

# **Magnetic Solid Phase Microextraction on a Microchip Combined with Electrothermal Vaporization – Inductively Coupled Plasma Mass Spectrometry for Determination of Cd, Hg and Pb in Cells**

Beibei Chen<sup>1</sup>, Shujing Heng<sup>1</sup>, Hanyong Peng<sup>1</sup>, Bin Hu<sup>1\*</sup>, Xu Yu<sup>1</sup>, Zhiling Zhang<sup>1</sup>, Daiwen Pang<sup>1</sup>, Xin Yue<sup>2</sup>, Ying Zhu<sup>2</sup>

<sup>1</sup>*Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China*

<sup>2</sup>*College of Life Sciences, Wuhan University, Wuhan 430072, China*

\*Corresponding author: Dr. Bin Hu, E-mail: binhu @ whu.edu.cn, Fax: 86-27-68754067

Supplementary material.

The present document provides further information on the paper mentioned above.

## **Experimental**

Fabrication of PDMS Microfluidic Devices.

Synthesis of  $\gamma$ -MPTS Modified Silica Coated MNPs.

## **Results and Discussion**

**Chip-based Magnetic Solid Phase Microextraction (MSPME).**

Effect of Sample Flow Rate and Sample Volume.

Effect of the Eluent Concentration.

Effect of Eluent Flow Rate and Volume.

Effect of Co-existing Ions.

**Table S1, S2**

**Figures S1 to S6**

## Experimental

**Fabrication of PDMS Microfluidic Devices.** Soft lithography and rapid prototyping with PDMS technology are employed for fabrication of microfluidic devices. A transparent mask patterned with a high resolution laser printer was used to make a master on a silicon wafer with AZ 9260 photoresist. Before PDMS casting, the master was exposed to trimethylchlorosilane vapor for 3 min to avoid the adhesion between PDMS and silicon wafer. For the upper thick layer with flow channels, GE RTV 615 (PDMS) component A and B were mixed at a ratio of 10:1 and cast on the master after air bubbles disappeared. Then two permanent magnets were placed parallelly on both sides of the central channels. After incubated at 80°C for 1.5 h, the solidified PDMS was peeled off and drilled on demand. For the lower thin layer with controlling channels, GE RTV 615 (PDMS) component A and B were mixed at a ratio of 20:1. After air bubbles disappeared, the mixture was spin-coated onto the master (~30 μm thick) and then incubated at 80°C for 30 min. Afterwards, these two layers were treated with oxygen plasma, and the upper layer was aligned onto the lower layer and bonded at 80°C for 30 min. After the ensemble was peeled off and the holes for valves were frilled, the ensemble and a clean glass were exposed to oxygen plasma and then bonded together.

**Synthesis of  $\gamma$ -MPTS Modified Silica Coated MNPs.** The synthesis of  $\gamma$ -MPTS modified silica coated MNPs was the same as in our previous work.<sup>21</sup> Briefly, 11.68 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 4.30 g  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  were dissolved in 200 mL high purity deionized water with nitrogen gas protection and vigorously stirring at 85°C for 2h. Then 20 mL 30% (v/v)  $\text{NH}_3 \cdot \text{H}_2\text{O}$  was added to generate  $\text{Fe}_3\text{O}_4$  MNPs which were subsequently washed and stored in high purity deionized water. Afterwards, 20 mL 40 g L<sup>-1</sup>  $\text{Fe}_3\text{O}_4$  MNPs dispersion, 80 mL 10% (v/v) TEOS and 60 mL glycerol were mixed in a 250 mL round-bottom flask and the pH was adjusted to 4.5 using acetic acid, and the mixture was stirred at 90°C for 2 h under a nitrogen atmosphere. The obtained silica coated MNPs were washed with high purity deionized water, ethanol, again high purity deionized water and stored in high purity deionized water. Then proper amount

of silica coated MNPs was added to ethanol containing 1% (v/v)  $\gamma$ -MPTS and 16 mmol L<sup>-1</sup> acetic acid (pH 4.5), and the mixture was reacted at 60°C for 2 h under stirring and a nitrogen atmosphere. After that, the resulting MNPs were washed with high purity deionized water and ethanol, then dried at room temperature under vacuum. The diameter of the prepared  $\gamma$ -MPTS modified silica coated MNPs was estimated to be 50-70 nm by TEM characterization.

## Results and Discussion

### Chip-based Magnetic Solid Phase Microextraction (MSPME).

**Effect of Sample Flow Rate and Sample Volume.** The sample flow rate influences the retention of target metals on the magnetic solid phase packed-column. To optimize the sample flow rate, 0.5 mL of sample solution was passed through the microchannel with the flow rates ranging from 5 to 20  $\mu\text{L min}^{-1}$ . The experimental results shown in Figure S-1 indicated that quantitative adsorption could be obtained for Cd, Hg and Pb at the sample flow rate less than 15  $\mu\text{L min}^{-1}$ . Thus, a flow rate of 15  $\mu\text{L min}^{-1}$  was used in subsequent experiments.

To study the effect of the sample volume on the adsorption efficiency of target metals, various sample solution (volume of 0.4-1.5 mL) containing 10  $\mu\text{g L}^{-1}$  Cd, Hg and Pb were passed through the microchannel at a flow rate of 15  $\mu\text{L min}^{-1}$  and the adsorption efficiencies were determined. As could be seen in Figure S-2, the quantitative adsorption could be obtained for Cd, Hg and Pb with the sample volumes ranging from 0.4 to 1.5 mL. Thus, the theoretic enrichment factor of 150-fold could be achieved with 1.5 mL sample solution loading and 10  $\mu\text{L}$  0.2 mol L<sup>-1</sup> HNO<sub>3</sub>-2% (m/v) thiourea as the eluent. However, the available biological samples are generally limited, and larger amount of sample volume would require longer analytical time. To balance the enrichment factor, throughput and limited volume of biological samples, a sample volume of 0.5 mL was selected.

**Effect of the Eluent Concentration.** As shown in Figure 3, the adsorption of Hg and Pb was so strong that it is difficult to elute them quantitatively by applying strong acid

alone. To overcome this problem, a mixed solution of HNO<sub>3</sub> and thiourea was studied as the eluent. By fixing the thiourea concentration at 2% (m/v), the effect of HNO<sub>3</sub> concentrations, varying in 0.01-0.5 mol L<sup>-1</sup>, on the relative signal intensity of the target Cd, Hg and Pb was investigated. As shown in Figure S-3, the concentration of HNO<sub>3</sub> in the whole tested range had no obvious influence on the elution of Hg; whereas the relative signal intensity of Cd and Pb was increased with the HNO<sub>3</sub> concentration increasing from 0.01 to 0.1 mol L<sup>-1</sup>, and then kept relatively stable with further increase of HNO<sub>3</sub> concentration. By keeping the HNO<sub>3</sub> concentration at 0.2 mol L<sup>-1</sup>, the effect of thiourea concentration on the relative signal intensity of Cd, Hg and Pb was studied and the results suggested that no obvious fluctuation for Cd signal intensity in the whole tested thiourea concentration range (shown in Figure S-4). However, the addition of thiourea greatly improved the signal intensity of Hg and Pb. Thus, 0.2 mol L<sup>-1</sup> HNO<sub>3</sub>-2% (m/v) thiourea was employed for the elution of target metals in subsequent experiments.

**Effect of Eluent Flow Rate and Volume.** The effect of eluent flow rate and volume on the relative signal intensity of target metals was also examined, respectively. The results indicated that the eluent flow rates, ranging in 1-4 μL min<sup>-1</sup>, had no obvious influence on the relative signal intensity of Cd, Hg and Pb (shown in Figure S-5). By keeping the eluent flow rate at 4 μL min<sup>-1</sup>, five portions of 10 μL 0.2 mol L<sup>-1</sup> HNO<sub>3</sub>-2% (m/v) thiourea (totally 50 μL) was continuously used to elute the target Cd, Hg and Pb, and each fraction was analysed by ETV-ICP-MS subsequently. We found that 10 μL eluent was sufficient to elute all the target Cd, Hg and Pb, and no obvious memory effect was observed (Figure S-6).

**Effect of Co-existing Ions.** Under the optimized conditions, 0.5 mL solutions containing 10 μg L<sup>-1</sup> Cd, Hg and Pb and a certain amount of foreign ion such as K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> (the concentration is 100-50,000 folds of the target metal) were subjected to the chip-based MSPME-ETV-ICP-MS and the interference caused by coexisting ions was investigated. Taking the recoveries of the target metals kept above 85% as the criteria of non-interference, the tolerance limits were 50,000-fold for K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>,

20,000-fold for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ , and 200 fold for  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Zn}^{2+}$ , respectively (Table S-1). For example, the reported elements concentration to Cd concentration in human liver <sup>1,2</sup> are lower than the tolerance folds in this method, which indicate that the method has a good tolerance to matrix interference and is suitable for target metals analysis in biological samples.

## References.

1. P. Q. Zhang, C. Y. Chen, M. Horvat, R. Jacimovic, I. Falnoga, M. Logar, B. Li, J. J. Zhao and Z. F. Chai, *Anal. Bioanal. Chem.*, 2004, **380**, 773-781.
2. S. Caroli, A. Alimonti, E. Coni, F. Petrucci and O. Senofonte, *Crit. Rev. Anal. Chem.*, 1994, **24**, 363-398.

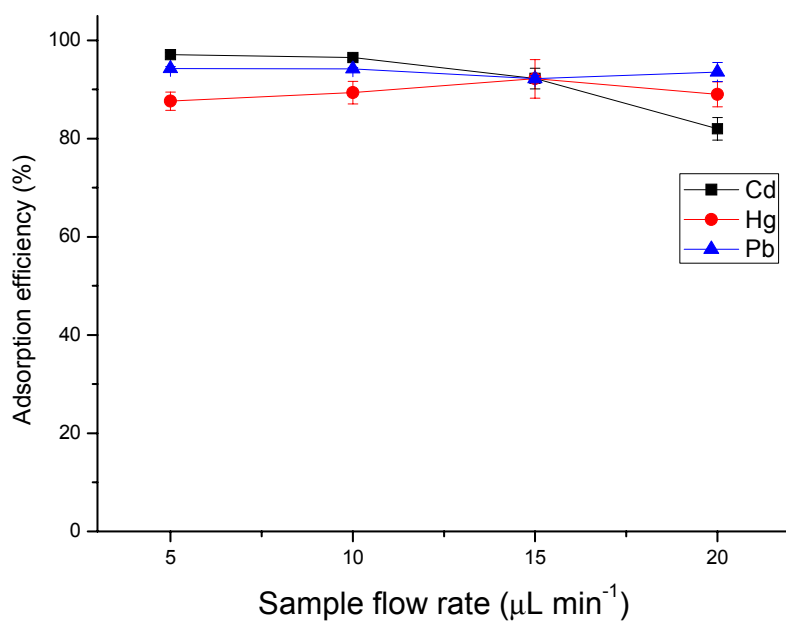
**Table S1** The blank values

Blank value	Cd	Hg	Pb
High purity water (CPS)	14±5.7	325±186	67±39
PBS buffer (CPS)	24±18	211±37	663±551
Cell rupture solution (CPS)	16±2.4	772±186	245±36
0.2 mol L <sup>-1</sup> HNO <sub>3</sub> -2% (m/v) thiourea (CPS)	53±13	1349±252	4902±173
γ-MPTS modified MNPs (CPS)	143±30	1665±293	8308±507
Procedural blank (CPS)	248±27	1567±128	8606±673
Procedural blank (ng L <sup>-1</sup> )	0.41±0.05	4.30±0.35	6.76±0.53

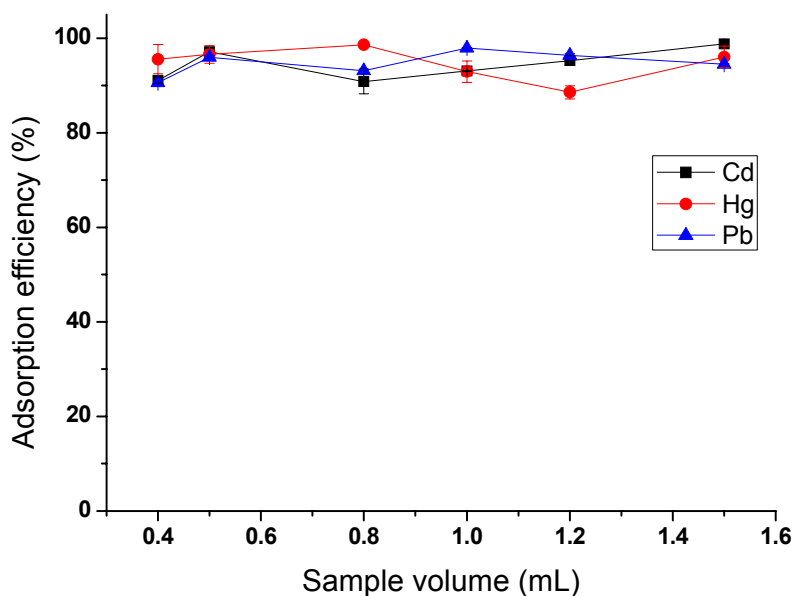
**Table S2** Tolerance folds of coexisting ions to the target elements

Coexisting ions	Tolerance folds <sup>a, b</sup>	Reported elements concentration to Cd <sup>c</sup> in human liver
K <sup>+</sup>	50,000	~1377 <sup>[1]</sup>
Na <sup>+</sup>	50,000	~563 <sup>[1]</sup>
Ca <sup>2+</sup>	20,000	~54 <sup>[1]</sup>
Mg <sup>2+</sup>	20,000	~23 <sup>[2]</sup>
Cu <sup>2+</sup>	200	~2 <sup>[2]</sup>
Fe <sup>3+</sup>	200	~119 <sup>[2]</sup>
Zn <sup>2+</sup>	200	~31 <sup>[2]</sup>
Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup>	50,000	--
SO <sub>4</sub> <sup>2-</sup>	20,000	--

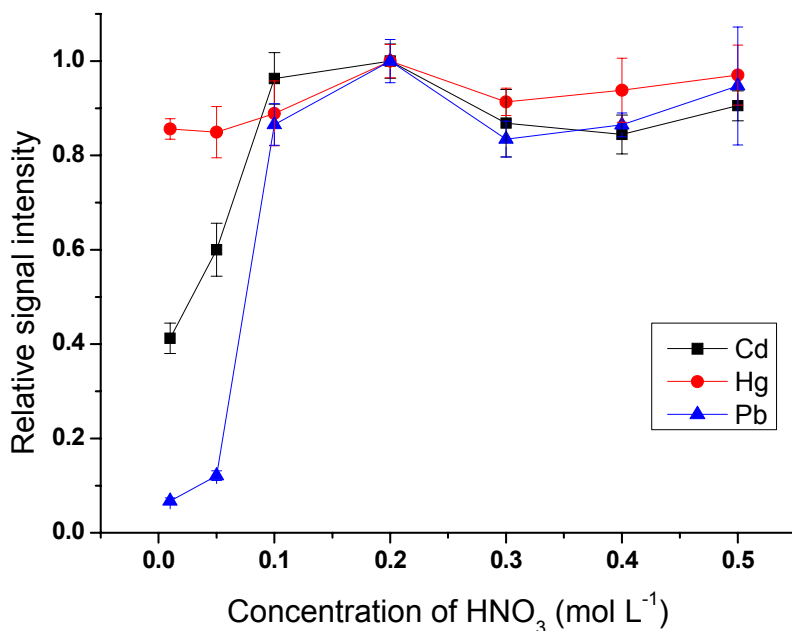
a: Tolerance folds =  $c_M/c_{\text{Cd, Hg, Pb}}$ , M: coexisting ions; b:  $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; c:  $c_{\text{Cd}} = 5.92 \mu\text{g g}^{-1}$



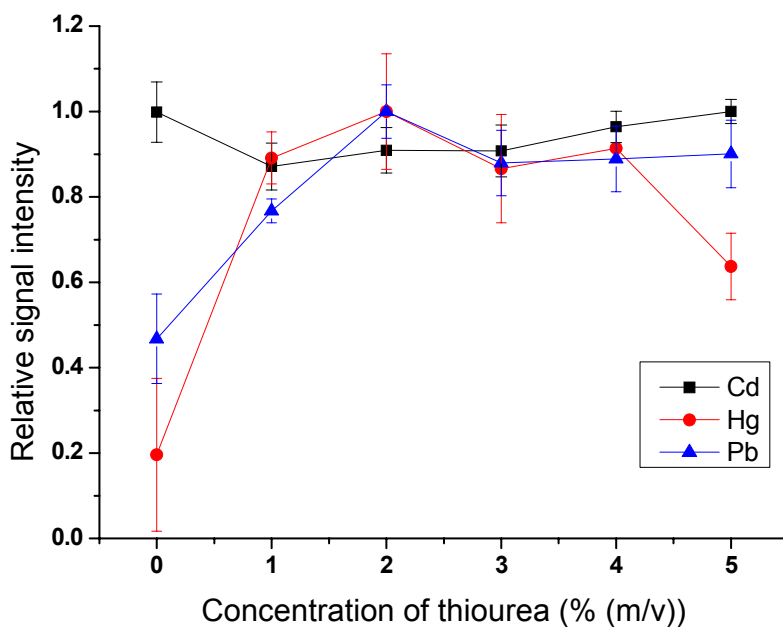
**Fig. S1.** Effect of sample flow rate on the extraction of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample volume: 0.5 mL)



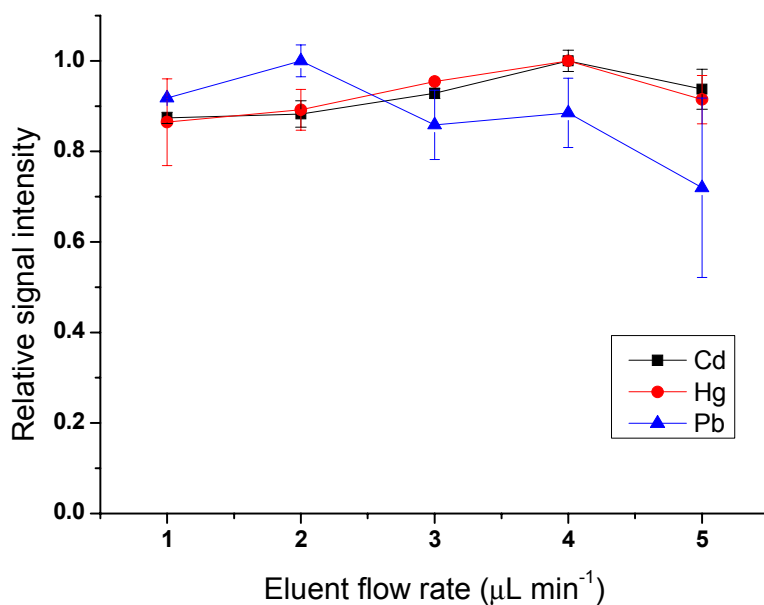
**Fig. S2.** Effect of sample volume on the extraction of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample flow rate:  $15 \mu\text{L min}^{-1}$ )



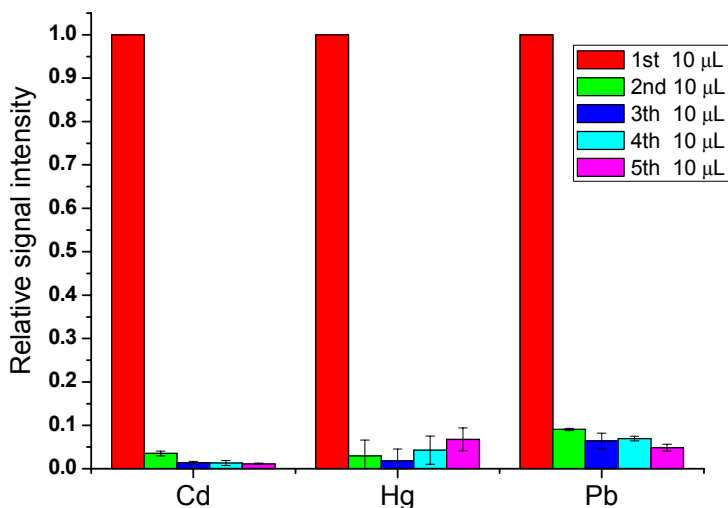
**Fig. S3.** Effect of the concentration of HNO<sub>3</sub> on the relative signal intensity of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample flow rate:  $15 \mu\text{L min}^{-1}$ ; sample volume: 0.5 mL; 2% (m/v) thiourea in elution; eluent flow rate:  $2 \mu\text{L min}^{-1}$ ; eluent volume: 100  $\mu\text{L}$ )



**Fig. S4.** Effect of the concentration of thiourea on the relative signal intensity of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample flow rate:  $15 \mu\text{L min}^{-1}$ ; sample volume: 0.5 mL;  $0.2 \text{ mol L}^{-1}$  HNO<sub>3</sub> in elution; eluent flow rate:  $2 \mu\text{L min}^{-1}$ ; eluent volume: 100  $\mu\text{L}$ )



**Fig. S5.** Effect of eluent flow rate on the relative signal intensity of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample flow rate:  $15 \mu\text{L min}^{-1}$ ; sample volume: 0.5 mL; eluent:  $0.2 \text{ mol L}^{-1} \text{ HNO}_3$ -2% (m/v) thiourea ; eluent volume: 20  $\mu\text{L}$ )



**Fig. S6.** Effect of eluent volume on the relative signal intensity of Cd, Hg and Pb. ( $c_{\text{Cd, Hg, Pb}} = 10 \mu\text{g L}^{-1}$ ; pH: 7.4 (PBS buffer); sample flow rate:  $15 \mu\text{L min}^{-1}$ ; sample volume: 0.5 mL; eluent:  $0.2 \text{ mol L}^{-1} \text{ HNO}_3$ -2% (m/v) thiourea ; eluent flow rate:  $4 \mu\text{L min}^{-1}$ )