Electronic Supplementary Material (ESI) for Analyst.

Chip-based array magnetic solid phase microextraction on-line coupled with inductively coupled plasma mass spectrometry for the determination of trace heavy metals in cells

Han Wang, Zhekuan Wu, Beibei Chen, Man He, Bin Hu*
Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

Supplementary materials

Abbreviations

MNPs	magnetic nanoparticles
APTES	(3-aminopropyl) triethoxysilane
TEOS	tetraethoxysilane
PDMS	polydimethylsilicone
FBS	fetal bovine serum
PBS	phosphate buffer solution
FT-IR	fourier transform infrared spectroscopy
XPS	X-ray photo electron spectroscopy
TEM	transmission electron microscope
ICP-MS	inductively coupled plasma mass spectrometry

Preparation of Fe_3O_4@SiO_2@APTES MNPs

The Fe_3O_4 MNPs were prepared through a solvothermal reaction. Briefly, 11.68 g $FeCl_3 \cdot 6H_2O$ and 4.30 g $FeCl_2 \cdot 4H_2O$ were dissolved in 200 mL high purity deionized water with nitrogen gas protection and vigorously stirring at 85^{o} C. Then 40 mL 30% (v/v) $NH_3 \cdot H_2O$ was added to generate Fe_3O_4 MNPs which were subsequently washed

and stored in high purity deionized water. Afterwards, 4.0 g of prepared Fe_3O_4 MNPs were dispersed in the mixture of isopropanol (100 mL), deionized water (12 mL) and concentrated ammonia aqueous solution (7 mL), followed by the addition of 8 mL TEOS. After stirring at room temperature for 12 h, the Fe_3O_4@SiO_2MNPs were then washed with high purity deionized water and ethanol. And then 4.0 g of prepared Fe_3O_4@SiO_2 MNPs were dispersed in the mixture of ethanol (200 mL), glycerol (100 mL), deionized water (5 mL), followed by the addition of 5 mL APTES. After stirring at 85^o C with nitrogen gas protection for 3 h, the Fe_3O_4@SiO_2@APTES MNPs were washed with high purity deionized water and ethanol.

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 50XT 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 preparation of the upper thick layer (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 permanent magnets were placed parallel on both sides of the central channels. After incubated at 75^o C for 3 h, the solidified PDMS was peeled off and drilled on demand. For the preparation of the lower thin layer (controlling channels), GE RTV 615 (PDMS) component A and B were mixed at a ratio of 15:1. After air bubbles disappeared, the mixture was spin-coated onto the master (~ 50 μm thick) and then incubated at 75^{o}Cfor 30 min. Afterwards, these two layers were treated with oxygen plasma, and the upper layer was aligned onto the lower layer and bonded at 75^{o}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. And the schematic layout of the fabricated chip layer by layer was shown in Fig. S6.

Preparation of magnetic solid phase packed-column on the chip

25 mg Fe_3O_4@SiO_2@APTES MNPs were dispersed in 1 mL high purity deionized water containing 1gL^{-1} CTAB to avoid aggregation[25]. The mixture was sonicated for 15 min to disperse homogeneously. Then the dispersion was introduced into the channel from inlet of A1-A5 (Fig.1) at a flow rate of 4 mL min^{-1} for 5 min with B2-B8 being switched off by the controlling microvalves. When reaching the magnetic zone, the Fe_3O_4@SiO_2@APTES MNPs self-assembled into a solid phase packed-column in the microfluidic channel under the magnetic field. As can be seen in Fig. 1(c), the Fe_3O_4@SiO_2@APTES MNPs were steadily trapped in the channels observed under the microscope. Then the flow rate was decreased to 1 mL min^{-1} for 5 min to consolidate the column. After that, the self-assembled magnetic solid phase packed-column on the chip was dried at room temperature prior to further experiments.

Sample preparation

HepG2, Jurkat T and MCF7 cells were maintained in a humidified incubator containing 5% CO_2 and 95% air at 37 °C. HepG2 and MCF7 cells were incubated in DMEM medium, supplemented with 10% FBS and Jurkat T cells were incubated in RPMI-1640 medium, supplemented with 10% FBS.

After centrifugation (1500 rpm, 5 min), the supernatant was removed and the cells were washed with PBS twice. Then the amount of cells was counted, and the density of cells solution were diluted to 120,000 HepG2 cells, 600,000 Jurkat T cells and 160,000 MCF7 cells per millilitre (in PBS), respectively. Each 0.5 mL of cell dispersion solution was subjected to ultrasonication for 30 min, and then introduced into microextration channel on chip directly.

To investigate the extractable species of target metal in the proposed system, only HepG2 cell was used. The density of cell lysis samples is 2×10^6 per millilitre, and 30

μL sample was injected into the SEC column after filtration. The target metal ions prepared in eluent solution involved a mixed standard solution (10, 30, 0.5, 0.5, 0.5 and 0.5 ng mL^{-1} for Cu, Zn, Cd, Pb and Bi, respectively) in 0.5 mol L^{-1} HNO_3 containing 2% thiourea and 30 μL was injected into the SEC/RP-HPLC column after filtration.

As a comparison, the cell samples were also analyzed by conventional ICP-MS with acid digestion. After centrifugation (1500 rpm, 5 min), the supernatant was removed and the cells were washed with PBS twice. Then the amount of cells was counted, and 0.5 mL of cell dispersion was put into PTFE digestion vessels. After adding 2.0 mL of HNO_3, the vessels were put on an electric hot plate (120 ºC) for 2 h and maintained at 80 ºC. When the samples were nearly dryness, the digest was transferred and diluted with 5% HNO_3 to 1.0 mL. The obtained solution was subjected to ICP-MS measurement directly.

Optimization of microextraction conditions

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 microextraction channels with the flow rates ranging from 4 to 25 μL min^{-1}. The experimental results in Fig. S5 indicate that quantitative adsorption can be obtained for Cu, Zn, Cd, Hg, Pb and Bi with a sample flow rate less than 8 μL min^{-1}. Thus, a flow rate of 8 μL 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 0.1-2.0 mL) containing Cu, Zn, Cd, Hg, Pb and Bi each at 6 ng were passed through the microextraction channels on chip at a flow rate of 8 μL min^{-1} and the adsorption efficiencies were determined. As can be seen in Fig. S6, a quantitative adsorption can be obtained for Cu, Zn, Cd, Hg, Pb and Bi with the sample volume ranging from 0.1 to 2.0 mL. In consideration of the enrichment factor,

sample throughput and available amount of cell samples, a sample volume of 0.5 mL was selected in subsequent experiments.

As shown in Fig. 2, the adsorption of the target metals on Fe_3O_4@SiO_2@APTES is decreased rapidly with decreasing the sample pH when the pH is lower than 6, therefore, acid was chosen to elute the retained metals from the Fe_3O_4@SiO_2@APTES. However, the preliminary studies indicate that the adsorption of Hg and Bi is so strong that it is difficult to elute them quantitatively even by using strong acid alone. To overcome this problem, a mixed solution of HNO_3 and thiourea was studied as the eluent. By fixing the HNO_3 concentration at 1.5 mol L^{-1}, the effect of thiourea concentration in the range of 0-4 % (m/v) on the elution of target metal was investigated. As shown in Fig. S7, thiourea with a concentration of higher than 2 % presents a quantitative recovery for Hg and Bi, whereas no obvious effect of thiourea on the elution of Cu, Zn, Cd and Pb is observed in the tested concentration range. At last, the concentration of thiourea was selected as 2% (m/v).

Then the effect of HNO_3 concentration on the elution of target metals was studied by fixing the concentration of thiourea at 2% (m/v) and the results are shown in Fig. S8. As can be seen, Cu, Zn, Cd, Hg, Pb and Bi can be eluted quantitatively in the HNO_3 concentration range of 0.2-1.5 mol L^{-1}. Consequently, 0.5 mol L^{-1} HNO_3 containing 2% (m/v) thiourea was selected as the eluent in further experiments.

The effect of eluent flow rate in the range of 3-10 μL min^{-1} on the desorption efficiency of target metals was examined by online ICP-MS detection mode. The experimental results show that no obvious influence of the eluent flow rate on the desorption efficiency of Cu, Zn, Cd, Hg, Pb and Bi was observed in the eluent flow rate range of 3-6 μL min^{-1}. Subsequently, 5 μL min^{-1} was selected as the eluent flow rate.

By keeping the eluent flow rate of 5 μL min^{-1}, the effect of eluent volume on the desorption of target metals from microchannels was studied with eluent volume varying in the range of 3-35 μL. It was found that 7 μL eluent was enough for a

quantitative desorption of all the analytes. Therefore, the eluent volume of 7 μL was selected for further experiments.

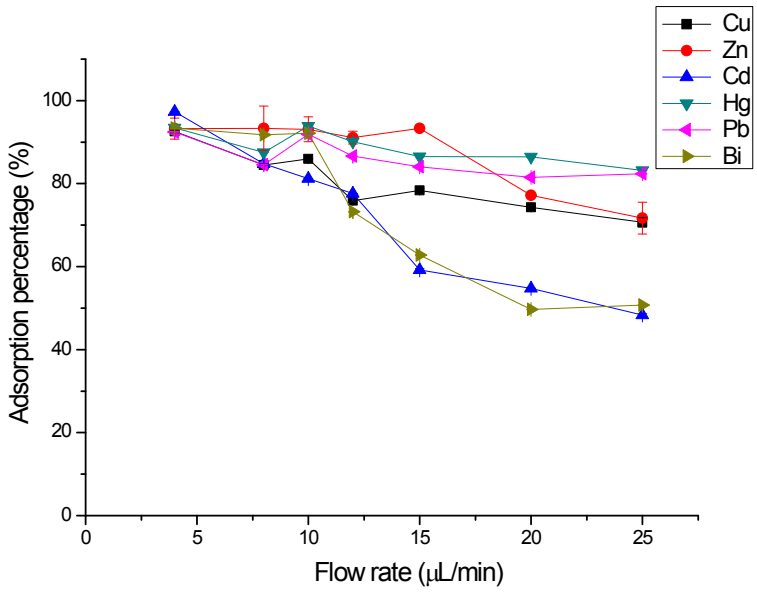


Fig. S1 Effect of flow rate on the extraction of Cu, Zn, Cd, Hg, Pb and Bi. ($C_{Cu, Zn, Cd, Hg, Pb, Bi}$ = 10 μg L^{-1}; sample volume: 0.5 mL)

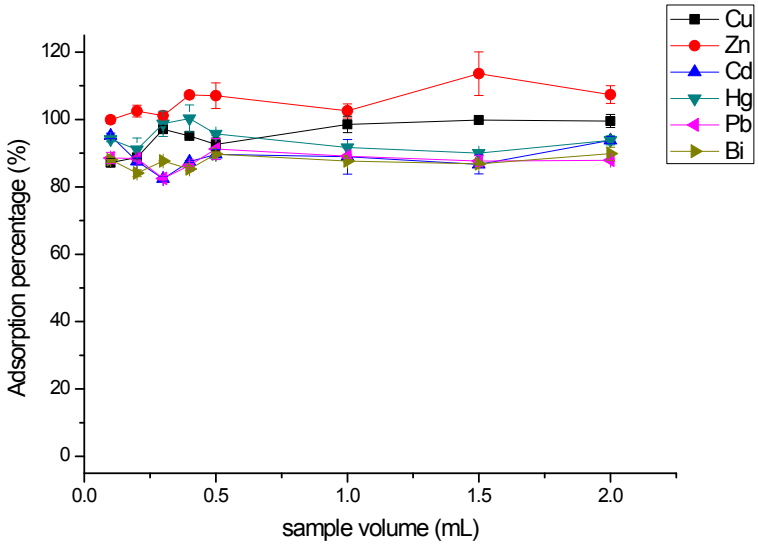
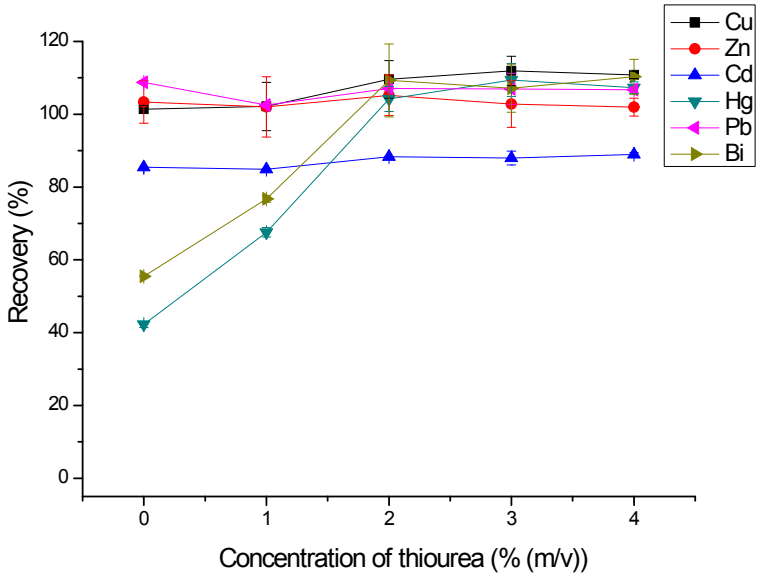


Fig. S2 Effect of sample volume on the extraction of Cu, Zn, Cd, Hg, Pb and Bi. (sample flow rate: 8 μL min^{-1})



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138 **Fig. S3** Effect of concentration of thiourea on the recovery of Cu, Zn, Cd, Hg, Pb and
139 Bi. ($C_{Cu, Zn, Cd, Hg, Pb, Bi}$ = 10 μg L^{-1}; sample flow rate: 8 μL min^{-1}; sample volume: 0.5
140 mL; 1.5 mol L^{-1} HNO_3 in the eluent; eluent flow rate: 3 μL min^{-1}; eluent volume: 0.1
141 mL)

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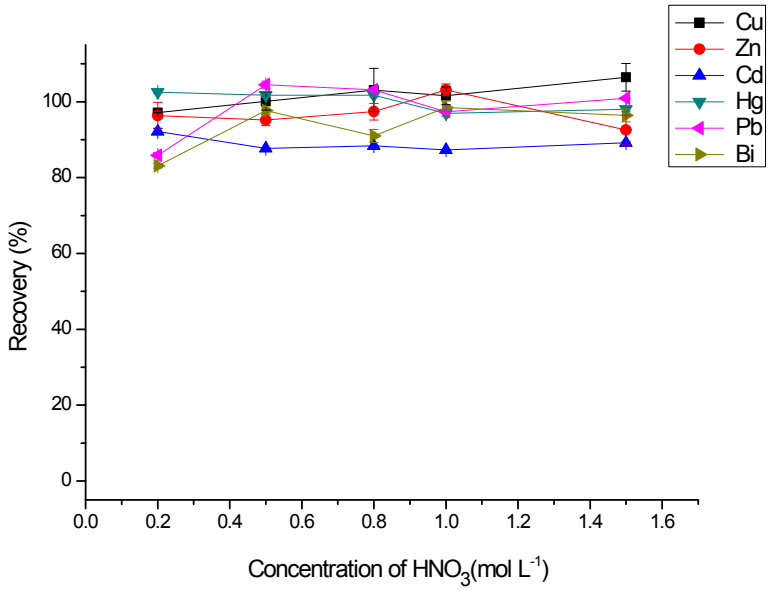

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144 **Fig. S4** Effect of concentration of HNO_3 on the recovery of Cu, Zn, Cd, Hg, Pb and
145 Bi.($C_{Cu, Zn, Cd, Hg, Pb, Bi}$ = 10 μg L^{-1}; sample flow rate: 8 μL min^{-1}; sample volume: 0.5
146 mL; 2% thiorea in the eluent; eluent flow rate: 3 μL min^{-1}; eluent volume: 0.1 mL)

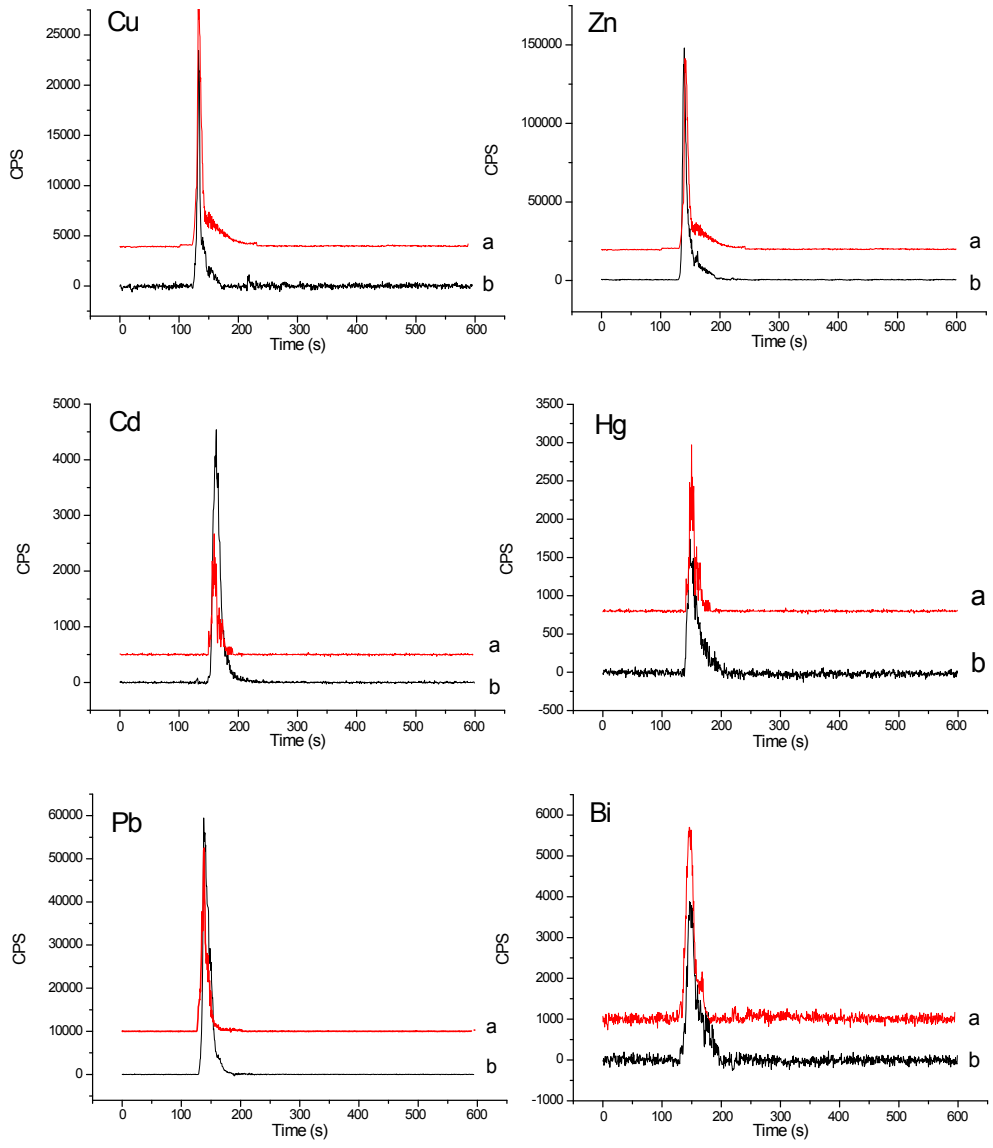
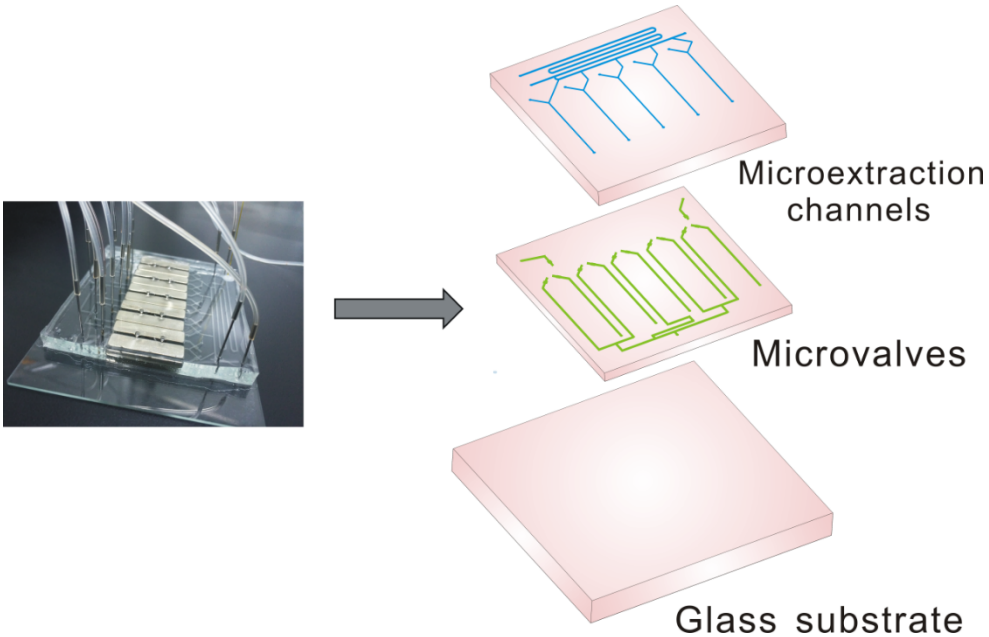


Fig. S5 HPLC-ICP-MS chromatograms for the eluent from the chip-based MSPME of cell lysis solution (a) and the mixed standard solution of target metal ions prepared in 0.5 mol L^{-1} HNO_3 containing 2% thiourea (elution solution) (b).



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172 **Fig. S6** The schematic layout of the fabricated chip layer by layer.

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174 **Table S1 Tolerance folds of coexisting ions to the target elements (c=10 μg L^{-1})**

Coexisting ions	Tolerance concentration (μg mL^{-1})
K^+	5000
Na^+	5000
Ca^{2+}	2000
Mg^{2+}	2000
Fe^{3+}	5
Al^{3+}	5
Cl^-	5000
NO_3^-	5000
SO_4^{2-}	2000
HCO_3^-	5000
$PO4^{3-}$	5000

175 Note: The tolerance to the co-existing ions was evaluated in the presence of all tested ions.

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179 **Table S2 Processes of chip-based MSPME**

Microvalve (open)	Process	Solution
B1	Sample introduction	Sample solution
B2	Elution of channel 1	Eluent
B7	Wash the channel	5%(v/v) nitric acid
B8	Separate the liquid	N_2
B3	Elution of channel 2	Eluent
B7	Wash the channel	5%(v/v) nitric acid
B8	Separate the liquid	N_2
B4	Elution of channel 3	Eluent
B7	Wash the channel	5%(v/v) nitric acid
B8	Separate the liquid	N_2
B5	Elution of channel 4	Eluent
B7	Wash the channel	5%(v/v) nitric acid
B8	Separate the liquid	N_2
B6	Elution of channel 5	Eluent
B7	Wash the channel	5%(v/v) nitric acid
B8	Separate the liquid	N_2

180 Note: The eluent was 0.5 mol L^{-1} HNO_3 containing 2% thiourea

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