



Novel Hg²⁺-imprinted polymers based on thymine–Hg²⁺–thymine interaction for highly selective preconcentration of Hg²⁺ in water samples

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HIGHLIGHTS

- Novel functional monomer T-IPTS tailor-made for Hg²⁺ was designed and synthesized.
- Hg²⁺ imprinted polymers, Hg-IIP-T, was prepared based on T–Hg²⁺–T interaction.
- Hg-IIP-T shows excellent selectivity towards Hg²⁺.
- Potential applications: preconcentration and/or removal of Hg²⁺ from polluted water.

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ABSTRACT

A novel functional monomer T-IPTS, 3-isocyanatopropyltriethoxysilane (IPTS) bearing thymine (T) bases, was synthesized for imprinting Hg²⁺. Then a novel Hg²⁺ ionic imprinted polymers (IIPs) based on thymine–Hg²⁺–thymine (T–Hg²⁺–T) interactions, i.e. Hg-IIPs-T, were prepared by sol–gel process for the first time in this work. The Hg-IIPs-T exhibited excellent selectivity towards Hg²⁺ over Cd²⁺, Zn²⁺, Pb²⁺, Co²⁺, Mn²⁺, Mg²⁺ and Ca²⁺, due to the specific T–Hg²⁺–T interactions with high selectivity and high affinity. Accordingly, Hg-IIPs-T were used as solid-phase extraction (SPE) sorbents for preconcentration of trace Hg²⁺ in water samples, and satisfactory recoveries ranging from 95.2 to 116.3% were obtained. Also, under optimized conditions, preconcentration factor and detection limit were achieved of 200 and 0.03 µg L^{−1}, respectively. The IIPs-T-SPE proved to be a rapid and high-effective cleanup and enrichment method for trace Hg²⁺ in water samples. More importantly, these results indicated that devising and synthesizing new functional monomers tailor-made for template would become a general promising way to improve the selectivity and stability of IIPs.

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1. Introduction

Molecular imprinting is a versatile technique to create molecular recognition system in three-dimensional cross-linked polymers, namely molecularly imprinted polymers (MIPs) [1–3]. MIPs are synthesized in the presence of template molecules, functional monomers and cross-linking agents by covalent, non-covalent or semi-covalent methods. Binding sites with molecular recognition properties are formed after template molecules have been removed from the polymerized material, leaving behind cavities for the subsequent rebinding process that are complementary

in size and shape to the template molecules [1–3]. In the past few decades, MIPs have aroused extensive attentions and been widely applied in many fields, such as solid-phase extraction (SPE) [4,5] and chemical sensors [6,7], owing to their desired selectivity, physical robustness, thermal stability, as well as low cost and easy preparation. Up to date, most of organic molecules [8,9], inorganic metal ions [10–12] and biological macromolecules [13,14] have been used as templates to prepare MIPs. However, only a small quantity of functional monomers can be used in molecular imprinting, for example, methacrylic acid (MAA), acrylic amide (AA) and 4-vinylpyridine (4-VP), to some extent which restricts the selectivity and the further applications of MIPs. So, it is imperative to devise and synthesize new functional monomers for specific templates imprinting, and thereby for enhanced selective recognition and detection capabilities based on MIPs.

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Ionic imprinted polymers (IIPs) are an important branch of MIPs and are similar to MIPs, but they recognize inorganic ions after imprinting, especially metal ions [3,15,16]. IIPs are often puzzled by the problem of low selectivity because many metal ions have the same charge, similar ionic radius and properties. Mercury ion (Hg^{2+}), an environmentally and toxicologically important element, being a research hotspot [17–20], also has been used as template to prepare Hg^{2+} -IIPs for preconcentration of trace Hg^{2+} [21–23]. However, the most commonly used functional monomers for Hg -IIPs, such as 4-VP and 3-mercaptopropyltrimethoxysilane (MPTAS), can provide coordination for many ions and thereby results in the low selectivity [21–23]. Recently, for many selective and sensitive chemosensors toward Hg^{2+} , thymine (T) bases in DNA sequences have often been used as ligand because they can form stable $\text{T-Hg}^{2+}\text{-T}$ complexes while other metal ions do not show any evident interaction with T [24–26]. The special $\text{T-Hg}^{2+}\text{-T}$ interaction has been widely utilized for Hg^{2+} determination, such as colorimetric sensing based on the aggregation of gold nanoparticles [27], fluorescence analysis [28] and electrochemistry sensor [29].

Inspired by the $\text{T-Hg}^{2+}\text{-T}$ interaction, we proposed to synthesize a new functional monomer, 3-isocyanatopropyltriethoxysilane (IPTS) bearing T bases (T-IPTS), with T group as recognition element for the imprinting of Hg^{2+} . Then a novel Hg -IIPs based on $\text{T-Hg}^{2+}\text{-T}$ interactions, Hg -IIPs-T (that is IIPs-T, for simplicity), were prepared by sol-gel process. To the best of our knowledge, this work is the first demonstration of $\text{T-Hg}^{2+}\text{-T}$ application in IIPs. To further confirm the feasibility of the IIPs-T for the specific uptake of Hg^{2+} , they were used as SPE sorbent for preconcentration of Hg^{2+} from water samples. Satisfactory recoveries were obtained. Compared to the IIPs based on traditional functional monomer of 4-VP, *i.e.* IIPs-VP, the novel IIPs-T showed significantly higher selectivity and binding capacity. The excellent selectivity of Hg -IIPs-T can be expected by the specific identification between T and Hg^{2+} .

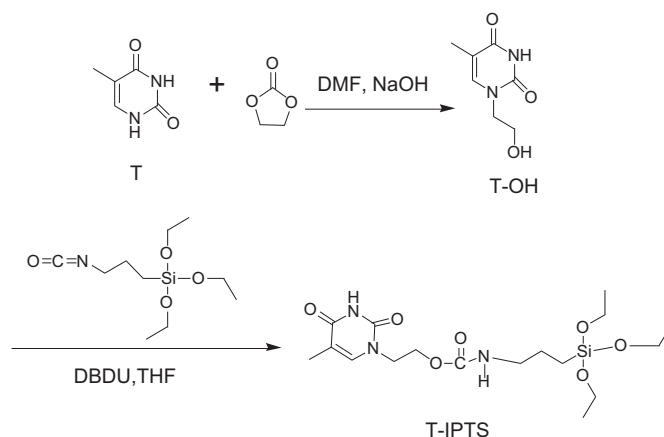
2. Materials and methods

2.1. Materials

Thymine (T), ethylene carbonate, 3-isocyanatopropyltriethoxysilane (IPTS), dibutyltin dilaurate (DBDU), tetraethoxysilane (TEOS) and 4-vinylpyridine (4-VP) were obtained from Sigma-Aldrich (Shanghai, China) and used as received. Tetrahydrofuran (THF, 99%, Tianjin Jiangtian Chemicals, China) was refluxed over sodium and then distilled. Ethylene glycol dimethacrylate (EGDMA, 98%, Sigma-Aldrich) was purified by distillation under vacuum. Azobisisobutyronitrile (AIBN, chemical purity, Tianjin Jiangtian Chemicals, China) was recrystallized from ethanol. Mercury chloride (HgCl_2), dimethyl formamide (DMF), NaOH, HCl and other affiliated reagents were all obtained from Sinopharm Chemical Reagent (Shanghai, China). Ultrapure water used was 18 M Ω purified with a Mili-Q system (Millipore, USA). All pH measurements were carried out by using a pHs-3TC digital pH meter equipped with a combined glass-calomel electrode (Shanghai Tianda, China).

2.2. Instrumentation

Atomic fluorescence spectrometry (AFS, AFS-2202E, Beijing Haiguang, China) was used for the determination of metal ion concentrations. Scanning electron microscope (SEM, Hitachi S-4800, Japan) was used for morphological evaluation. All samples were sputter-coated with gold before SEM analysis. Fourier transform infrared (FT-IR) spectrometer (Thermo Nicolet Corporation, USA) was employed to examine the infrared spectra of samples using a pressed KBr tablet. ^1H NMR and ^{13}C NMR spectra were taken on



Scheme 1. Synthesis route for T-IPTS.

a Bruker 500 MHz spectrometer (Bruker, Germany). Mass spectra (MS) were obtained on a LCQ Fleet (Thermo Fisher Scientific, USA) with an electrospray ionization (ESI) interface.

2.3. Synthesis and characterization of the functional monomer T-IPTS

A new functional monomer T-IPTS based on thymine was synthesized using thymine as a precursor, as shown in Scheme 1. N-(2-hydroxy) thymine (T-OH) was firstly synthesized according to our previous work [30]. Briefly, thymine (4.66 g, 37 mmol) was suspended in DMF (50 mL) and then ethylene carbonate (4.9 g, 111 mmol) was added. After addition of NaOH (35 mg), the mixture was refluxed for 6 h. DMF was removed in vacuum and the residue was yellow ropy liquid. The residue was washed with methanol and after filtering, the filtrate was dried in vacuum. Then the residue was crystallized from dichloromethane to give T-OH as white solid. Characterization data of T-OH were as follows: ^1H NMR (500 MHz, DMF- d_7) δ (ppm): 11.12 (s, 1H, NH), 7.43 (s, 1H, CH), 4.87 (s, 1H, OH), 3.66–3.68 (t, 2H, CH_2), 3.54–3.56 (t, 2H, CH_2), and 1.72–1.80 (t, 3H, CH_3).

And then T-IPTS was synthesized by virtue of semi-covalent imprinting technique [31]. Briefly, T-OH (0.85 g, 5 mmol) was dissolved in 50 mL of dried THF. To the solution, IPTS (2.47 g, 10 mmol) was added dropwise at room temperature, followed by the addition of 0.3 mL DBDU, and then the reaction mixture was refluxed for 24 h under nitrogen atmosphere. After reaction, the solvent was evaporated and the T-IPTS complex was finally obtained and the product was isolated by column chromatography on silica gel (ethyl acetate/petroleum ether, 5:1, v/v). T-IPTS was characterized by ^1H NMR, ^{13}C NMR, MS and FT-IR. ^1H NMR (500 MHz, DMF- d_7) δ (ppm): 11.12 (s, 1H, NH), 8.31 (s, 1H, NH), 7.38 (s, 1H, CH), 4.32–4.34 (t, 2H, CH_2), 3.41–3.46 (q, 6H, CH_2), 3.31 (t, 2H, CH_2), 2.49–2.50 (t, 2H, CH_2), 1.74 (s, 3H, CH_3), 1.23 (t, 2H, CH_2), 1.12–1.14 (t, 2H, CH_2), 1.04–1.06 (t, 9H, CH_3). ^{13}C NMR (500 MHz, DMF- d_7) δ (ppm): 164.69, 156.66, 151.32, 142.05, 108.67, 61.51, 58.12, 47.40, 43.45, 18.58, 15.08, 14.32, 7.63. MS (ESI $^+$): m/z $\text{C}_{17}\text{H}_{31}\text{N}_3\text{O}_7\text{Si}$, calcd. 417.19, found 417.17. FT-IR: 1712, the newly produced ester carbonyl.

2.4. Preparation of Hg -IIPs

Hg -IIPs based on $\text{T-Hg}^{2+}\text{-T}$ interactions, *i.e.* IIPs-T for simplicity, were prepared *via* sol-gel process by using the prepared T-IPTS as function monomers, and TEOS as cross-linkers. Before polymerization, T-IPTS (0.834 g, 2 mmol) and HgCl_2 (0.271 g, 1 mmol) were dissolved in 50 mL of ethanol to form $\text{T-Hg}^{2+}\text{-T}$ template-monomer

complex. Then, TEOS (2.080 g, 10 mmol) and aqueous ammonia (5 mL, 14%) were sequentially added into the above solution. The sol mixture was stirred at room temperature for 12 h followed by stirring at 60 °C for 6 h to obtain high cross-linking density. The resultant Hg-IIPs were isolated by centrifugation and washing with ethanol for 3 times. To remove the template ions, the obtained IIPs were washed with copious amounts of 0.5 mol L⁻¹ HCl. The final product was recovered by filtration, washed with ultrapure water up to the eluent up to pH 6–7 and dried under vacuum at 60 °C for 24 h. The non-imprinted polymers (NIPs) as control materials were prepared using the same procedure but without the addition of template ions. For comparison, Hg-IIPs using 4-VP as functional monomers, namely IIPs-VP for simplicity, were also prepared by bulk polymerization as reported [21].

2.5. Batch experiments

Estimation of molecular recognition properties was made. The batch experiments were investigated as follows: the obtained Hg-IIPs (30 mg) were equilibrated with 10 mL of aqueous solutions containing Hg²⁺ varying from 0 to 80 mg L⁻¹ with pH 8.0 (0.1 mol L⁻¹ phosphate buffer) at room temperature for 24 h. Then the samples were centrifuged and the supernatant solutions were collected. The amount of Hg²⁺ in solution after treatment with IIPs was determined by AFS. The adsorption capacity of Hg²⁺ was calculated from the subtraction of the concentration between initial sample and the supernatant liquid. The batch operation was used for the studies of static/dynamic adsorption capacity, and the selectivity and the interference test. For the dynamic adsorption experiment, the binding kinetics was tested by monitoring the temporal amount of Hg²⁺ in solutions. Selectivity experiments were carried out by using Cd²⁺, Pb²⁺ and Zn²⁺ as comparison ions and interference tests were investigated by using 10 times Fe³⁺, Co²⁺, Mn²⁺, Cd²⁺, Mg²⁺, Ca²⁺, Zn²⁺, Pb²⁺, Na⁺, and K⁺ as co-existent ions.

2.6. Hg²⁺ adsorption–desorption studies

The reusability of Hg-IIPs was tested by the adsorption–desorption studies according to references [11,12,32]. In brief, adsorbed Hg²⁺ ions were desorbed by treatment with 0.1 mol L⁻¹ HCl. After the Hg²⁺-adsorbed IIPs were placed in the desorption medium and stirred at room temperature for 2 h, the final Hg²⁺ concentration in aqueous phase was determined as described above. In order to test the reusability of Hg-IIPs, the adsorption–desorption studies were repeated five times by using the same imprinted polymers.

2.7. Sample collection

Tap water sample was collected from our laboratory after flowing for about 5 min, and river water sample was collected from Xin'an River in Yantai city of China. The water samples were collected in Nalgene bottles and filtered through 0.45 µm pore size membrane to remove the suspended particles, and then the filtrates were alkalified to pH 8.0 with 0.1 mol L⁻¹ NaOH and kept in a refrigerator at 4 °C for use. Recoveries were investigated by spiking the Hg²⁺ standards into water samples at three concentrations (10, 20 and 50 µg L⁻¹), and each concentration was analyzed five replicates, respectively.

2.8. Column experiments

The prepared Hg-IIPs of 500 mg were slurred with ultrapure water and poured into a pyrex glass column with 4.0 mm i.d. The column was preconditioned successively with 0.1 mol L⁻¹ HCl,

ultrapure water and a blank solution. Then 50 mL solution containing Hg²⁺ at three concentrations (10, 20 and 50 µg L⁻¹) was passed through the column at a flow rate of 1.0 mL min⁻¹. The column was washed with 10 mL ultrapure water and the adsorbed Hg²⁺ was eluted with 5 mL of 0.1 mol L⁻¹ HCl, and then the extractants were detected by AFS.

3. Results and discussion

3.1. Synthesis of the functional monomer T-IPTS

To be successfully applied in molecular imprinting, an ideal functional monomer must have two kinds of groups: one is polymerizable group; the other is functional group, which can selectively interact with the template covalently or non-covalently. Considering that the specific T–Hg²⁺–T interactions with high selectivity and high affinity, this work aimed to develop new Hg-IIPs based on T–Hg²⁺–T interactions, so T was used as the functional groups in the new monomer. The polymerizable group is vinyl for free radical polymerization or silane coupling agent for sol–gel process. Herein, silane coupling agent was introduced to the new functional monomer (synthesis process is shown in Scheme 1) because sol–gel process was used in preparation of IIPs. T-IPTS was synthesized through the coupling reaction between the isocyanato group of IPTS and the hydroxyl moiety of T–OH, forming a urethane bond. This method was similar to the semi-covalent imprinting technique reported in references [31,33,34]. The formation of the urethane bonds had been confirmed by the MS, ¹H NMR and ¹³C NMR measurements.

3.2. Preparation and characterization of the IIPs-T

Hg-IIPs based on T–Hg²⁺–T interactions, Hg-IIPs-T, that is IIPs-T for simplicity, were prepared by sol–gel process using T-IPTS as function monomers, and TEOS as cross-linkers, as shown in Scheme 2. First, functional monomer T-IPTS and template Hg²⁺ were preassembled, and T–Hg²⁺–T interactions were produced between the monomer and template; then polymerization was performed in the presence of cross-linker TEOS and catalyst aqueous ammonia. After polymerization, template was removed by washing with copious amounts of 0.5 mol L⁻¹ HCl. Then recognition site specific for Hg²⁺ was formed in IIPs-T.

The sol–gel process was adopted because of its distinct advantages: (1) the ease fabrication; (2) the eco-friendly reaction solvent of ultrapure water or ethanol, which is quite different from the general solvents for free radical polymerization, such as chloroform, acetonitrile and toluene; (3) the mild polymerization conditions that allow specific reagents to be readily introduced within the highly cross-linked host structure without the problem of thermal or chemical decomposition.

For comparison, Hg-IIPs were also prepared using 4-VP as functional monomer by bulk polymerization, Hg-IIPs-VP, that is IIPs-VP for simplicity. Fig. 1 shows their morphologies of the two kinds of IIPs and their corresponding NIPs. The IIPs-T by sol–gel process exhibited regular sphere morphology with the diameter about 300 nm (Fig. 1A), unlike the irregular bulk particles of IIPs-VP (Fig. 1B). For the control polymers, similar morphologies were obtained, as seen in Fig. 1C and D. For the irregular bulk particles, extraction of the original template located in the interior area of the polymer is quite difficult, which would result in incomplete template removal, small binding capacity and slow mass transfer. Then regular sphere particles with nano-sized diameter were expected to improve binding capacity. So just considering from the polymer morphology, the IIPs-T have some advantages over IIPs-VP in improving the imprinting capacity towards Hg²⁺.

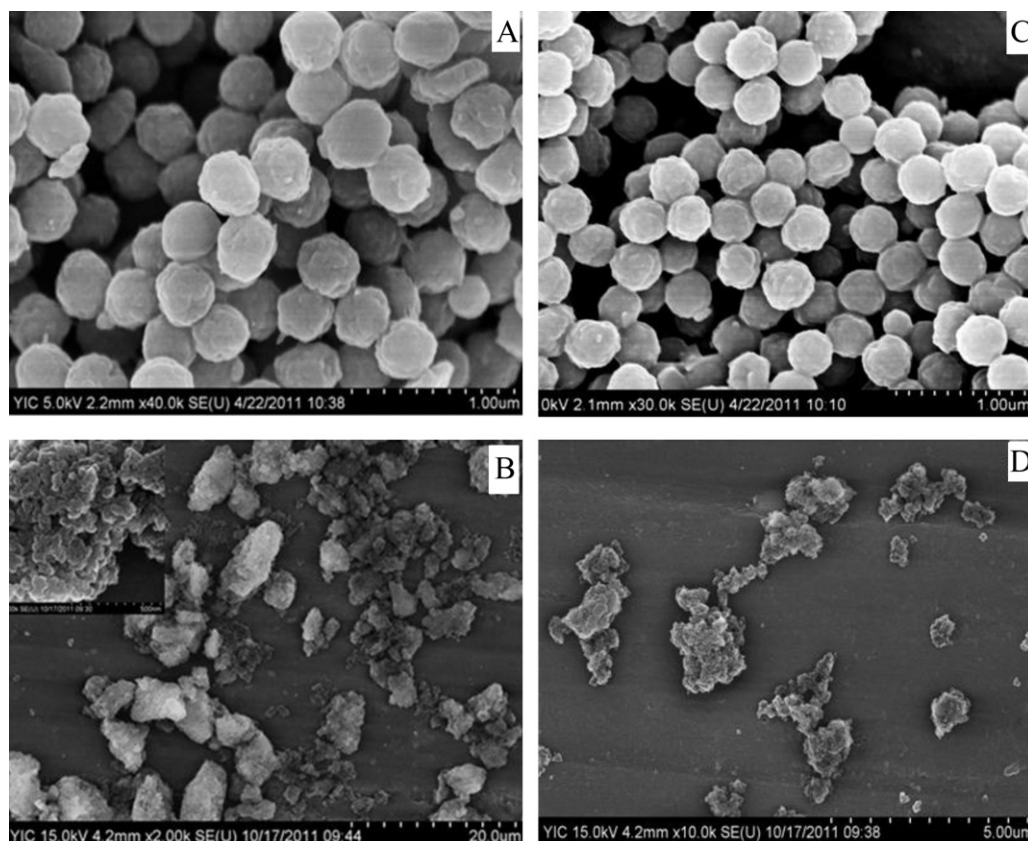


Fig. 1. SEM images for IIPs-T by sol-gel process (A), IIPs-VP by bulk polymerization (B) and their corresponding NIPs (C and D).

3.3. Binding studies of the IIPs-T

Before carrying out adsorption experiments, the effect of varying pH values on Hg^{2+} uptake was investigated by using the batch procedure. It is obvious from Fig. 2, that the adsorption capacity of IIPs-T for Hg^{2+} is dependent on pH values. The adsorption capacity was low below pH 4.0 but increased rapidly; after pH 6.0, the increase is relatively very low and the adsorption capacity approached the maximum at pH 8.0; after that, the capacity decreased continuously (Fig. 2). The influence of pH on T- Hg^{2+} -T interaction has been reported as following: at a pH below 7.0, protonation of the nitrogen atoms of the T base reduces its affinity with Hg^{2+} , while at a relatively higher pH (>10.0), Hg^{2+} may be complexed by OH^- , which in turn, may reduce its complex with the T [35]. The binding affinity of T with Hg^{2+} had significant effect on the binding capacity of IIPs-T. Higher binding affinity of T- Hg^{2+} -T resulted in higher binding capacity of IIPs-T. So, pH 8.0 was chosen as the optimal pH value for IIPs-T. As seen from Fig. 2, for IIPs-VP, similar change trend of adsorption capacity was obtained and pH 8.0 was also the optimum pH because it was also nitrogen atoms coordinated with Hg^{2+} . Therefore, pH 8.0 was selected as optimum for further experiments.

Then static adsorption experiments were performed in order to evaluate the adsorption capacities of Hg-IIPs at concentrations of 0–80 mg L^{-1} from aqueous solutions with pH 8.0. As can be seen from Fig. 3A, the amounts of Hg^{2+} adsorbed per unit mass of IIPs increased with the increase of initial concentrations of Hg^{2+} . The adsorption capacity of IIPs-T was about 2 times of IIPs-VP, and was about 4 times of NIPs for three replicate measurements. In order to determine the binding rate of Hg^{2+} on the IIPs, dynamic binding experiments were carried out. Fig. 3B shows that the adsorption amounts of Hg^{2+} onto both IIPs-T and IIPs-VP increased with time.

Excitedly, the IIPs-T offered more rapid adsorption for Hg^{2+} than that of the latter. One hour (1 h) was enough as an equilibration time for IIPs-T while 3 h was required for IIPs-VP. From the static adsorption and dynamic binding experiments, we can see that, IIPs-T own higher binding capacity and faster mass transfer than IIPs-VP. Therefore, the superior adsorption capacity of IIPs-T could be attributed to two reasons. One is that the T- Hg^{2+} -T complex was more stable and the interaction between T and Hg^{2+} was more intense than that between VP and Hg^{2+} . The other is that IIPs-VP were prepared by bulk polymerization, and average particle sizes were larger than that of IIPs-T prepared by sol-gel process (Fig. 1).

3.4. Ionic selectivity and recognition reliability of the IIPs-T

Selectivity is a primary concern in this work, so we demonstrated the selectivity of the prepared IIPs-T by carrying out two groups of experiments. One was to test adsorption capacity and selectivity coefficient of the IIPs-T toward Zn^{2+} , Cd^{2+} and Pb^{2+} , respectively, at the same concentration as Hg^{2+} . The other is to test adsorption capacity of IIPs-T toward Hg^{2+} in the presence of Hg^{2+} and 10 times various possible interference ions.

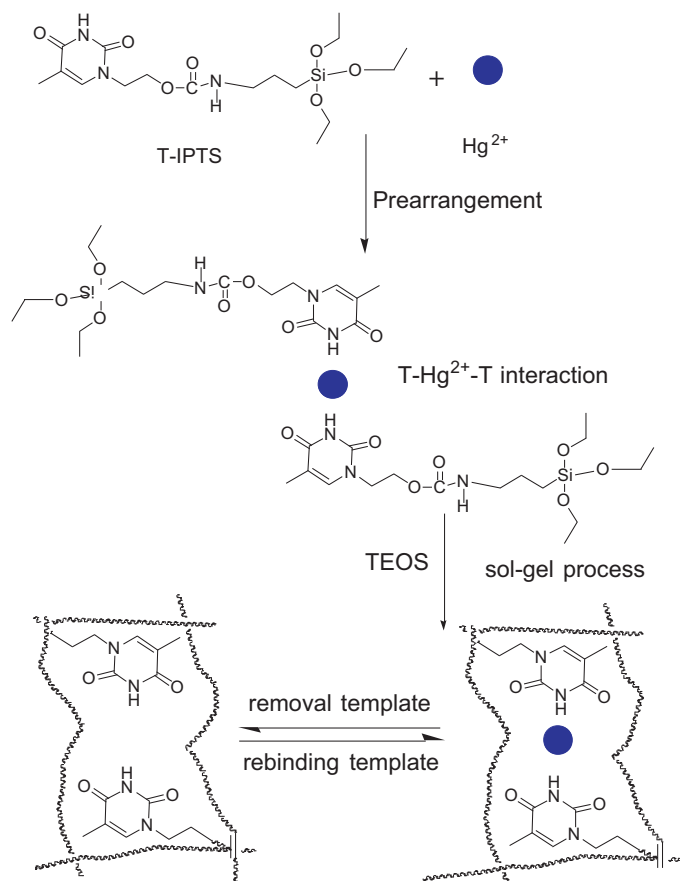
Among these metal ions of As, Hg, Se, Te, Bi, Sn, Cd, Zn and Pb, which can be detected by AFS, Cd^{2+} , Zn^{2+} and Pb^{2+} are commonly present in water samples. So in order to evaluate the selective binding capacities of IIPs, competitive adsorption of Cd^{2+} , Zn^{2+} and Pb^{2+} on Hg-IIPs was investigated. The results of competitive ion binding tests were summarized in Table 1. As can be seen, both the IIPs-T and IIPs-VP had higher binding capacities for Hg^{2+} than that for the other ions. Furthermore, the IIPs-T displayed higher binding capacity for Hg^{2+} than the IIPs-VP, for example, with the selective coefficient (46.6) about 10 times higher than that of IIPs-VP (4.78) (Table 1), which is very likely owing to the highly selective

Table 1
Selectivity of the prepared IIPs and NIPs.^a

Metal ions	IIPs-T		NIPs-T		IIPs-VP		NIPs-VP	
	Q	k	Q	k	IIPs	k	NIPs	k
Hg ²⁺	2.8	–	0.28	–	1.10	–	0.29	–
Cd ²⁺	0.07	38.8	0.08	3.43	0.23	4.78	0.13	2.12
Zn ²⁺	0.06	46.6	0.07	3.68	0.23	4.78	0.14	2.03
Pb ²⁺	0.10	28.0	0.11	2.49	0.40	2.75	0.21	1.40

Experimental conditions: IIPs or NIPs, 30 mg; C₀ = 20 mg L⁻¹; V, 10 mL; sample solution, pH 8.0; adsorption time, 24 h; room temperature.

^a Selective coefficient, $k = Q_{\text{template}}/Q_{\text{competitive ion}} \cdot Q_{\text{template}}$ and $Q_{\text{competitive ion}}$ are adsorption capacities of the template and the competitive ions on IIPs or NIPs, respectively.



Scheme 2. Schematic illustration for the preparation of IIPs-T by sol-gel process.

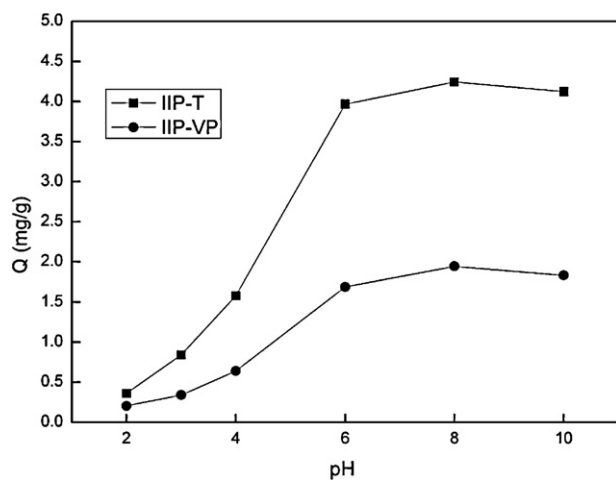


Fig. 2. Effect of pH on adsorption of Hg²⁺ on IIPs-T and IIPs-VP particles. Experimental conditions: IIPs, 30 mg; C₀ = 40 mg L⁻¹; V, 10 mL; adsorption time, 24 h; room temperature.

T-Hg²⁺-T interactions. All the results indicated that it was Hg²⁺ rather than Zn²⁺, Cd²⁺ or Pb²⁺ could specifically bind T and thereby the IIPs-T. Therefore, the prepared IIPs-T had a superior selectivity specialism for Hg²⁺.

For further study of the selectivity and reliability of the Hg-IIPs, the adsorption capacities of Hg²⁺ on Hg-IIPs in the presence of other possible interference ions, including Fe³⁺, Co²⁺, Mn²⁺, Cd²⁺, Mg²⁺, Ca²⁺, Zn²⁺, Pb²⁺, Na⁺ and K⁺, respectively, were evaluated. As shown in Fig. 4, for the IIPs-T, those interference ions did not cause significant reduction of Hg²⁺ adsorption capacity, and it could be estimated that only <10% of binding sites were occupied by those 10 times interference ions; in contrast, for the IIPs-VP, the binding capacity for Hg²⁺ reduced almost 40–60%. This revealed that the IIPs-T based on T-Hg²⁺-T interactions had higher selectivity for

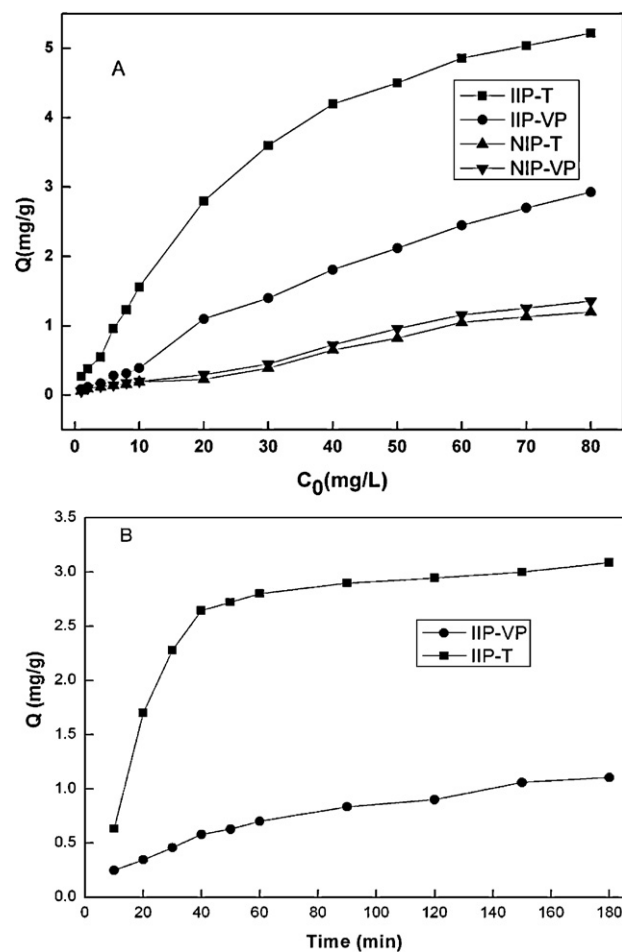


Fig. 3. (A) Binding isotherms of IIPs and NIPs for Hg²⁺. Experimental conditions: volume, 10 mL; mass of polymers, 30 mg; adsorption time, 24 h; sample solution, pH 8.0. (B) Dynamic uptake of Hg²⁺ onto Hg-IIPs. Experimental conditions: volume, 10 mL; mass of polymers, 30 mg; C₀ = 40 mg L⁻¹; sample solution, pH 8.0.

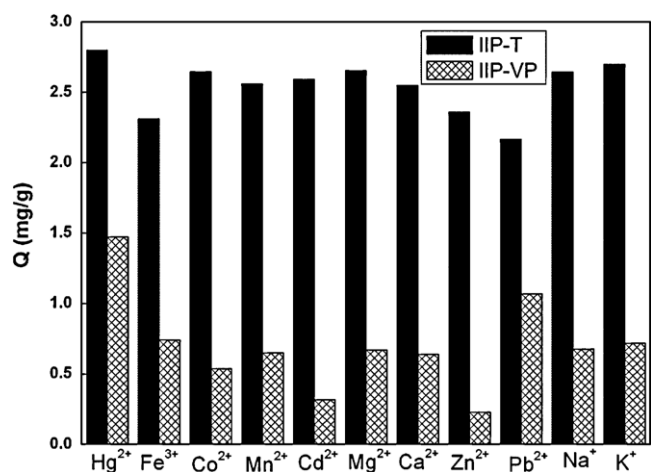


Fig. 4. Adsorption capacities for Hg²⁺ in the presence of 20 mg L⁻¹ Hg²⁺ and 200 mg L⁻¹ other metal ions, including Fe³⁺, Co²⁺, Mn²⁺, Cd²⁺, Mg²⁺, Ca²⁺, Zn²⁺, Pb²⁺, Na⁺ and K⁺, respectively. Other experimental conditions: IIPs, 30 mg; V, 10 mL; sample solution, pH 8.0; room temperature.

Hg²⁺ than the IIPs-VP. All the results indicated that the IIPs-T were unaffected and highly selective and reliable for Hg²⁺ recognition.

3.5. Reusability of the IIPs-T

Reusability is an important index for the novel IIPs-T, which is very likely to be a key factor in improving the economical efficiency. Desorption of the adsorbed Hg²⁺ from the IIPs-T was also studied in a batch experimental set-up. When 0.1 mol L⁻¹ HCl was used as desorption medium, desorption ratio was higher than 95% after 2 h treatment. In order to show the reusability of the IIPs-T, adsorption–desorption cycle was repeated five times by using the same imprinted polymers. The results showed that the prepared novel IIPs-T could be used repeatedly without significant decrease in their adsorption capacities for Hg²⁺.

3.6. Variable optimization of the IIPs-T-SPE

Based on the above results, the IIPs-T is employed as SPE sorbents for preconcentration of Hg²⁺. In order to attain ideal extraction recoveries in a short time, various influence factors of SPE were studied and optimized, such as flow rate of loading sample, loading volume and the elution conditions including elution volume, eluent flow rate and eluent concentration.

The flow rate and volume of loading sample solution through the packed column are very important parameters affecting the adsorption amounts and analytical time. The effect of flow rate in the range of 0.5–2.0 mL min⁻¹ was investigated. In general, analyte adsorption amounts and recovery values are decreased with the increase of sample flow rate. Here, a flow rate <0.5 mL min⁻¹ was not chosen to avoid a long time of analysis. The results showed that Hg²⁺ was adsorbed quantitatively by the IIPs-T (>95%) at a flow rate of 0.5–1.0 mL min⁻¹. However, at a flow rate over 1.0 mL min⁻¹, there was a decrease in percentage adsorption (<95%), because Hg²⁺ could not equilibrate properly with the sorbent. Thus, to achieve a more rapid operation with higher recovery, a sample rate of 1.0 mL min⁻¹ was finally selected as an optimum value. On the other hand, in order to determine the loading volume that can be concentrated with the acceptable level of recoveries, different volumes (20, 50, 80, 100 and 150 mL) of solutions containing 0.1 mg L⁻¹ of Hg²⁺ at pH 8.0 were passed through the IIPs-T column. The recovery value was higher than 95% while the sample volume was less than 80 mL. Considering the higher recovery and shorter analytical time, so, 1.0 mL min⁻¹ and 50 mL were chosen

Table 2

IIPs-T-SPE recoveries (%) and relative standard deviations (RSD, %) obtained from analysis of water samples spiked with Hg²⁺.^a

Samples	Added (μg L ⁻¹)	Found (μg L ⁻¹)	Recovery ^b ± RSD ^c
River water	0.00	0.45	–
	10.0	11.2	107.5 ± 2.4
	20.0	19.9	97.3 ± 3.2
	50.0	48.05	95.2 ± 2.9
Tap water	0.00	0.00	–
	10.0	10.4	104.0 ± 5.2
	20.0	19.12	95.6 ± 3.6
	50.0	58.1	116.3 ± 2.6

^a Experimental conditions: loading volume, 50 mL; loading rate, 1 mL min⁻¹; washing, 10 mL of pure water; eluting, 5 mL of 0.1 mol L⁻¹ HCl.

^b Average value from five individual experiments.

^c *n* = 5.

as the optimal flow rate and loading volume of sample solution, respectively, in the following work.

It is well known that the elution conditions, including elution volume, eluent flow rate and eluent concentration, have great influence on the recovery of Hg²⁺. In order to find optimum elution conditions, different eluent volumes from 2 to 10 mL, different flow rates between 0.5 and 2 mL min⁻¹, and different eluent concentrations varying from 0.05 to 0.5 mol L⁻¹ HCl were investigated. At last, 5 mL of 0.1 mol L⁻¹ HCl at 1.0 mL min⁻¹ was used for elution.

3.7. Analytical figures of merit for Hg²⁺ determination by using IIPs-T-SPE

Under the above optimized conditions, the analytical figures of merit of IIPs-T-SPE with AFS for Hg²⁺ determination were investigated. The calibration curve was obtained with correlation coefficient of *R* = 0.996 in the dynamic linear range of 0.08–50 μg L⁻¹. The relative standard deviation (RSD) for the determination of 10 μg L⁻¹ Hg²⁺ was 3.4% (*n* = 5), which indicated that the IIPs-T-SPE-AFS possessed good precision for the analysis of trace Hg²⁺ in solution samples. The limit of detection (LOD) based on three times the standard deviation and for 10 replicate measurements of blank solution, was attained of 0.03 μg L⁻¹.

The preconcentration factor was also investigated by the optimized column procedure using increasing volume of Hg²⁺ solution within 100–1500 mL and keeping the total amount of loaded Hg²⁺ constant to 1.0 μg. The maximum sample volume could be up to 1000 mL with 95% recovery. Consequently, by considering the sample solution volume of 1000 mL and the final elution volume of 5 mL, the preconcentration factor of 200 was easily available.

3.8. Applications of the IIPs-T-SPE to water samples

To evaluate the practicality of the developed method, IIPs-T-SPE was further applied for the preconcentration of trace Hg²⁺ in river and tap water samples. The recovery was investigated by spiking the Hg²⁺ standard at three levels (10, 20 and 50 μg L⁻¹) and analyzing five replicates for each concentration. As shown in Table 2, recoveries were in the range of 95.2–116.3%, with the RSD of 2.4–5.2%, indicating the obtained IIPs-T based on T–Hg²⁺–T interaction were greatly applicable for the effective preconcentration and quantitative determination of trace Hg²⁺ in real water samples. The result also suggested that matrix effects could be remarkably reduced by virtue of the IIPs-T-SPE procedure.

Also, it was observed that the endogenous content of Hg²⁺ was detected at 0.45 μg L⁻¹ in the tested river water sample. The value is lower than the maximum contaminant level (MCL) for Hg²⁺ in drinking water, namely 2 μg L⁻¹ formulated by the United States Environmental Protection Agency and 1 μg L⁻¹ by the

Table 3
Comparison of method performance for determination of Hg²⁺ by SPE.

Sorbent	Detection technique	pH	Linear range (μg L ⁻¹)	RSD (%)	LOD (μg L ⁻¹)	PF ^a	Recovery (%)	Ref.
Hg-IIPs-T	AFS	8	0.08–50	3.4	0.03	200	95.2–116.3	This work
Hg-imprinted copolymer	CV-AAS ^b	8	0.13–25	2.4	0.05	200	94–105	[21]
Home-made C ₁₈ micro-column	HPLC/CV-AFS ^c	3–5	10–1000	<6	0.8	1000	85–90	[36]
C ₁₈ -EPT ^d	CV-AAS	3.5	0.02–1.90	1.1	0.0106	380	97–101.5	[37]
C ₁₈ -IIDE ^e	CV-AAS	3–7	–	–	0.005	>150	98.8–102.0	[38]
4-bp ^f modified C ₁₈	CV-AAS	4.5	0.0062–0.31	<4.5	0.00187	128	89	[39]
C ₁₈ -CBT ^g	HPLC-UV	4	–	1.0	0.0013	100	90–95	[40]
Poly-allylthiourea	CV-AAS	2	–	1.9	0.08	160	96–105	[41]
2-MBI ^h modified								
Agar mini-column	CV-AAS	2.5	0.040–2.40	2.6	0.02	–	95.0–99.1	[42]
DPC ⁱ doped Fe ₃ O ₄	CV-AAS	6–8	–	2.2	0.16	100	97.2–100	[43]
Silica gel-OHC ^j	ICP-AES ^k	3	–	<4.0	0.10	50	97.2–100.8	[44]
Sulfoxide	ICP-MS ^l	–	–	–	0.0015	–	99.0–100.7	[45]
AC-ATSC ^m	ICP-OES ⁿ	3	–	1.9	0.12	–	97.6–100.6	[46]

^a Preconcentration factor.

^b Cold vapor atomic absorption spectrometry.

^c Cold vapor atomic fluorescence spectrometry.

^d 1,3-Bis(2-ethoxyphenyl)triazene.

^e Isopropyl 2-[(isopropoxycarbothioly)disulfany]ethane thioate.

^f 1,4-Bis(4-pyridyl)-2,3-diaza-1,3-butadiene.

^g 1,3-Bis(2-cyanobenzene)triazene.

^h 2-Mercaptobenzimidazole.

ⁱ 1,5-Diphenylcarbazide.

^j 2-(2-Oxoethyl)hydrazine carbothioamide.

^k Inductively coupled plasma atomic emission spectrometry.

^l Mass spectrometry.

^m 1-Acylthiosemicarbazide-modified activated carbon.

ⁿ Optical emission spectrometry.

Health Department of the Chinese Government. Therefore, the validated IIPs-T-SPE coupled to AFS method are an ideal alternative to simultaneous enrichment, separation and determination of Hg²⁺ in complicated water samples for environmental health and safety purpose.

3.9. Comparison with other methods

The method performance of the developed IIPs-T-SPE-AFS toward Hg²⁺ in aqueous standard solutions and real water samples was compared with other reported SPE-based methods by using various sorbents coupled with various detection techniques [21,36–46], listed in Table 3. As can be seen from the table, as a new material, the presented IIPs-T are ideal candidates for SPE sorbents for preconcentration of Hg²⁺. The attained LOD, RSD, preconcentration factor and recovery of the method are also comparable. In addition, the high selectivity and reusability of the IIPs-T are another obvious advantages compared with other methods.

4. Conclusions

In summary, the new Hg-IIPs based on T-Hg²⁺-T interaction were synthesized and successfully applied to water samples for highly selective preconcentration of Hg²⁺. The most important characteristic of Hg-IIPs-T is their excellent selectivity towards Hg²⁺ over other metal ions. The novelty of this work is also reflected by the fact that it provides the grounds for development of novel ion adsorption sorbents and the design of new ion-separation matrices. The novel Hg-IIPs-T were validated practically applicable through the analysis of spiked river and tap water samples. Enrichment and determination of trace Hg²⁺ in real water matrices has been fulfilled via the prepared Hg-IIPs-T. Furthermore, the prepared Hg-IIPs-T open attractive perspectives for removal: as ideal sorbents for Hg²⁺, they can easily extract and remove Hg²⁺ from Hg²⁺-contaminated water, and therefore realize water purification and pollution abatement. Besides used as sorbents in sample pretreatment, the newly obtained Hg-IIPs-T might

also hold a promising potential for application in environmental detection as recognition element in some chemosensors.

On the other hand, Hg²⁺ imprinting is just an analytical model of proof-of-concept; we purpose to develop a general-applicable platform. For example, Ag⁺ also can be imprinted based on cytidine-Ag-cytidine (C-Ag-C) interactions by using the new functional monomer bearing C group. With the appropriate choice of specific DNA bases or reasonable utilization of chelate functional groups, and furthermore, with the delicate design and synthesis of new functional monomers, the heavy metal ion detection IIPs-based platform can be developed for the rapid, selective and sensitive routing monitoring of water quality. In addition to improving the selectivity of IIPs and thereby MIPs, devising and synthesizing new functional monomers are effective way to imprint various analytes, and therefore enrich the research contents as well as broaden the applications of molecular imprinting techniques.

Conflict of interest

The authors have declared no conflict of interest.

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References

- [1] B. Sellergren, Imprinted polymers with memory for small molecules proteins, or crystals, *Angew. Chem. Int. Ed.* 39 (2000) 1031–1037.
- [2] C. Alexander, H.S. Andersson, L.I. Andersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J.O. Mahony, M.J. Whitcombe, Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003, *J. Mol. Recognit.* 19 (2006) 106–180.

- [3] L.X. Chen, S.F. Xu, J.H. Li, Recent advances in molecular imprinting technology: current status, challenges and highlighted applications, *Chem. Soc. Rev.* 40 (2011) 2922–2942.
- [4] M. Lasáková, P. Jandera, Molecularly imprinted polymers and their application in solid phase extraction, *J. Sep. Sci.* 32 (2009) 799–812.
- [5] S.F. Xu, L.X. Chen, J.H. Li, W. Qin, J.P. Ma, Preparation of hollow porous molecularly imprinted polymers and their applications to solid-phase extraction of triazines in soil samples, *J. Mater. Chem.* 21 (2011) 12047–12053.
- [6] L. Vial, P. Dumy, Artificial enzyme-based biosensors, *New J. Chem.* 33 (2009) 939–946.
- [7] J.H. Li, Z. Zhang, S.F. Xu, L.X. Chen, N. Zhou, H. Xiong, H.L. Peng, Label-free colorimetric detection of trace cholesterol based on molecularly imprinted photonic hydrogels, *J. Mater. Chem.* 21 (2011) 19267–19274.
- [8] Y. Xie, H. Li, L. Wang, Q. Liu, Y. Shi, H. Zheng, M. Zhang, Y. Wu, B. Lu, Molecularly imprinted polymer microspheres enhanced biodegradation of bisphenol A by acclimated activated sludge, *Water Res.* 45 (2011) 1189–1198.
- [9] X.L. Song, J.H. Li, J.T. Wang, L.X. Chen, Quercetin molecularly imprinted polymers: preparation, recognition characteristics and properties as sorbent for solid-phase extraction, *Talanta* 80 (2009) 694–702.
- [10] N. Hoai, D. Yoo, D. Kim, Batch and column separation characteristics of copper-imprinted porous polymer micro-beads synthesized by a direct imprinting method, *J. Hazard. Mater.* 173 (2010) 462–467.
- [11] D. Singh, S. Mishra, Synthesis, characterization and removal of Cd(II) using Cd(II)-ion imprinted polymer, *J. Hazard. Mater.* 164 (2009) 1547–1551.
- [12] G. Bayramoglu, M.Y. Arica, Synthesis of Cr(VI)-imprinted poly(4-vinyl pyridine-co-hydroxyethyl methacrylate) particles: Its adsorption propensity to Cr(VI), *J. Hazard. Mater.* 187 (2011) 213–221.
- [13] M.J. Whitcombe, I. Chianella, L. Larcombe, S.A. Piletsky, J. Noble, R. Porter, A. Horgan, The rational development of molecularly imprinted polymer-based sensors for protein detection, *Chem. Soc. Rev.* 40 (2011) 1547–1571.
- [14] A. Bossi, F. Bonini, A.P.F. Turner, S.A. Piletsky, Molecularly imprinted polymers for the recognition of proteins: the state of the art, *Biosens. Bioelectron.* 22 (2007) 1131–1137.
- [15] M. Saraji, H. Yousefi, Selective solid-phase extraction of Ni(II) by an ion-imprinted polymer from water samples, *J. Hazard. Mater.* 167 (2009) 1152–1157.
- [16] Y. Liu, Z.C. Liu, J. Gao, J.D. Dai, J. Han, Y. Wang, J.M. Xie, Y.S. Yan, Selective adsorption behavior of Pb(II) by mesoporous silica SBA-15-supported Pb(II)-imprinted polymer based on surface molecularly imprinting technique, *J. Hazard. Mater.* 186 (2011) 197–205.
- [17] J.H. Li, W.H. Lu, J.P. Ma, L.X. Chen, Determination of mercury (II) in water samples using dispersive liquid–liquid microextraction and back extraction along with capillary zone electrophoresis, *Mikrochim. Acta* 175 (2011) 301–308.
- [18] G.Q. Wang, Z.P. Chen, W.H. Wang, B. Yan, L.X. Chen, Chemical redox-regulated mesoporous silica-coated gold nanorods for colorimetric probing of Hg²⁺ and S²⁻, *Analyst* 136 (2011) 174–178.
- [19] T.T. Lou, Z.P. Chen, Y.Q. Wang, L.X. Chen, Blue-to-Red colorimetric sensing strategy for Hg²⁺ and Ag⁺ via redox-regulated surface chemistry of gold nanoparticles, *ACS Appl. Mater. Interfaces* 3 (2011) 1568–1573.
- [20] S. Clemens, M. Monperrus, O.F.X. Donard, D. Amouroux, T. Guerin, Mercury speciation in seafood using isotope dilution analysis: a review, *Talanta* 89 (2012) 12–20.
- [21] Y. Liu, X. Chang, Y. Guo, S. Meng, Highly selective determination of inorganic mercury(II) after preconcentration with Hg(II)-imprinted diazaminobenzene-vinylpyridine copolymers, *Anal. Chim. Acta* 538 (2005) 85–91.
- [22] Z. Fan, Hg(II)-imprinted thiol-functionalized mesoporous sorbent micro-column preconcentration of trace mercury and determination by inductively coupled plasma optical emission spectrometry, *Talanta* 70 (2006) 1164–1169.
- [23] T. Alizadeh, M.R. Ganjali, M. Zare, Application of an Hg²⁺ selective imprinted polymer as a new modifying agent for the preparation of a novel highly selective and sensitive electrochemical sensor for the determination of ultratrace mercury ions, *Anal. Chim. Acta* 689 (2011) 52–59.
- [24] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, Mercury(II)-mediated formation of thymine–Hg(II)–thymine base pairs in DNA duplexes, *J. Am. Chem. Soc.* 128 (2006) 2172–2173.
- [25] Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, 15N–15N J-coupling across Hg(II): direct observation of Hg(II)-mediated T–T base pairs in a DNA duplex, *J. Am. Chem. Soc.* 129 (2007) 244–245.
- [26] X. Liu, C. Qi, T. Bing, X. Cheng, D. Shangguan, Highly selective phthalocyanine–thymine conjugate sensor for Hg²⁺ based on target induced aggregation, *Anal. Chem.* 81 (2009) 3699–3704.
- [27] D. Li, A. Wiecekowska, I. Willner, Optical analysis of Hg²⁺ ions by oligonucleotide–gold-nanoparticle hybrids and DNA-based machines, *Angew. Chem. Int. Ed.* 47 (2008) 3927–3931.
- [28] C. Chiang, C. Huang, C. Liu, H. Chang, Oligonucleotide-based fluorescence probe for sensitive and selective detection of mercury(II) in aqueous solution, *Anal. Chem.* 80 (2008) 3716–3721.
- [29] S. Liu, H. Nie, J. Jiang, G. Shen, R. Yu, Electrochemical sensor for mercury(II) based on conformational switch mediated by interstrand cooperative coordination, *Anal. Chem.* 81 (2009) 5724–5730.
- [30] L. Chen, T.T. Lou, C.W. Yu, L.X. Chen, N-1-(2-mercaptoethyl)thymine modification of gold nanoparticles: a highly selective and sensitive colorimetric chemosensor for Hg²⁺, *Analyst* 136 (2011) 4770–4773.
- [31] C.D. Ki, C. Oh, J.Y. Chang, The use of a thermally reversible bond for molecular imprinting of silica spheres, *J. Am. Chem. Soc.* 124 (2002) 14838–14839.
- [32] E. Birlık, A. Ersöz, E. Açıkkalp, A. Denizli, R. Say, Cr(III)-imprinted polymeric beads: sorption and preconcentration studies, *J. Hazard. Mater.* 140 (2007) 110–116.
- [33] C. Zhai, Q. Lu, X. Chen, Y. Peng, L. Chen, S. Du, Molecularly imprinted layer-coated silica nanoparticles toward highly selective separation of active diosgenin from dioscorea nipponica makino, *J. Chromatogr. A* 1216 (2009) 2254–2262.
- [34] X. Wang, L. Wang, X. He, Y. Zhang, L. Chen, A molecularly imprinted polymer-coated nanocomposite of magnetic nanoparticles for estrone recognition, *Talanta* 78 (2009) 327–332.
- [35] H. Wang, Y. Wang, J. Jin, R. Yang, Gold nanoparticle-based colorimetric and turn-on fluorescent probe for mercury(II) ions in aqueous solution, *Anal. Chem.* 80 (2008) 9021–9028.
- [36] J. Margetínová, P. Houserová-Pelclová, V. Kubň, Speciation analysis of mercury in sediments, zoobenthos and river water samples by high-performance liquid chromatography hyphenated to atomic fluorescence spectrometry following preconcentration by solid phase extraction, *Anal. Chim. Acta* 615 (2008) 115–123.
- [37] M.K. Rofouei, A. Sabouri, A. Ahmadelinezhad, H. Ferdowsi, Solid phase extraction of ultra traces mercury(II) using octadecyl silica membrane disks modified by 1,3-bis(2-ethoxyphenyl)triazene (EPT) ligand and determination by cold vapor atomic absorption spectrometry, *J. Hazard. Mater.* 192 (2011) 1358–1363.
- [38] M. Shamsipur, A. Shokrollahi, H. Sharghi, M.M. Eskandari, Solid phase extraction and determination of sub-ppb levels of hazardous Hg²⁺ ions, *J. Hazard. Mater.* B117 (2005) 129–133.
- [39] M. Soleimani, M.S. Mahmodi, A. Morsali, A. Khani, M.G. Afshar, Using a new ligand for solid phase extraction of mercury, *J. Hazard. Mater.* 189 (2011) 371–376.
- [40] T. Hashempour, M.K. Rofouei, A.R. Khorrami, Speciation analysis of mercury contaminants in water samples by RP-HPLC after solid phase extraction on modified C₁₈ extraction disks with 1,3-bis(2-cyanobenzene)triazene, *Microchem. J.* 89 (2008) 131–136.
- [41] W. Qu, Y. Zhai, S. Meng, Y. Fan, Q. Zhao, Selective solid phase extraction and preconcentration of trace mercury(II) with poly-allylthiourea packed columns, *Mikrochim. Acta* 163 (2008) 277–282.
- [42] N. Pourreza, K. Ghanemi, Determination of mercury in water and fish samples by cold vapor atomic absorption spectrometry after solid phase extraction on agar modified with 2-mercaptobenzimidazole, *J. Hazard. Mater.* 161 (2009) 982–987.
- [43] Y. Zhai, S. Duan, Q. He, X. Yang, Q. Han, Solid phase extraction and preconcentration of trace mercury(II) from aqueous solution using magnetic nanoparticles doped with 1,5-diphenylcarbazine, *Microchim. Acta* 169 (2010) 353–360.
- [44] X. Chai, X. Chang, Z. Hu, Q. He, Z. Tu, Z. Li, Solid phase extraction of trace Hg(II) on silica gel modified with 2-(2-oxoethyl)hydrazine carbothioamide and determination by ICP-AES, *Talanta* 82 (2010) 1791–1796.
- [45] Y. Zhu, B. Ahmady, M. Numata, K. Chiba, Solid phase extraction using a sulfoxide adsorbent for preconcentration and separation of Hg(II) in natural water followed by ICP-MS measurements, *Anal. Sci.* 28 (2012) 417–421.
- [46] R. Gao, Z. Hu, X. Chang, Q. He, L. Zhang, Z. Tu, J. Shi, Chemically modified activated carbon with 1-acylthiosemicarbazide for selective solid-phase extraction and preconcentration of trace Cu(II), Hg(II) and Pb(II) from water samples, *J. Hazard. Mater.* 172 (2009) 324–329.