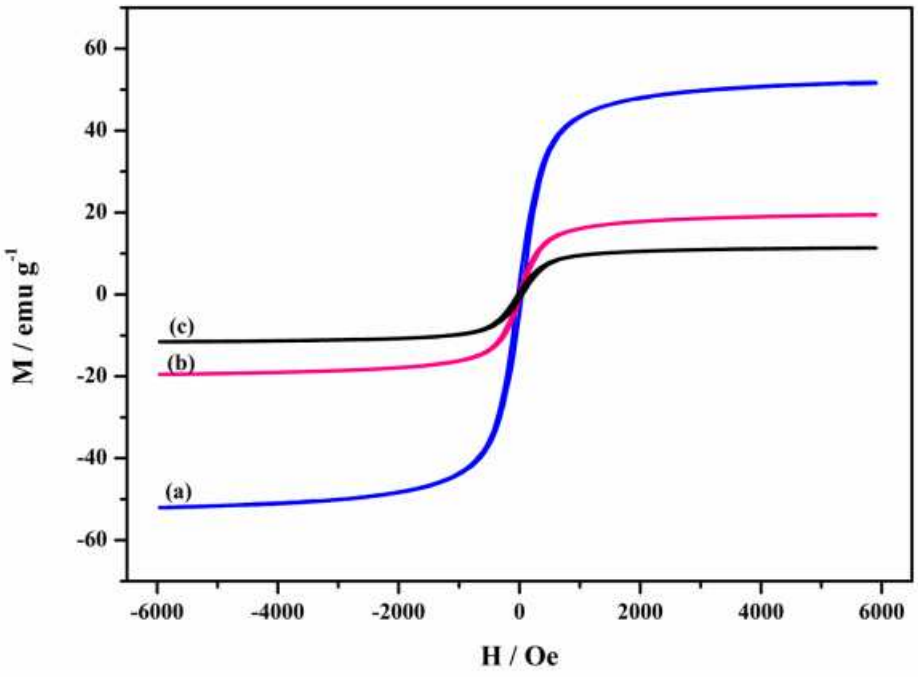

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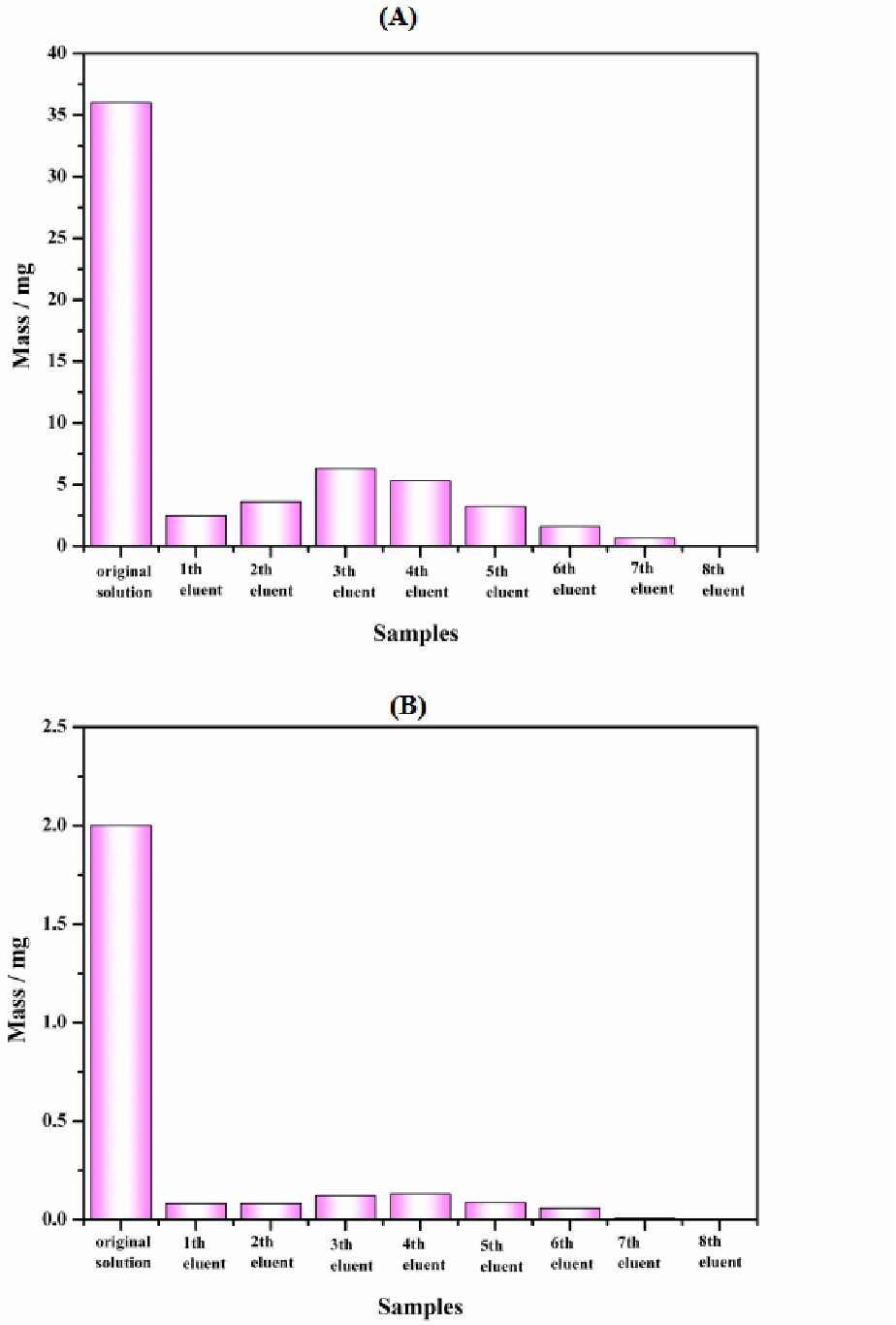

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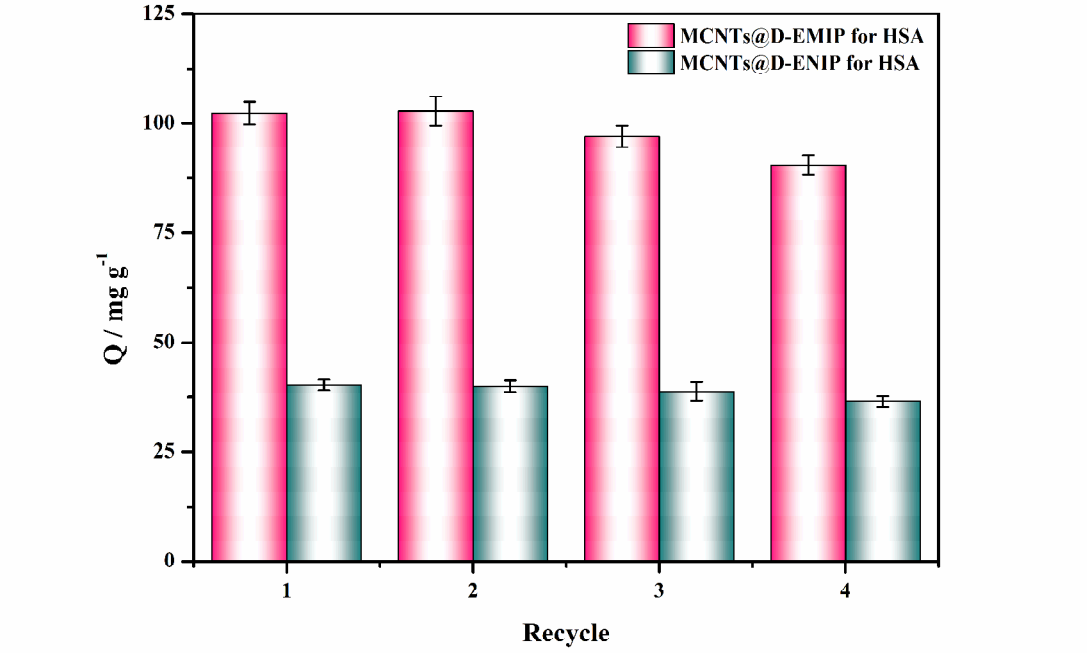

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	HSA	Trf	BSA	Mb	Cyt C
Q_{MIP} / mg g^{-1}	103.67	68.48	25.24	12.71	21.49
Q_{NIP} / mg g^{-1}	40.31	31.55	15.96	8.76	18.11
IF	2.57	2.17	1.58	1.45	1.19
$K(IF_{HSA}/IF_{com})$			1.63	1.77	2.16