Magnetic quantitative immunoanalysis of carcinoembryonic antigen by ICP-MS with mercury labels

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Supplementary material.

Preparation of nanostructured silica-coated magnetite

3.5 g IDA was dissolved in 10 mol L^{-1} sodium hydroxide aqueous solution (50 mL), and the solution was adjusted to pH 11. After magnetic stirring for 1.0 h in an ice-bath, 1.0 g GLYMO was added dropwise under magnetic stirring within 0.5 h. The mixture was heated to 65 ^{o}C and maintained for 6 h, then the reaction system was cooled down to 0 ^{o}C in ice-bath. The above procedure was repeated twice. Finally, the obtained IDA-derived silane coupling agent solution (GLYMO-IDA) was adjusted to pH 6 with concentrated HCl for next grafting reaction on the surface of magnetite nanoparticles (MNPs) [1].

The MNPs were prepared by the conventional co-precipitation method with minor modifications [2]. $FeCl_3 \cdot 6H_2O$ (11.68 g) and $FeCl_2 \cdot 4H_2O$ (4.30 g) were dissolved in 200 mL deionized water under nitrogen gas with vigorous stirring at 85 °C. Then, 30 mL 30% $NH_3 \cdot H_2O$ was added to the solution. The color of bulk solution turned from orange to black immediately. After cooling to room temperature, the suspension was washed sequentially with deionized water (6×250 mL) and ethanol (3×250 mL). The cleaned magnetite was stored in ethanol at a concentration of 40 g L^{-1}. Then, the magnetite suspension prepared above (20 mL) was placed in a 500 mL round-bottom flask and a magnetic separation was processed. The supernatant was removed, and 10 mL TEOS was added, followed by glycerol (60 mL) and ethanol (100 mL) addition. The pH of the suspension was adjusted to 4.6 using glacial acetic acid, and the mixture was then stirred and heated to 90 °C, maintained for 2 h under a nitrogen atmosphere. Then the suspension was cooled down to room temperature, washed with ethanol (3×500 mL), and then dried in vacuum at 60 ^{o}C.

0.20 g of prepared MNPs were washed with high purity deionized water, and then homogeneously dispersed in 100 mL ethanol, followed by a dropwise addition of 10 mL GLYMO-IDA. After stirring at room temperature for 6 h, the product of Fe_3O_4@SiO_2@IDA microspheres was collected with a magnet and washed repeatedly with ethanol and water, and finally dried in vacuum at 60 ^{o}C.

Elution conditions

In order to completely dissociate the mercury labeled sandwich type immunocomplex and released mercury from magnetic nanoparticles, the effect of different eluents (pH 2.0, including formic acid, nitric acid, acetic acid, citric acid and hydrochloric acid) on the concentration of released mercury was studied. The experimental results in Fig. S6 indicated that the diluted nitric acid (pH 2.0) was the most effective to remove mercury from the captured mercury labels. Consequently, diluted nitric acid was selected as the eluent in this study.

The pH effect of eluent (optimized by different concentration of nitric acid) on the signal intensity of ^{202}Hg was also studied and the experimental results were given in Fig. S7. As could be seen, the mercury labeled sandwich type immunocomplex could be released easily and quantitatively when the pH value of nitric acid was lower than 1.5. Accordingly, diluted nitric acid of pH 1.0 was selected as the eluent.

To study the effect of the elution volume on the signal intensity of released mercury, six portions of 15 μL of eluent (pH 1.0, totally 90 μL) were used to continuously release Hg^{2+} from the mercury labeled immunocomplex adsorbed on the MNPs, and the ^{202}Hg in the six eluents (each 15 μL) were determined by ICP-MS. As shown in Fig. S8, 45 μL of eluent was sufficient to release Hg^{2+} quantitatively. Finally, 50 μL of eluent (pH 1.0) was employed as the optimized elution conditions.

The effect of elution time in the range of 1-30 min on the signal intensity of released mercury was investigated by using 50 μL HNO_3 (pH 1.0) as the eluent. The results represented in Fig. S9 indicated that no obvious fluctuation of ^{202}Hg intensity was observed after 5 min elution. Consequently, 10 min was selected for the following experiments.

Reference

1. X. Q. Xu, C. H. Deng, M. X. Gao, W. J. Yu, P. Y. Yang, and X. M. Zhang, *Adv. Mater.,* 2006, **18**, 3289-3294.

2. H. Deng, X. L. Li, Q. Peng, X. Wang, J. P. Chen, and Y. D. Li, *Angew. Chem.-Int. Edit.*, 2005, **44**, 2782-2785.

Fig. S1. Scheme about the synthesis of MNPs-anti-CEA conjugate. (Monoclonal CEA antibody (L1C00205) was conjugated to the carboxyl-modified magnetic nanoparticles with coupling reagents EDC and NHS)

Fig. S2 The effect of blocking time in the range of 0-120 min on the immunoassay was studied, sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S3. The effect of total volumes of PBST (50-300 μL, 50 μL used each time) on the elimination of nonspecific adsorption. sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S4. The effect of incubation time (5 to 60 min) on the signal intensity of mercury in the eluent for immunoreactions between first antibody and CEA. sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S5. Effect of incubation time (5 to 60 min) of the second step for immunoreactions between second antibody and CEA. sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S6. The effect of different eluents (pH 2.0, including formic acid, nitric acid, acetic acid, citric acid and hydrochloric acid) on the concentration of released mercury. sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S7. The pH effect of eluent (optimized by different concentration of nitric acid) on the signal intensity of mercury, sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S8. The effect of the elution volume on the signal intensity of released mercury, sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL

Fig. S9. The effect of elution time in the range of 1-30 min on the signal intensity of released mercury. sample volume: 50 μL; CEA: 20 μg L^{-1}; blocking buffer: 50 μL; eluent: 50 μL HNO_3 (pH 1.0)

Fig. S1

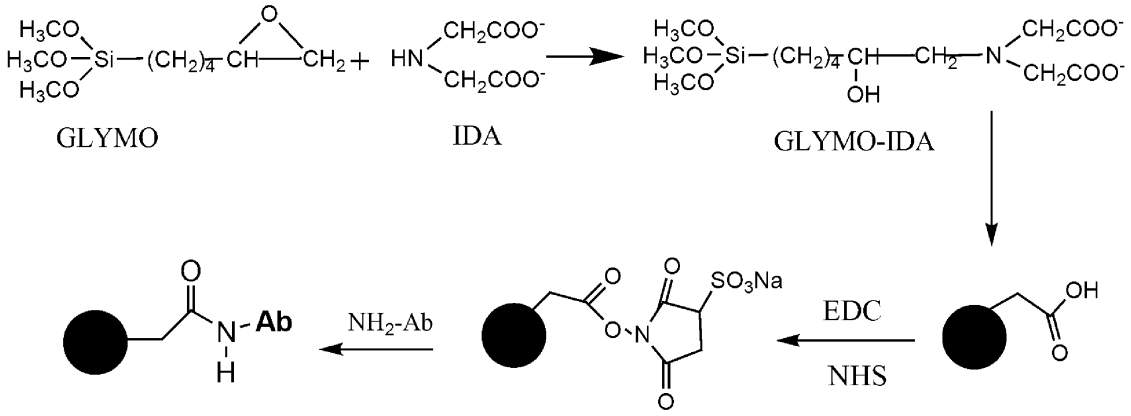

Fig. S2

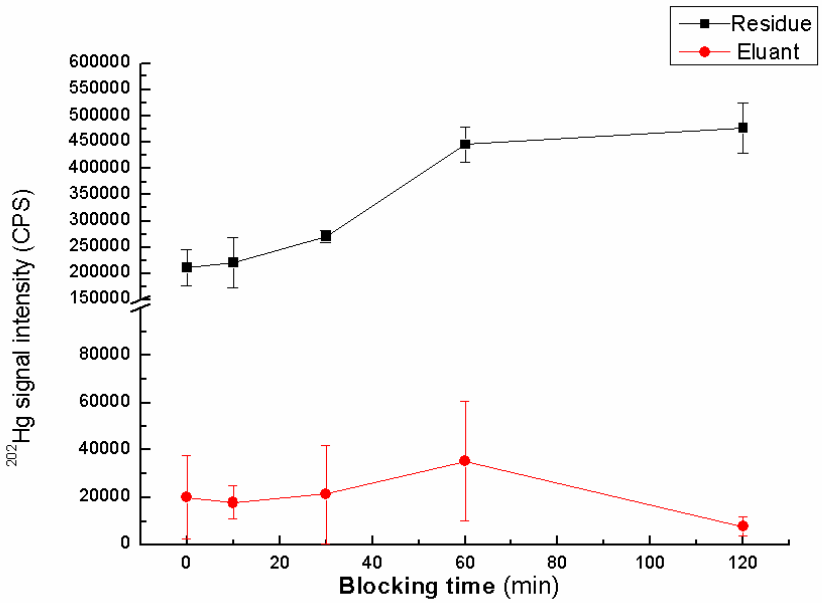

Fig. S3

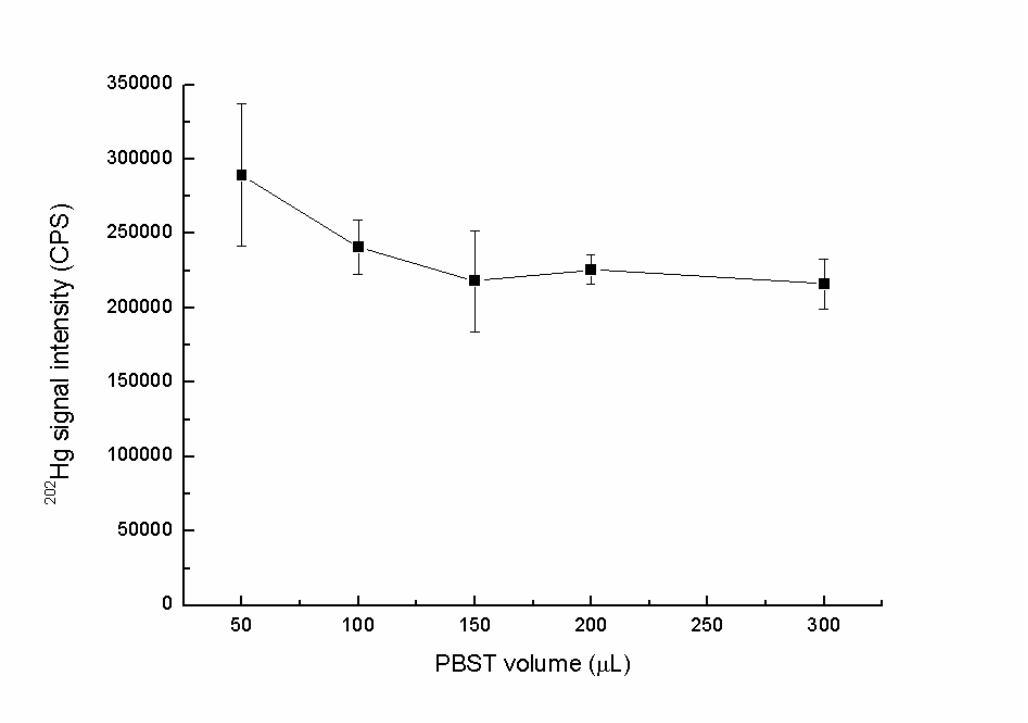

Fig. S4

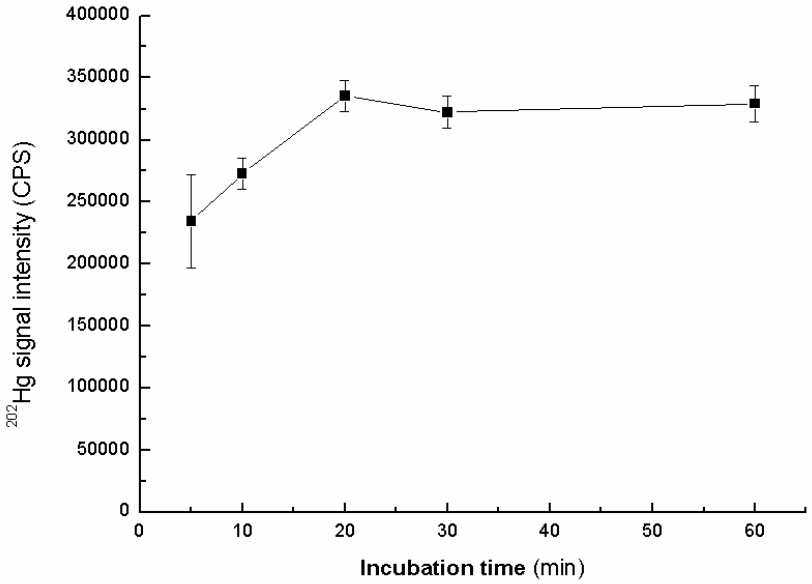

Fig. S5

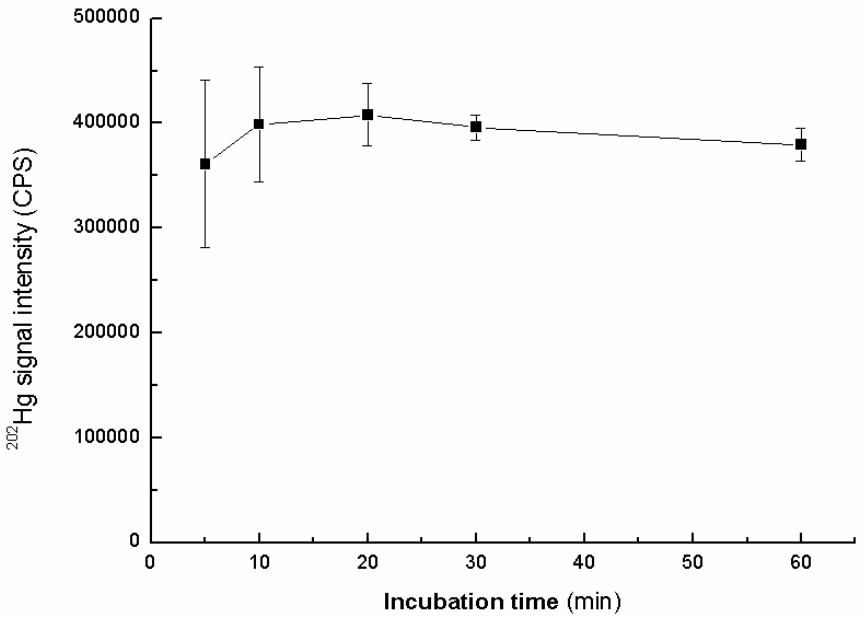

Fig. S6

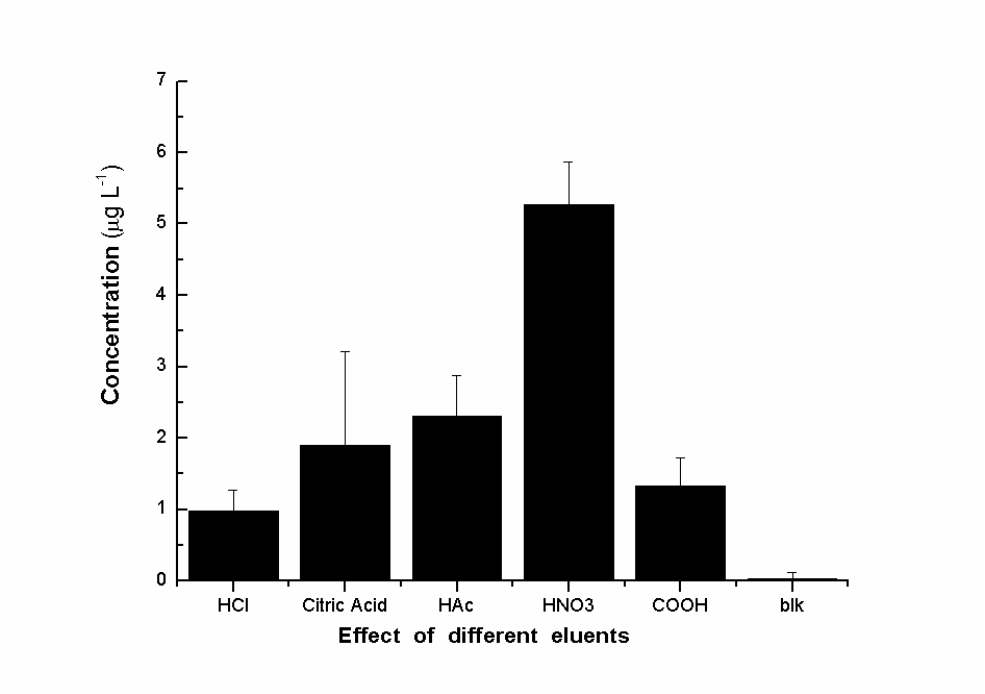

Fig. S7

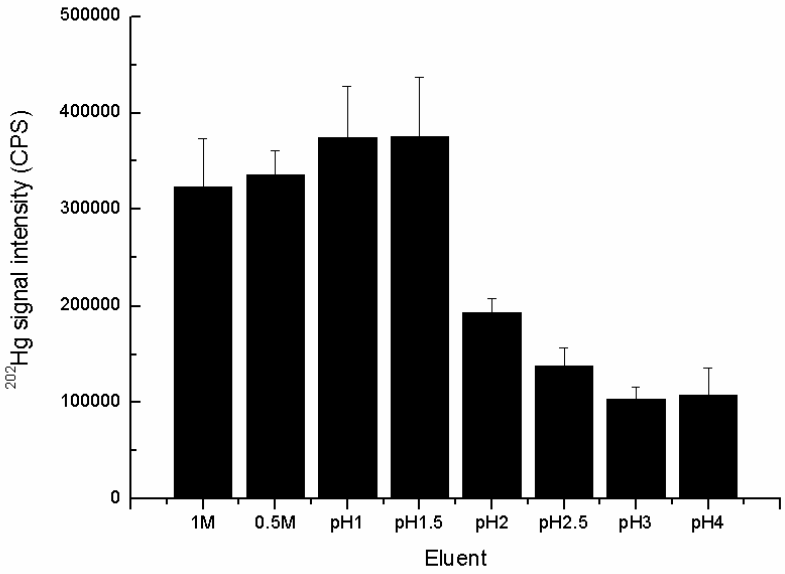

Fig. S8

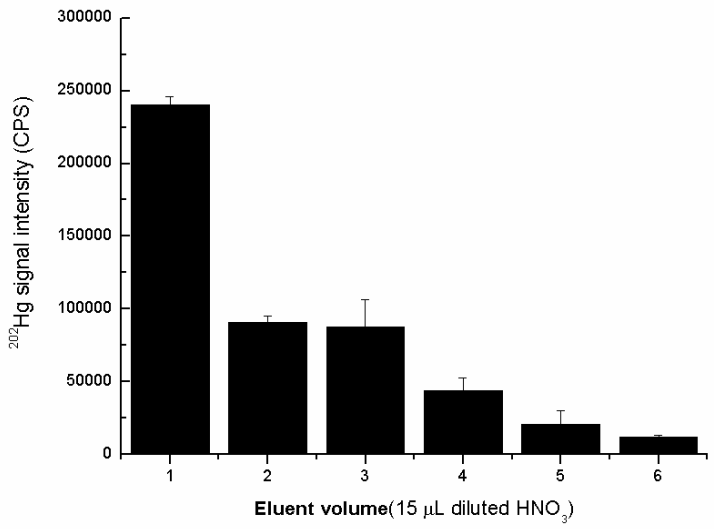

Fig. S9