# Fabrication of magnetic $\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathbf{P}($ DVB-co-MAA) microspheres using hollow microspheres as templates for protein separation 

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Fig. S1. (A and B) SEM images and TEM images of DVB cross-linked hollow P (DVB-co-MAA) microspheres;
(C and D) SEM images and TEM images of MBA cross-linked hollow P (MBA-co-MAA) microspheres.


Fig. S2. (A) SEM images of $\mathrm{P}(\mathrm{DVB}-\mathrm{co}-\mathrm{MAA})$ hollow microspheres (B) $\mathrm{P}($ HEMA-DVB-MAA) (HEMA:MAA=1:1) hollow microspheres, (C) P(HEMA-DVB-MAA) (HEMA:MAA=6:1) hollow microspheres.


Fig. S3 (A) Particle size distribution of $\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathrm{P}(\mathrm{DVB}-\mathrm{co}-\mathrm{MAA})$ microspheres; (B) Particle size distribution of $\mathrm{Fe}_{3} \mathrm{O}_{4}$ magnetic nanoparticles.


Fig. S4. (A-D) The photos of $\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathrm{P}(\mathrm{DVB}-\mathrm{co}-\mathrm{MAA})$ microspheres suspended in water, after standing for $0 \mathrm{~h}, 4 \mathrm{~h}, 8 \mathrm{~h}$, and 12 h , respectively.

Table 1. Carboxyl content and zeta potential of $\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathbf{P}(\mathrm{DVB}-c o-\mathrm{MAA})$ microspheres, $\mathrm{Fe}_{3} \mathrm{O}_{4} @$ P(HEMA-DVB-MAA)(HEMA:MAA=1:1) microspheres and $\mathrm{Fe}_{3} \mathrm{O}_{4} @$ P(HEMA-DVB-MAA)
(HEMA:MAA=6:1)microspheres

| Microsphere | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ |
| :---: | :---: | :---: | :---: |
| Carboxyl content $(\mathrm{mmol} / \mathrm{g})$ | 0.9005 | 0.4565 | 0.3412 |
| Zeta potential (mv) | -47.87 | -26.55 | -13.32 |



Fig. S5. The hydrodynamic diameter and zeta potential of $\mathrm{Fe}_{3} \mathrm{O}_{4} @$ P(DVB-co-MAA) microspheres. The test was repeated three times.


Fig. S6. SDS-PAGE analysis of magnetic $\mathrm{Fe}_{3} \mathrm{O}_{4} @$ P (DVB-co-MAA) microspheres' protein separation. (Separation results of BSA and LYZ solutions. Lane M: protein molecular weight marker; lane 1: original solution before separation; lanes 2 and 3: supernatant and eluted protein after separation at $\mathrm{pH}=4.7$, respectively. Lanes 4 and 5: supernatant and eluted protein after separation at $\mathrm{pH}=9$, respectively.)


Fig. S7. Reuse of Microsphere $1\left(\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathrm{P}(\mathrm{DVB}-\mathrm{co}-\mathrm{MAA})\right.$ ), Microsphere $2\left(\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathrm{P}(\mathrm{HEMA}\right.$-DVB-MAA) (HEMA : MAA=1:1)), and Microsphere 3 ( $\mathrm{Fe}_{3} \mathrm{O}_{4} @ \mathrm{P}(\mathrm{HEMA}-\mathrm{DVB}-\mathrm{MAA})$ (HEMA : MAA=6:1)).

