



Development of metal complex imprinted solid-phase microextraction fiber for 2,2'-dipyridine recognition in aqueous medium

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ABSTRACT

In this paper, a novel metal complex imprinted polymer (CIP) coated solid-phase microextraction (SPME) fiber was prepared which could recognize the complex template [Cu(OAc)₂(2,2'-dipyridine)] in aqueous medium. The saturating adsorption capacity of CIP-coated fiber was 2.2 and 2.6 times greater than those of molecularly imprinted polymer (MIP) coated fiber and nonimprinted polymer (NIP) coated fiber, respectively. Extraction conditions that influenced the recognition performance of CIP-coated fiber were investigated including pH, extraction solvent, metal ion species, etc. The ligand selectivity was also evaluated and discussed. The results demonstrated that CIP-coated fiber had better binding affinity for 2,2'-dipyridine compared to its structure analogues. The recognition ability of CIP coating was stable and effective in aqueous medium while MIP coating showed weak imprinting effect due to disturbance from protic solvent. 2,2'-dipyridine extracted by CIP-coated fiber using HPLC/UV detection resulted in a linear range of 10–200 µg/L with a detection limit of 2.0 µg/L. The proposed method was successfully applied to the analysis of 2,2'-dipyridine in spiked tap water, laboratory wastewater and human urine samples with recoveries 80.3–103.3% and RSDs 5.5–8.9%.

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1. Introduction

Molecular imprinting (MI) is a technique to prepare selective binding sites in polymer matrix using target molecule as templates for imprinted cross-linked polymers. Removal of the template results in recognition ability which is determined by several factors such as the size and shape of the cavity complementary to the template molecule and rebinding interactions between the functional monomer and the template [1]. In non-covalent imprinting the molecularly imprinted polymer (MIP) can be established relying on various interactions like hydrogen bond which is frequently employed. In virtue of its notable features, such as lock-key pre-organization, selective identification, broad applicability and good mechanical and chemical stability, MIP has been widely used in many fields [2]. However, its recognition capability has limitation due to the weak nature of hydrogen bond. The imprinting effect may be weakened or even damaged by strong polar solvents because the hydrogen bond is unstable in these environments [3]. Imprinted polymers obtained from organic solvents may have poor recognition ability for the target molecule within aqueous environment, which causes limitation on application of MIPs in bioseparation field. Thus, the preparation and applica-

tion of MIPs are limited in organic solvents, while many biological molecules are lack of solubility in organic media [4]. Moreover, mimicking natural systems have been attracting great interest and natural recognitions are usually carried out in aqueous conditions. Consequently, it is significant to develop water-compatible MIPs that can be extended to more application fields. One of the virtual methods is to prepare MIPs via metal ion coordination.

Metal ion coordination plays an important role in biological recognition systems and many pharmaceuticals show biological activities when they are in the form of metal complexes [5]. The research on metal ion mediated imprinting has been drawing attention since the first report by Fujii et al. [6] in 1984. According to this approach, metal ion is employed as an assembled pivot that organizes the functional monomer and the template. The complex constructed with metal ion, generally a transition metal ion, and the target molecule can be regarded as a “complex template”. Compared to the traditional interactions utilized in MIP, there are several advantages associated with the metal coordinate interactions. The coordination bond has higher strength, specificity, and directionality which make it more like a covalent interaction than hydrogen bond or electrostatic interaction in water [7]. Thus, it is more stable in water or other protic solvents. Also, it is possible to alter the affinity and selectivity by replacing the imprinted metal ion with other metal ions. Up to now, the complex imprinted polymers

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(CIPs) are mainly applied in catalyst [8–10], separation [11,12], recognition [13,14] and sensors [15,16]. Thin coatings of molecularly imprinted, metal-complexing polymers were grafted to activated silica beads suitable for HPLC separation [11]. By replacing Cu(II) in imprinted polymers with weaker-binding Zn(II), the ligand-exchange supports showed great separating ability between bis-imidazole templates from highly similar substrates. A thermosensitive macroporous hydrogel targeting lysozyme was developed based on metal coordinate interaction which improved the selectivity and adsorption capacity [13]. The interaction of the imprinted hydrogel to lysozyme could be switched between coordinate effect and electrostatic effect by adding or not adding Cu(II) ion. This imprinted hydrogel showed high selectivity in purifying lysozyme from the mixture of proteins and egg white. Vinyl-substituted zinc(II) porphyrin, as functional monomer, was used to improve the binding ability and selectivity of (–)-cinchonidine imprinted polymer with fluorescence detection [14]. In consideration of the merits of CIP like high sensitivity and selectivity, the application of this technique in sample preparation should be noticed.

The combination of MIP and solid-phase microextraction (SPME) was firstly proposed in 2001 by Koster et al. [17] and Mullett et al. [18]. Zhu et al. [19,20] reported the preparation and application of MIP-coated stir bar for the first time using monochrotophos and L-glutamine as templates. Xu et al. [21] developed a MIP coated stir bar with ractopamine as template by glass capillary filling with magnetic core as substrate. In recent years, the investigation of MIP coating associated with fiber SPME [22–26] gained more and more attention thanks to its simplicity, flexibility, sensitivity and selectivity. Turiel et al. [22] used silica capillaries as molds in preparing molecularly imprinted polymeric fibers (monoliths) via the silica being etched away after polymerization. Prasad et al. [23] developed MIP–carbon consolidated composite sensors by mechanically withdrawing the monolithic fiber from capillaries. Li and co-workers [24–26] prepared a series MIP modified silica SPME fibers through chemical immobilization. Target analytes also reported, included bisphenol A [27], folic acid [28], tryptophan [29] and diethylstilbestrol [30] in various complicated matrix. To the best of our knowledge, there is still no published report on the combination of CIP and SPME.

2,2'-dipyridine (2,2'-dpy) is frequently used as metal chelating ligand like the ruthenium/dipyridyl chemiluminescence system. 2,2'-dpy is harmful to human being which makes it a potential pollutant to environment. Meanwhile, 2,2'-dpy play an important role in therapy research of vasospasm [31,32]. Yu et al. [33] investigated the effect of 2,2'-dpy on cerebral vasospasm in a double-injection rabbit model of subarachnoid haemorrhage (SAH). It turned out 2,2'-dpy might attenuate cerebral vasospasm. So the analysis of 2,2'-dpy in biological or environmental system is very important. In this work, a novel metal complex imprinted polymer (CIP) coated solid-phase microextraction was prepared on the surface of silanized silica fiber. The CIP coating could recognize 2,2'-dpy with Cu(II) ion as a bridge in aqueous medium. Binding characteristics, imprinting effect and ligand selectivity of CIP-coated SPME fiber were evaluated by online SPME–HPLC procedure. Effects of recognition conditions were also investigated and discussed. The prepared CIP-coated SPME fiber showed preponderant adsorption capacity and selectivity with little interference from protic solvent compared to MIP- and nonimprinted polymer (NIP)-coated SPME fiber. This preliminary study on the combination of CIP and SPME were indicative of a practicable protocol for preparing water-compatible MIPs for sample pretreatment. The prominent performance of the CIP illustrated its potential as a flexible, variable and selective method for future use in analysis of a wide range of different compounds.

2. Experimental

2.1. Materials

2,2'-dipyridine (2,2'-dpy) was from Yuanyue Chemical Industry Corporation (Shanghai, China). 4,4'-dipyridine and diphenyl were obtained from Jingchun reagent Co. Ltd. (Shanghai, China). 4-vinylpyridine (4-Vpy) was from Sigma–Aldrich (St. Louis, MO, USA). Copper acetate ($\text{Cu}(\text{OAc})_2$), 1,10-phenanthroline (1,10-phen), methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were provided by Damao Regent Plant and all of analytical grade (Tianjin, China). 4,4'-dimethyl-2,2'-dipyridine (4,4'-dimethyl-2,2'-dpy) was supplied by Tokyo chemical industry Co. Ltd. Ethylene glycol dimethacrylate (EGDMA) was from Corel Chemical Plant (Shanghai, China). Methanol of HPLC grade was from Merck (Darmstadt, Germany). 3-(Methacryloxy)propyltrimethoxysilane was purchased from Shengda Fine Chemical Industry Corporation (Beijing, China). All solutions used for HPLC were filtered through a 0.45 μm nylon filter before analysis. Other chemicals were of analytical grade. All aqueous solutions were prepared using ultra-pure water obtained from a Millipore purification system all through the experiment. The pH value of aqueous solutions was adjusted using hydrochloric acid (HCl) from Damao Regent Plant.

2.2. Instrumentals

A UV3150 spectrophotometer (Shimadzu, Japan) and pH meter (Shanghai Precision Scientific Instruments Co.) were used to determine the component of metal complex and for pH measurements, respectively. A HGC-12 nitrogen Evaporator (Quandao, Shanghai, China) was used for the polymerization. A XSG-409L biological microscope (Hanjin instrument and apparatus Co. Ltd., Shanghai, china) and an S-4300 scanning electron microscope (HITACHI, Japan) were used to investigate the surface morphology and coating thickness of polymer coated fibers. A NICOLET AVATAR 330 Fourier transform infrared (FT-IR) spectrometer and a thermal gravity (TG) analyzer (Netzsch-209, Bavaria, Germany) were applied to study the composition and the thermal stability (at a heating rate of 10 °C/min) of the coatings, respectively. A SPME–HPLC coupling device (Supelco, Bellefonte, PA, USA) was used for desorption. The HPLC system assembled from Shimadzu LC-20A (Shimadzu, Japan) consists of a model LC 20AB pump and a model SPD-20A UV detector.

2.3. Preparation of $[\text{Cu}(\text{OAc})_2(2,2'\text{-dpy})]$ imprinted polymer coated silica fiber

The CIP-coated SPME silica fiber was prepared by a similar procedure according to our previous work [34]. Firstly, the silica fibers were dipped in acetone for 3 h to remove the protective polyimide layer. After that they were activated with sodium hydroxide and then with HCl to expose hydroxyl. Thereafter, they were washed with water until pH 7.0 and then dried in an oven at 150 °C. In order to immobilize CIP on the modified fibers with chemical bond, the hydroxylated fibers were treated with 10% (v/v) 3-(methacryloxy)propyltrimethoxysilane solution in acetone for 1 h at room temperature. Finally the silanized fibers were rinsed with methanol and dried under a stream of nitrogen.

Based on Zeng et al.'s work [35], a modified synthetic process was applied to integrate CIP preparation and surface imprinted. 156.0 mg 2,2'-dpy, 182.0 mg $\text{Cu}(\text{OAc})_2$ and 0.210 mL 4-Vpy were dissolved in 10.0 mL ethanol. This solution was shaken for 12 h at room temperature to form stable pre-polymer solution before 7.56 mL EGDMA and 30.0 mg AIBN were dissolved adequately. Then 1.3 mL of the thoroughly mixed solution was moved into a small glass tube and deoxygenized with nitrogen for 2 min. One end of

the silanized fiber was immersed in the solution and the glass tube was sealed immediately to initiate the coating reaction at 65 °C. After the polymerization was accomplished, the fiber was cautiously pulled out with a thin layer of CIP coating immobilized on the surface. The above coating procedure was repeated until obtaining the wanted film thickness. Then the CIP-coated fibers were conditioned at 130 °C for 3 h under nitrogen protection. At last the CIP-coated fibers were washed with 10% (v/v) acetic acid solution in methanol, 0.1 mol/L ethylenediaminetetraacetic acid (EDTA) aqueous solution (adjusted pH to 7.0 with NaOH), water and methanol successively to remove the molecular template, Cu²⁺, ungrafted functional monomer and cross linker. The MIP-coated and the NIP-coated silica fibers were prepared following the same procedure as above without addition of Cu(OAc)₂ and in the absence of both Cu(OAc)₂ and 2,2'-dpy. The obtained coatings were prepared with 1.0 cm long by scraping the uniform coatings from the top. Then the uncoated end of fiber was attached into the inner tube of a 10 cm long hollow stainless steel tube with adhesive.

2.4. Adsorption study

All SPME fibers were conditioned in 0.1 mol/L HCl, methanol and water in turn for 20 min before use. In a typical process, 3.0 mL 2,2'-dpy-Cu(OAc)₂ (dpy-Cu) standard aqueous solution (pH 6.0) and a magneton were added into a 5-mL glass vial. A SPME fiber was immersed into the solution to extract analytes for 60 min at a stir speed of 750 rpm and then pulled out and washed with methanol for 1 min. After that the fiber was immersed into the SPME-HPLC interface chamber to desorb the analytes with mobile phase as desorption solution for 15 min. Subsequently, all desorption solution was injected into the HPLC system for analysis. The adsorbed amounts of 2,2'-dpy or its analogues were calculated by respective area of chromatographic peak and relative standard curves. The same fiber conditioned process mentioned above was run before the fiber was applied to next extraction.

2.5. Ligand selectivity investigation

In order to evaluate the ligand selectivity of CIP-, MIP- and NIP-coated fibers, feeding solutions with concentration of 500 μg/L [Cu(OAc)₂(ligand)] were prepared. Equilibrium adsorbed amounts of single 2,2'-dpy, 1,10-phen, 4,4'-dimethyl-2,2'-dpy, 4,4'-dpy or diphenyl on coatings were studied with extraction time and desorption time of 1.5 h and 20 min, respectively. The selectivity factor (α) of certain fiber for 2,2'-dpy to analogue was calculated by the equation as follows:

$$\alpha = \frac{n_{\text{template}}}{n_{\text{analogue}}} \quad (1)$$

where n_{template} is the adsorbed amount of the template on a certain fiber and n_{analogue} is the adsorbed amount of single analogue on the same fiber, respectively.

2.6. HPLC conditions

Chromatographic separation was run in a Diamonsil C18 (250 mm × 4.6 mm, 5 μm particle size, Dikma) column. The buffer solution was made as 0.1% triethylamine (v%) in water. The mobile phase was methanol/buffer (50:50, v/v) at the flow rate of 1.0 mL/min for the detection of 2,2'-dpy, 4,4'-dpy and 1,10-phen. While for 4,4'-dimethyl-2,2'-dpy and diphenyl, the mobile phase was methanol/buffer (60:40, v/v) and (80:20, v/v), respectively, both at the flow rate of 1.0 mL/min with methanol/buffer (50:50, v/v) as desorption solvent. Ultraviolet detector was set at 280 nm for 2,2'-dpy and 4,4'-dimethyl-2,2'-dpy, 260 nm for 4,4'-dpy, 1,10-phen and diphenyl, respectively.

2.7. Calibration and spiked sample preparation

Calibration curve was established by the standard curve method. Stock solutions of dpy-Cu were prepared at the concentration of 1000 mg/L (count as 2,2'-dpy) in water (pH 6.0). Standard solutions were made by diluting the stock solution serially with water (pH 6.0). All solutions were sealed and refrigerated at 4 °C until used for calibration, limit of detection (LOD) and linearity tests.

Tap, laboratory wastewater and human urine were selected for real sample analysis. Each water sample was spiked with 2,2'-dpy at concentration of 20 μg/L and 100 μg/L, respectively. Then the water sample was filtered through a 0.45 μm nylon filter. Consequently, Cu(OAc)₂ was added to obtain the same concentration as 2,2'-dpy. At last the pH of spiked water sample was adjusted to be 6.0 before analysis. Because the pH of urine sample was closed to 6.0, spiked urine was used without adjusting pH value.

3. Results and discussion

3.1. Preparation of CIP-coated SPME fiber

Molecular imprinting effect lies in the reservation of cavity complementary to the template. It is necessary to understand the structure of the target analyte. In this work, the complex template included Cu(OAc)₂ and 2,2'-dpy connected to each other with coordinate interaction. In order to characterize their complexation, a series solutions in methanol were prepared with constant molar sum of Cu(OAc)₂ and 2,2'-dpy (C_{total}) and different mole of 2,2'-dpy ($C_{2,2'\text{-dpy}}$) in them. The UV spectra were recorded and illustrated in Fig. S1. It could be seen that as the molar fraction of Cu(OAc)₂ increased, the absorption at 280 nm showed a red shift to 300 nm whose absorption intensity increased gradually, which indicated the decrease of free 2,2'-dpy and the increase of complex constructed with Cu(OAc)₂ and 2,2'-dpy. After the molar ratio of Cu(OAc)₂ was greater than 0.5, the absorption band at 300 nm stopped shifting and the absorption intensity started to decrease. The absorbance values at 300 nm were plotted against the molar ratio of 2,2'-dpy (Fig. S2). It could see that the absorption curve reached its maximum value when the molar ratio of 2,2'-dpy was 0.5, corresponding to 1:1 coordination ratio between 2,2'-dpy and Cu(OAc)₂. Thus it was inferred that a complex of equal mole 2,2'-dpy and Cu(OAc)₂ was formed, which was consistent with the result of Zeng et al. [35], during the CIP-coated procedure.

In this study, the coating procedure was based on chemical connection between silanized fibers and CIP to enhance stability. As shown in Fig. 1, 2,2'-dpy (molecular template) and equimolar Cu(OAc)₂ were pre-organized to form the complex template [Cu(OAc)₂(2,2'-dpy)]. Then the 4-Vpy (functional monomer) was added to chelate with [Cu(OAc)₂(2,2'-dpy)] through coordinate interaction. Thereafter the free-radical polymerization was carried out in the presence of cross linker and initiator, meanwhile the silanized fiber was dipped in this solution. CIP was bonded to the fiber surface via the participation of silanized fiber in the polymerization process. The obtained thickness of CIP coating was 12.0 μm under the observation of biological microscope. To investigate the repeatability of this method, 10 CIP-coated fibers were prepared in one batch and RSD of film thickness was 8.4%.

3.2. Characteristics of CIP-coated SPME fiber

Fig. 2 exhibited the scanning electron micrograph of CIP, MIP and NIP coatings under the magnification of 10,000. It could be observed that CIP coating (Fig. 2(c)) possessed dense, smooth and homogeneous surface with numerous micropores which were estimated from the measure scale to be about 0.5 μm in diameter. These pore

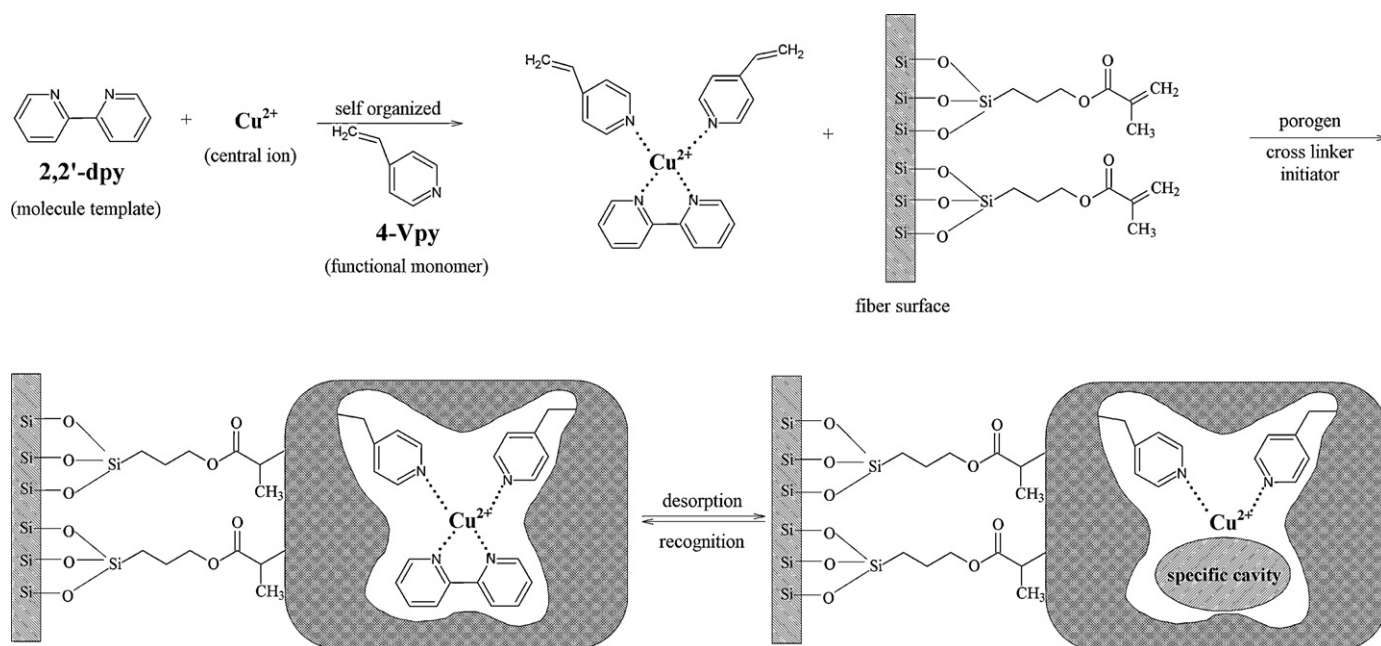


Fig. 1. Schematic representation of $[\text{Cu}(\text{OAc})_2(2,2'\text{-dpy})]$ imprinted SPME fiber coating process.

structures would increase surface area and benefit extraction performance of CIP-coated fiber. On the other hand, the morphological structures of MIP (Fig. 2(b)) and NIP (Fig. 2(a)) coatings, which were porous, loose and layer upon layer, were quite different from that of CIP coating. It might be expected that homogeneous polymers would have lower surface areas leading to decreased binding capacity. But the homogeneous structure did not undermine the binding ability of CIP coating in this study which was further tested by adsorption capacity assays and selectivity experiments.

The infrared (IR) spectra of CIP, MIP and NIP coatings were investigated and shown in Fig. S3. The IR spectrum of CIP coating after eluting complex template was similar to those of MIP coating after eluting template and of NIP coating. The broad absorption bands at 3441 cm^{-1} corresponded to O–H stretching vibration. Other absorption peaks match all three coatings: 2987 and 2957 cm^{-1} (stretching vibration of C–H bonds on benzene ring from 4-Vpy); 1729 cm^{-1} (stretching vibration of C=O bonds on carbonyl groups); and 1638 and 1456 cm^{-1} (stretching vibration of C=C bonds on ben-

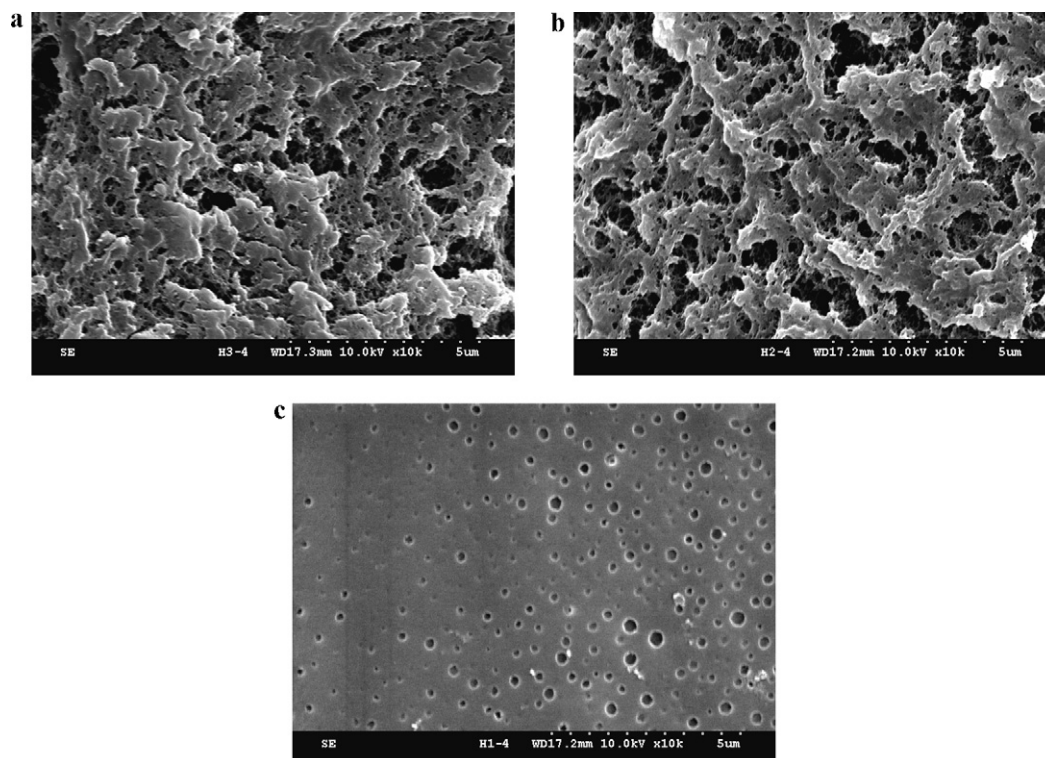


Fig. 2. Scanning electron micrographs of SPME coatings under the magnifications of 10,000. NIP coating (a), MIP coating (b) and CIP coating (c) after eluting template.

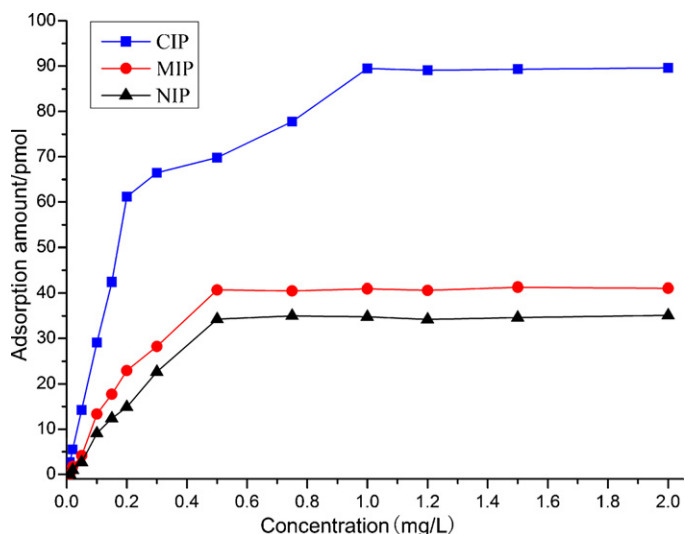


Fig. 3. Adsorption capacity isotherms of CIP-, MIP- and NIP-coated fibers under a series of dpy-Cu concentrations.

zene ring), 1388 cm^{-1} (bending vibration of C–H bonds on methyl groups). The IR profiles suggested that the addition of template and metal did not change the chemical constitution of CIP coating or MIP coating.

The thermogravimetric analysis of CIP, MIP and NIP coatings revealed that the obvious mass loss appeared to start at around $300\text{ }^{\circ}\text{C}$ (Fig. S4), and all had two fastest mass loss peaks judging from the derivative thermogravimetric (DTG) curves. The first was around $330\text{ }^{\circ}\text{C}$ and the second was around $420\text{ }^{\circ}\text{C}$. The solvent resistance test indicated that after immersed in water, methanol, acetonitrile, ethylacetate or n-hexane for 12 h with a magneton at a stir speed of 500 rpm, all coatings did not show any desquamating or cracking under naked eye and microscope observation. It could be concluded that these coatings had good thermal stability and solvent resistance. In addition, CIP coating maintained good surface quality and showed no obvious damage after used for more than 70 times.

3.3. Adsorption capacity assays

A series of dpy-Cu standard aqueous solutions with concentration from 10 to $2000\text{ }\mu\text{g/L}$ were prepared to evaluate the adsorption capacities of CIP-, MIP- and NIP-coated fibers. Fig. 3 showed the isotherms of the adsorption amounts to the concentrations of dpy-Cu. It could be observed that the adsorption amounts increased gradually along with the increase of dpy-Cu concentrations. It was found that CIP-coated fiber had better affinity to dpy-Cu, concluded from the phenomenon that higher adsorption rate was observed for CIP-coated fiber than MIP- and NIP-coated fibers. Meanwhile the adsorption capacities of CIP-coated fiber were the greatest at all concentrations. The maximum adsorption capacities of MIP- and NIP-coated fibers were 40.6 pmol and 34.4 pmol , respectively. On the other hand, the maximum adsorption capacity of CIP-coated fiber was 89.3 pmol , which was 2.2 and 2.6 times as much as those of MIP- and NIP-coated fibers, respectively.

Though the MIP coating was imprinted with 2,2'-dpy, the adsorption capacity of MIP-coated fiber was closed to that of NIP-coated fiber. This might be due to the fact that for MIP coating 2,2'-dpy and 4-Vpy interacted with each other through hydrogen bond during the polymerization process and the analytical step. The strength and stability of non-covalent bond directly impacted the imprinting effect of MIP-coated fiber. In this work, ethanol and

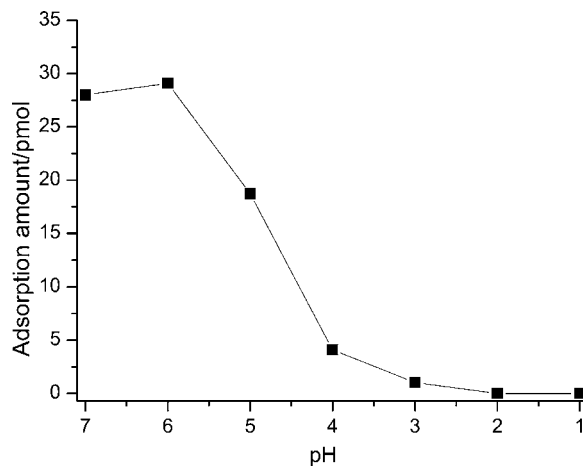


Fig. 4. Influence of pH on binding ability of CIP-coated fiber. Concentration: $100\text{ }\mu\text{g/L}$ 2,2'-dpy with equal mole of $\text{Cu}(\text{OAc})_2$.

water (pH 6.0) were selected to be porogen and extraction solvent, respectively. The strong polarity of solvent would disturb the hydrogen bond interaction between 2,2'-dpy and 4-Vpy resulting in weak imprinting effect of MIP-coated fiber. On the other hand, preparation of CIP coating based on metal coordination between $[\text{Cu}(\text{OAc})_2(2,2'\text{-dpy})]$ and 4-Vpy. The recognition target of CIP coating was the complex formed by $\text{Cu}(\text{OAc})_2$ and 2,2'-dpy rather than just 2,2'-dpy. That is to say, the polymerizing and recognizing processes of CIP coating utilized the metal coordination bond which was stronger and more stable than hydrogen bond in high polar environment. This would lead to better imprinting effect than that of MIP coating which was verified by the results of adsorption capacity assays mentioned above.

3.4. Influences of extraction conditions on recognition ability of CIP coating

3.4.1. Influence of pH values

Fig. 4 showed the bound amounts of dpy-Cu on CIP-coated fiber at different pH values. It was obvious that the binding capacity decreased as the pH decreased when pH was below 6.0, which could be attributed to the following two causes. Low pH environment would dissociate the dpy-Cu complex and then the specific recognition of CIP coating for the complex was weakened because 2,2'-dpy did not suit the cavity immobilized on fiber surface; at the same time, 2,2'-dpy was ionized in acid aqueous solution and tended to stay in the solution phase. Considering that Cu(II) ion may hydrolysis at pH 7.0 which depends on its concentration in the medium, pH 6.0 was chosen in this study.

3.4.2. Influence of extraction solvent

Water (pH 6.0), methanol, acetonitrile, and n-hexane were chosen to study the influence of extraction solvent on binding yields of CIP-coated fiber with $100\text{ }\mu\text{g/L}$ dpy-Cu solution (Fig. 5). The results illustrated that CIP-coated fiber had the best performance when water (pH 6.0) was used as extraction solvent. This phenomenon confirmed that aqueous environment did not interfere with the binding capacity of CIP-coated fiber. Water (pH 6.0) was determined as the optimized extraction solvent in this work. The coordinate interaction between complex template and CIP was stable and effective in protic environment. For further study of the impact of solvent on imprinting effect, the binding capacities of MIP-coated fiber to 2,2'-dpy in different solvents were also investigated and shown in Fig. 5 compared to the results of CIP-coated fiber. The molecular imprinting effect of MIP was weakened by high

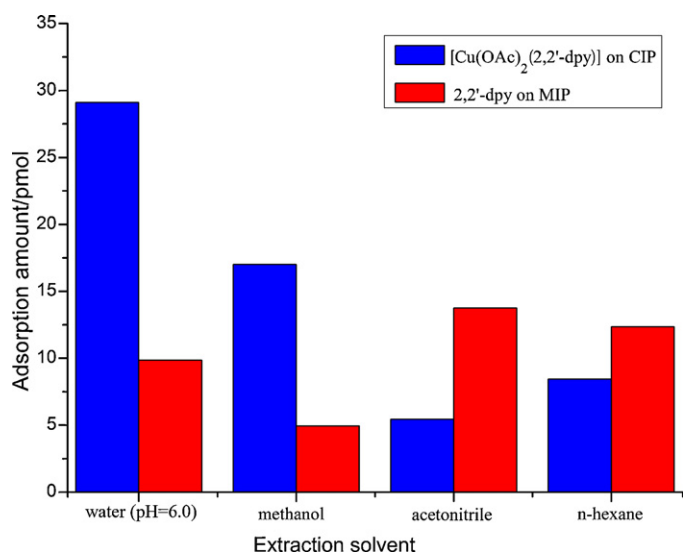


Fig. 5. Influence of extraction solvent on adsorption abilities of CIP- and MIP-coated fibers. Concentration: 100 $\mu\text{g/L}$ 2,2'-dpy with and without equal mole of $\text{Cu}(\text{OAc})_2$ for CIP and MIP coating, respectively.

polar environment such as water and methanol. It could be concluded that the addition of $\text{Cu}(\text{II})$ ion as a connecting bridge was necessary and effective.

3.4.3. Influence of extraction time and desorption time

100 $\mu\text{g/L}$ dpy–Cu aqueous solution was used to study the influences of extraction time and desorption time on binding capacities of CIP-coated fiber. Results showed that the amounts of bound analyte accelerated along with the increase of extraction time before reaching equilibrium at 60 min. The binding yield stayed almost the same when extraction time was more than 60 min. Time dependence of 2,2'-dpy desorption from CIP-coated fiber was investigated at 5, 10, 15, 20, 25, 30 min. It turned out that the equilibrium time of desorption was 15 min.

3.4.4. Influence of stir speed

The Influence of stir speed on binding yield of CIP-coated SPME fiber was studied at 0, 250, 500, 750, and 1000 rpm. It was found that the bound amount of dpy–Cu increased along with the acceleration of stir speed due to faster mass transfer. The adsorption of dpy–Cu reached equilibrium when stir speed extended to 750 rpm which was chosen as optimized stir speed.

3.4.5. Influence of $\text{Cu}(\text{II})$ ion

The impact of coordination bond in recognition process was illustrated in Fig. 6. Evidence of the bridge effect of $\text{Cu}(\text{II})$ ion was obtained via testing the adsorption performances of CIP-, MIP- and NIP-coated fibers with and without $\text{Cu}(\text{II})$ ion in feeding solutions. Seen from the results, in the presence of $\text{Cu}(\text{II})$ ion, all three fibers had higher adsorption capacities than when there was no $\text{Cu}(\text{II})$ ion. Meanwhile, CIP-coated fiber with fixed structure cavity complementary to the complex template turned out to have the greatest adsorption capacity, indicating that the introduction of $\text{Cu}(\text{II})$ ion in the polymerization and feeding solution enhanced the recognition ability of obtained polymer effectively. On the other hand, MIP-coater fiber imprinted with 2,2'-dpy also had imprinting effect but was weaker than that of CIP-coated fiber. NIP-coated fiber which had no imprinted cavity held the smallest adsorption capacity and was less effected by $\text{Cu}(\text{II})$ ion. It could be inferred that $\text{Cu}(\text{II})$ ion was an effective medium to bridge the complex template and the functional monomer.

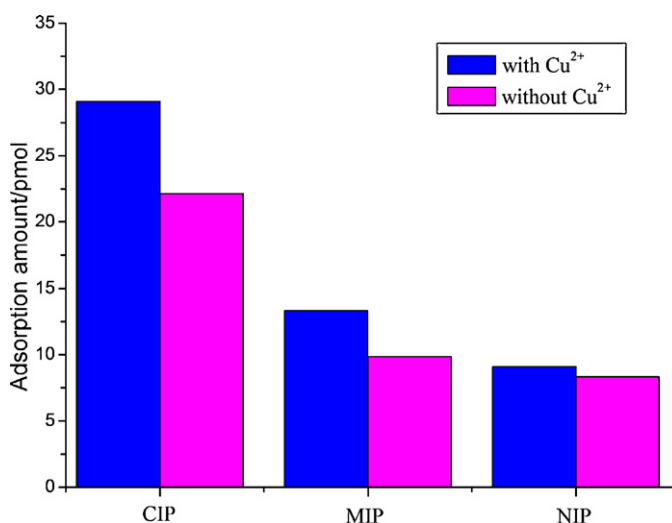


Fig. 6. Adsorption capacities of CIP-, MIP-, and NIP-coated fibers in the presence and absence of $\text{Cu}(\text{II})$ ion. Concentration: 100 $\mu\text{g/L}$ 2,2'-dpy with and without $\text{Cu}(\text{II})$ ion.

3.4.6. Influence of metal ion species

The complex template was constructed with two parts, $\text{Cu}(\text{II})$ ion and 2,2'-dpy. Both of them may contribute to the imprinting effect of obtained polymer coating. The influence of different metal ion species on binding ability of CIP-coated fiber was studied. As shown in Table 1, the amount of metal-2,2'-dpy complex bound to CIP-coated fiber was the greatest in the medium of $\text{Cu}(\text{II})$ ion. Considering that these divalent ions had just a little difference in radius, the dominant factor that affected corresponding binding capacity should be the specific cavity fixed according to the arrangement of the functional groups and three-dimensional structure of the complex template. When $\text{Cu}(\text{II})$ ion was replaced by other metal ion, the formed metal-2,2'-dpy complex would be inconsistent with the cavity on CIP-coated fiber, leading to decrease on binding capacity.

3.5. Ligand selectivity experiments

Fig. 7(a) illustrated the chemical structures of 2,2'-dpy, 1,10-phen, 4,4'-dimethyl-2,2'-dpy, 4,4'-dpy and diphenyl. The comparative adsorption capacities of CIP, MIP and NIP coatings for these ligands were also shown in Fig. 7(b). In case of CIP-coated fiber, the binding amount for 1,10-phen was only less than that for 2,2'-dpy because 1,10-phen has the most similar structure compared to 2,2'-dpy. As for 4,4'-dimethyl-2,2'-dpy, its approach to the imprinted cavity is limited by the steric hindrance of methyls near N atoms which coordinated with $\text{Cu}(\text{II})$ ion during recognition process. Meanwhile 4,4'-dpy is hard to coordinate with $\text{Cu}(\text{II})$ ion with bident, thus the formation of complex being consistent with the spatial arrangement of the 4-Vpy inside imprinted cavity was weak, resulting in low bound amount on CIP-coated fiber. Diphenyl has no atoms that can interact with $\text{Cu}(\text{II})$ ion or 4-Vpy effectively. Its binding on coating was mainly through unspecific adsorption

Table 1

Radii of different metal ions and the corresponding amounts of metal-2,2'-dpy bound to CIP-coated fiber.

Ion species	Ion radius (pm)	Corresponding bound amount (pmol)
Zn^{2+}	74	18.6
Co^{2+}	74	15.4
Cu^{2+}	72	29.1
Ni^{2+}	69	10.2

Concentration: 100 $\mu\text{g/L}$ 2,2'-dpy-metal in water (pH 6.0).

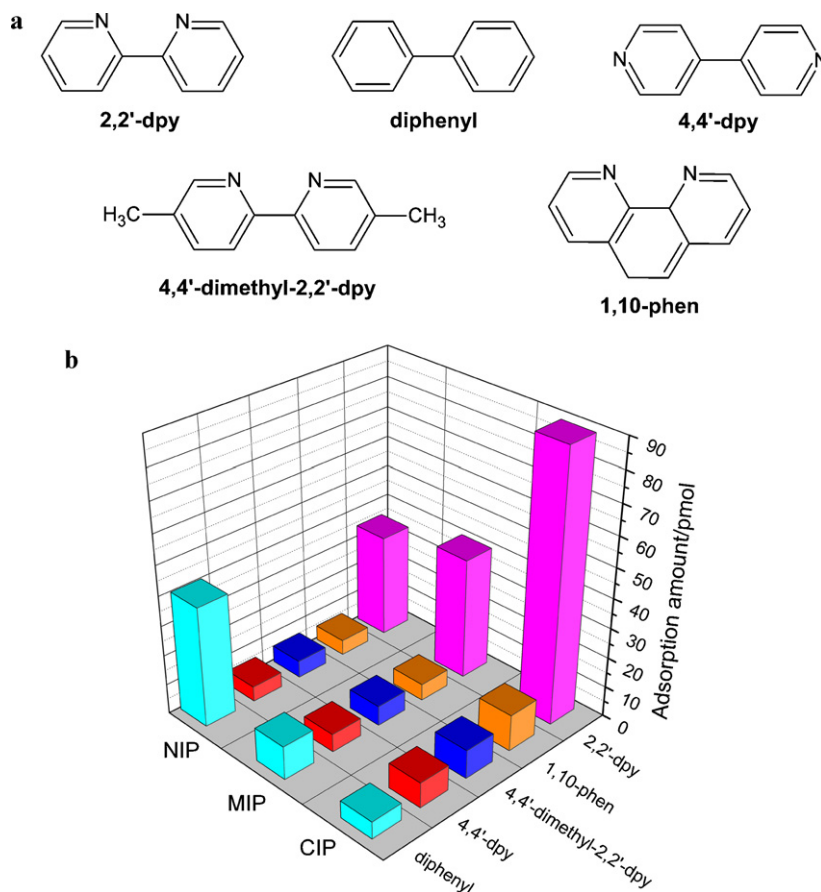


Fig. 7. Adsorption capacities of CIP-, MIP- and NIP-coated fibers for 2,2'-dpy and its analogues. Concentration: 500 $\mu\text{g/L}$ [$\text{Cu}(\text{OAc})_2$ -ligand], extraction time: 1.5 h, desorption time: 20 min. (a) Chemical structures of different ligands for selectivity experiment, (b) adsorption capacities of CIP-, MIP- and NIP-coated fibers for different ligands.

Table 2
Selectivity factors (α) of CIP-, MIP- and NIP-coated fibers for 2,2'-dpy to different ligands.

Coating	α (2,2'-dpy/analogue)			
	1,10-phen	4,4'-dimethyl-2,2'-dpy	4,4'-dpy	Diphenyl
CIP	7.4	9.4	10.6	15.7
MIP	7.2	6.2	6.8	3.6
NIP	6.7	6.0	6.9	0.8

such as hydrophobic force and π - π interaction. For MIP-coated fiber, the selectivity appeared to be poor and had little difference with NIP-coated fiber on adsorption capacities except referring to diphenyl. It could be concluded that the coordinate interaction and the specific recognition cavity were the major factors determining the imprinting effect and selectivity.

Table 2 gave out the selectivity factors (α) of three types of fibers to different ligands in the presence of $\text{Cu}(\text{II})$ ion. It further verified that CIP-coated fiber exhibited advantages for the recognition of [$\text{Cu}(\text{OAc})_2(2,2'\text{-dpy})$] inherited from the strong and oriented coordinate interaction.

Table 3
Recoveries of 2,2'-dpy in spiked tap water, laboratory wastewater and urine samples ($n = 3$).

Compound	Spiked($\mu\text{g/L}$)	Tap water		Laboratory wastewater		Urine	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2,2'-dpy	20	101.8	6.8	102.2	6.1	84.5	8.9
	100	95.5	6.0	103.3	5.5	80.3	8.1

3.6. Sample analysis

A new method of CIP-SPME/HPLC for 2,2'-dpy analysis was developed under optimized extraction conditions with a serial of standard solutions. The proposed method showed linear range of 10–200 $\mu\text{g/L}$ with a correlation coefficient of 0.9984. The limit of detection which was calculated by three times standard deviations of calibration curve divided by its slope was 2.0 $\mu\text{g/L}$ (RSD = 6.5%, $n = 6$). The precision of method was tested with six replicate analyses of a dpy-Cu standard solution at concentration of 20 $\mu\text{g/L}$ and turned to be 6.1% (single fiber) and 8.2% (fiber to fiber).

The CIP-SPME/HPLC method was applied to the analysis of 2,2'-dpy in tap water, laboratory wastewater and human urine samples. MIP- and NIP-coated fibers were also implemented for comparison. Fig. 8 demonstrated the chromatograms of determinations of 2,2'-dpy in tap water, laboratory wastewater and urine samples spiked with 20 $\mu\text{g/L}$ 2,2'-dpy by employing CIP-, MIP- and NIP-coated fibers. The results revealed that CIP-coated fiber had the highest enrichment effect and showed obvious advantages compared to the other two fibers. Better sensitivity was observed for CIP-coated fiber in aqueous system owing to the recognition ability for the complex template, while MIP-coated fiber showed weak imprinted

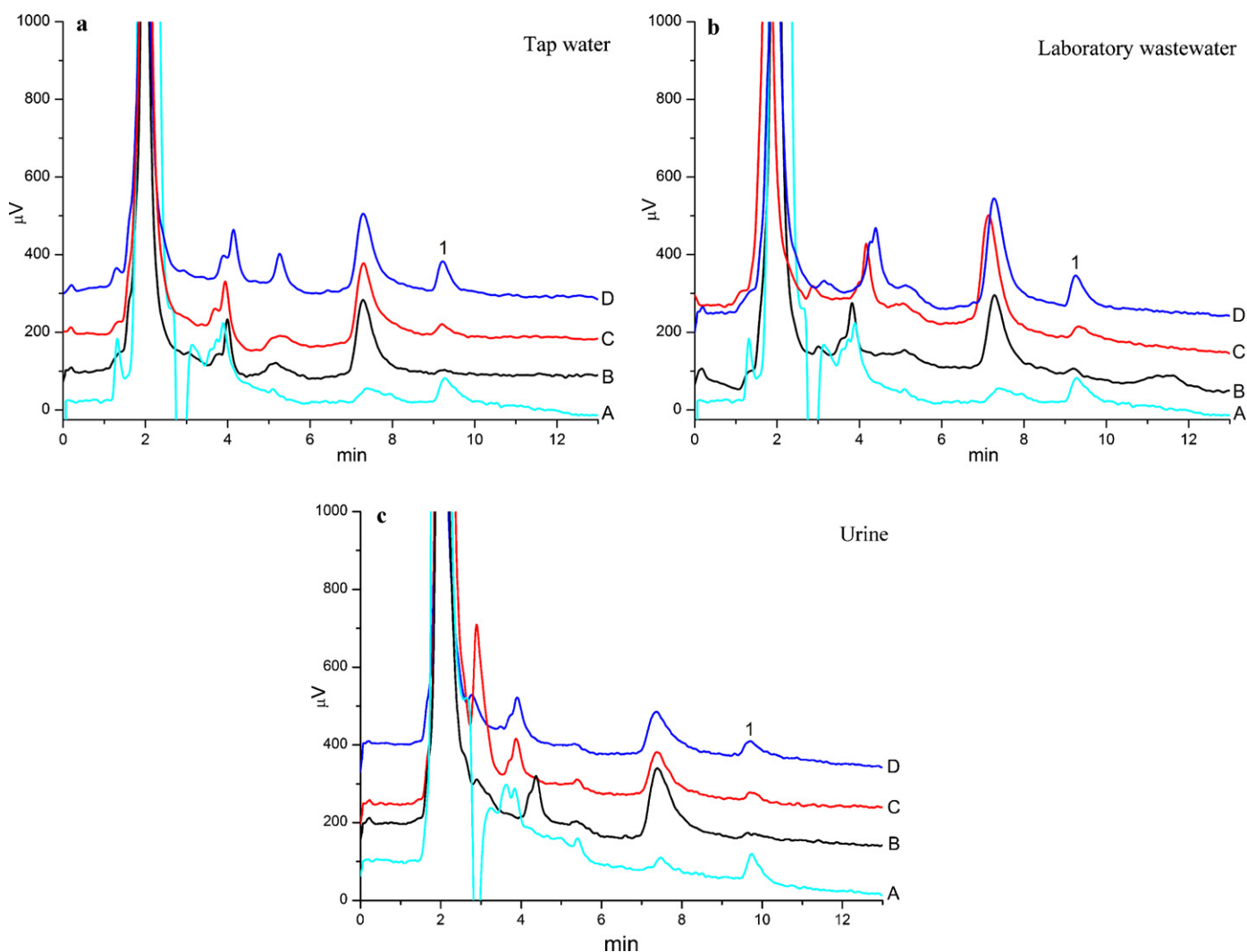


Fig. 8. Chromatograms of 2,2'-dpy in tap water, laboratory wastewater and urine samples. 100 $\mu\text{g/L}$ standard solution for direct injection (A), real water samples spiked with 20 $\mu\text{g/L}$ 2,2'-dpy and extracted by (B) NIP-, (C) MIP- and (D) CIP-coated fibers, respectively. (1) 2,2'-dpy.

effect. The recoveries for the spiked samples were 80.3–103.3% with RSD of 5.5–8.9% in three replicate analysis (Table 3). Though the recovery and RSD of urine sample analysis were worse than that of the other two sample analysis, the interfering peaks did not disturb the determination of 2,2'-dpy. It turned out that the proposed CIP-SPME-HPLC method could be applied to accurate and precise determination of 2,2'-dpy in aqueous samples.

4. Conclusions

In this study, the immobilization of complex imprinted polymer on silica fiber for selective recognition of $[\text{Cu}(\text{OAc})_2(2,2'\text{-dpy})]$ was firstly demonstrated and the binding characteristics of CIP-coated fiber were evaluated. In virtue of the bridge effect of $\text{Cu}(\text{II})$ ion, CIP-coated fiber exhibited higher adsorption capacity and ligand selectivity than MIP- and NIP-coated fibers. Through metal coordination, CIP-coated fiber could be applied in strong protic solvent such as methanol and water, where hydrogen bond will be disturbed or comparably less competitive. The proposed method was successfully applied to the analysis of 2,2'-dpy in tap water, laboratory wastewater and human urine samples. The recoveries for the spiked samples were 80.3–103.3% with RSD of 5.5–8.9%. The satisfactory results indicated the suitability and potential of CIP-SPME for sample pretreatment. CIP can be considered as a promising

solution to the problem of MIP that high polar environments may interfere with or even destroy the imprinting effect and selectivity of obtained polymers. Consequently, the application of MIP will be spread to more fields like biochemistry systems which are usually water-abundant. In addition, metal ion mediated recognition is a promising approach to mimic natural recognition process. Up to now, the research and application of CIP coating are still in initial stage. However, due to the simple variation of composition and structure of CIP, the technique presented here can be adapted to prepare a range of selective CIP-SPME coatings for aqueous sample analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.12.001.

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