Journal of Hazardous Materials 198 (2011) 175–181

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Synthesis by precipitation polymerization of molecularly imprinted polymer for the selective extraction of diclofenac from water samples

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ARTICLE INFO

Article history:
Received 11 July 2011
Received in revised form 30 August 2011
Accepted 6 October 2011
Available online 15 October 2011

Keywords:
Pharmaceuticals
Diclofenac
Molecularly imprinted polymer
Solid-phase extraction
Selective extraction
Environmental water samples

ABSTRACT

A molecularly imprinted polymer (MIP) was synthesized by precipitation polymerization using diclofenac (DFC) as a template, 2-vinylpyridine (2-VP) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linker, and toluene as porogen. The MIP showed outstanding affinity toward DFC in aqueous solution with a binding site capacity (Q_{max}) of 324.8 mg/g (1.09 mmol/g) and was used as solid-phase extraction (SPE) material for the quantitative enrichment of DFC in environmental water samples and off-line coupled to a reversed-phase HPLC/DAD. Various parameters including washing solvent, elution solvent and breakthrough volume affecting the extraction efficiency of the polymers have been evaluated to achieve the selective preconcentration of DFC from water samples and to reduce non-specific interactions. Recoveries of DFC extracted from tap water, river water and wastewater samples were higher than 95%, and no significant DFC recovery difference was obtained among the different water matrix. The stability of MIP was tested by consecutive percolation of water sample, and it was shown that the performance of the MIP did not vary even after 30 adsorption and desorption cycles. Furthermore, the MISPE was used for the analysis of DFC in river water and wastewater samples and revealed DFC concentrations of 0.69 ± 0.002 μg/L ($n = 3$) and 0.31 ± 0.004 μg/L ($n = 3$), respectively. The results were in good agreement with corresponding LC–MS/MS data.

1. Introduction

It is well established that the source, presence, and fate of pharmaceuticals in the aquatic environment are of great concern because of the potential impact on human health and the environment even at low concentration levels [1,2]. Currently more than 80 pharmaceutical compounds have been detected in sewage effluent, surface water, and groundwater [3]. A typical case is diclofenac (DFC), an important non-steroidal anti-inflammatory drug (NSAID) and widely used to reduce inflammation and as an analgesic in conditions such as in arthritis or acute injury [4]. Due to its wide use, DFC has been considered as one of the most frequently detected pharmaceutical residues in water bodies thus far. It has been detected in influents and effluents from water treatment plants at concentrations up to μg/L level [4,5], and also detected in a drinking water sample from a private water tap in Berlin [6]. Preliminary investigations concerning DFC impact on aquatic life indicated some adverse effects on rainbow trout exposed to water concentrations of 1.0–500 μg/L for 28 days [7]. A lethal impact of DFC has been reported on vulture populations on the Indian subcontinent [8,9].

In environmental aqueous samples pharmaceuticals including DFC are widely analyzed by gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography/MS (HPLC/MS) after derivatization [10–13]. Liquid chromatography (LC) methods have the advantage of avoiding an extra derivatization step. Furthermore, lower detection limits have been obtained with this technique. However, the major drawback of LC/MS in quantitative analysis is that it is sensitive to matrix effects, mainly ion suppression [14]. This is often the case with complex matrices, such as sewage or wastewater samples. In addition, HPLC/MS instrumentation is fairly expensive and rarely available in a common environmental laboratory presently. Therefore, sufficiently selective and sensitive analytical methods based on inexpensive instrumentation, such as HPLC with UV detection, are highly desirable for routine monitoring of DFC in water samples. Because the UV detection often lacks the required sensitivity, a preconcentration step should be involved before HPLC/UV detection.

Currently, solid-phase extraction (SPE) is the most widely used procedure to extract traces of organic compounds from environmental samples [15,16]. In addition, SPE could be automated or even performed on-line by direct connection to the chromatographic systems [17]. However, conventional SPE sorbents such as

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doi:10.1016/j.jhazmat.2011.10.027

C18 retain the analytes primarily by anion-exchange or reversed-phase adsorption and are thus rather nonspecific in nature [18]. Many interfering species might be co-eluted on conventional sorbents. Moreover, the high matrix loads would inevitably affect the performance of the extraction sorbent and result in frequent exchange of the SPE material. To overcome these problems, molecularly imprinted polymers (MIPs) with higher selectivity are increasingly developed and applied to different samples [19–21].

Molecular imprinting is an increasingly applied technique that allows the formation of selective recognition sites in a stable polymer matrix. Recently, because of their compatibility with organic solvents, MIPs have attracted considerable attention as SPE sorbents for the cleanup and preconcentration of target analytes prior to determination [22–24]. Molecularly imprinted solid-phase extraction (MISPE) has been applied to extract several pharmaceutically active compounds in different sample matrices [25–27]. Although, the potential of MIP for solid-phase extraction of DFC was well described by Sun et al. [28]. To the best of our knowledge, the method for the preparation of MIPs in that study is bulk polymerization, and the polymers in the form of bulk state need to be crushed and sieved to produce polymer particles with a desired size. As a result, large quantities of polymer particles are wasted, and some of the recognition cavities may be destroyed during these time-consuming processes leading to low capacity and poor site accessibility for the template [29]. Thus, we preferred to prepare MIPs directly in the form of spherical particles by precipitation polymerization, which is more suitable for SPE work.

The objective of this study was to prepare a molecularly imprinted polymer using DFC as the template by precipitation polymerization and to evaluate this polymer as a selective sorbent in SPE coupled off-line to a reversed-phase HPLC to selectively enrich DFC from a large volume of river and wastewater samples. A comparison of the MIP with conventional sorbent C18 was also discussed. The major advantages of this method are that MIP shows high selectivity and affinity to the target analytes and is very stable for a real environmental application. These advantages make the MISPE successfully avoid the problems in conventional nonspecific SPE.

2. Experimental

2.1. Chemicals

Diclofenac (DFC) sodium salt, carbamazepine (CBZ), 2-vinylpyridine (2-VP), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobisisobutyronitrile (AIBN) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN), methanol, toluene as well as acetic acid were from Tedia Company, Inc. (USA). Ultra–pure water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, USA). AIBN was recrystallized in methanol prior to use.

DFC was extracted into chloroform from an acidic solution in order to obtain its acid form. Standard stock solutions of DFC (1 g/L) and CBZ (1 g/L) were prepared in Millipore water and methanol:water (1:1, v:v) mixture, respectively, and stored at 4 °C.

2.2. Analytical methods

The HPLC analyses were carried out on an Agilent 1200 (Agilent Technologies, USA) HPLC system equipped with a G1329 auto sampler, a G1315D diode array detector (DAD) and a G1316A column oven. The UV detection wavelength was 272 nm and the column temperature was set at 30 °C. A SHMADZU C_{18} reversed-phase column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation. The mobile phase consisted of 60% methanol–acetonitrile mixed solution (1:1, 0.1% acetic acid) and 40% Millipore water (0.1% phosphoric acid). The flow rate was 1.0 mL/min, and the injection volume was 20 μL. Samples were filtered through a 0.45 μm syringe filter (Millipore) before injection. Quantification of CBZ and DFC was performed using an external standard method. The linear range was established between 0.1 and 1.0 mg/L with a correlation coefficient (R^2) of 0.9997. The limit of detection (LOD) was 0.01 mg/L for CBZ and DFC and the limit of quantitation (LOQ) was 0.1 mg/L for CBZ and DFC.

The LC–MS/MS analyses of DFC were performed on TSQ Quantum High Performance Liquid Chromatography coupled with Mass Spectrometry (Thermo Fisher Scientific, San Jose, CA, USA). The separation was performed on an Agilent Eclipse XDB C18 reversed phase column (150 mm × 2.1 mm, 5 μm), with the flow rate of 0.35 mL/min. Methanol (mobile phase A) and water with 0.1% (v/v) acetic acid (mobile phase B) were used for separation. The injection volume was 10 μL, and the column temperature was 30 °C. The gradient was held at 75% A for 5 min, and increased to 90% A within 5 min and held for 5 min, and then reset to initial conditions of 75% A in 5 min and held for 5 min. The mass spectrometer detection was operated in electrospray ionization negative ion mode with selected reaction monitoring (SRM). The first SRM (SRM1) monitored to quantify DFC was m/z 294 → 214, while the second SRM (SRM2) for analyte confirmation was m/z 294 → 249. Quantification of DFC was performed using an external standard method. The limit of detection (LOD) calculated by a signal-to-noise ratio (S/N) of 3 was 5 ng/L.

2.3. Preparation of MIP by precipitation polymerization

For the preparation of DFC-MIP, 200 mg (0.67 mmol) of DFC was mixed with 0.270 mL (2.56 mmol) of functional monomer 2-VP in a 250 mL screw-capped glass vial followed by addition of 60 mL of porogen toluene. An amount of 2.62 mL (13.88 mmol) of cross-linker EGDMA and 40 mg (0.24 mmol) initiator AIBN was then added to the above solution. The solution was purged with nitrogen in an ice bath for 5 min to remove oxygen, and sealed in the glass vial. The temperature was ramped from room temperature to 60 °C over a period of around 2 h and then held at this temperature for a further 24 h with stirring. The resultant microspheres were then sonicated in methanol/acetic acid solution (9:1, v:v) for 20 min, followed by centrifugation to remove solvent. This procedure was repeated several times until DFC could not be detected in the extraction solvent. Then, the microspheres were sonicated again in methanol three times for 10 min per cycle to remove residual acetic acid. Finally, the solvent was removed by centrifugation, and the microspheres were dried under vacuum at 60 °C and used for the subsequent studies. As a reference, non-imprinted polymer (NIP), which did not contain the template, was also prepared in parallel with the MIP by using the same synthetic protocol. To establish the reproducibility of the MIP preparation protocol, three batches of polymer following strictly the protocol outlined above were conducted.

2.4. Binding characteristics of the imprinted polymer

To evaluate the binding capacity of the MIP obtained, static adsorption test was carried out. 10 mg of polymer microspheres were added in 10-mL flask containing 5.0 mL DFC solutions of various concentrations (300–1000 mg/L). After shaken for 2 h at room temperature, the samples were centrifuged and filtered. The free DFC concentration in the filtrate was detected by HPLC. The adsorption capacity (Q) was calculated by subtracting the free concentrations from the initial concentrations. Meanwhile, the

maximum binding capacity (Q_{max}) and dissociation constant (K_d) were estimated by processing with the Scatchard equation:

$$\frac{Q}{C_{free}} = \frac{Q_{max} - Q}{K_d}$$

where Q and Q_{max} are the amount of DFC adsorbed onto unit mass of dry MIP and the maximum adsorption capacity of unit mass of dry MIP, respectively (mg/g), C_{free} is the concentration of DFC in equilibrium solutions (mg/L), and K_d is equilibrium dissociation constant of binding sites.

To investigate the binding kinetics of microspherical MIP, the binding efficiency of DFC at initial concentration of 300 mg/L (5 mL) to the MIP were measured as a function of time. The binding of DFC to NIP was also measured in the same way. All the experiments were performed in triplicate.

2.5. MISPE cartridges preparation and MISPE procedures

Empty SPE cartridges (63 mm × 9 mm i.d.) were dry-packed with 35 mg of MIP or NIP. PTFE frits (porosity 20 μm, Shenzhen Comma Biological Technology Co., Ltd., China) were placed above and below the sorbent bed. Prior to loading the sample, the cartridges were pre-conditioned with 5 mL of methanol followed by 5 mL of Millipore water at 0.1 mL/min. If not used immediately, preconditioned cartridges were sealed with appropriate stoppers and stored at 4 °C to prevent drying by solvent evaporation.

For condition optimum experiments, 3 mL sample of 0.5 mg/L DFC standard solution was loaded into the cartridge. The cartridge was dried by nitrogen for 20 min, and then it was washed with 2 mL of ACN/water (40:60, v:v). The analytes retained in the cartridge were eluted with 2 mL of methanol/acetic acid (9:1, v:v). Both the washing and elution fractions were collected and dried under a gentle nitrogen stream, the residues were reconstituted in 1 mL of methanol for the HPLC analysis.

2.6. Application of DFC-MIP to real water samples

River water sample was collected from Shajinggang River in Shanghai, and wastewater samples were collected from the effluent of the Quyang sewage treatment plant in Shanghai in May of 2011. All the water samples were filtered through a cellulose acetate filter to eliminate any solid impurities. The tap water sample was collected from the tap in the laboratory.

The cartridges were conditioned with 5 mL of methanol followed by 5 mL of Millipore water. 1000 mL of water samples were loaded onto the MISPE cartridges, and then it was washed with 2 mL of ACN/water (40:60, v:v). The DFC retained in the cartridges was eluted with 2 mL of methanol/acetic acid (9:1, v:v). The extracts were subsequently concentrated under a gentle nitrogen stream and reconstituted in 1 mL of methanol for the HPLC analysis.

3. Results and discussion

3.1. Binding characteristics of MIP

To characterize the adsorption behaviors of the MIP and NIP, batch binding tests were performed and the results were shown in Fig. 1. The DFC binding amount of both MIP and NIP increased with the increase in the initial DFC concentration, but MIP exhibited significantly higher affinity than the NIP all through (Fig. 1a). It may be explained that most of the functional monomers were orderly assembled onto DFC by electrostatic attraction when the MIP was synthesized and their positions were fixed by the polymerization. After the removal of template, cavities that could recognize the template via multiple point electrostatic interaction and shape complementarity were formed in the MIP. By contrast, the random

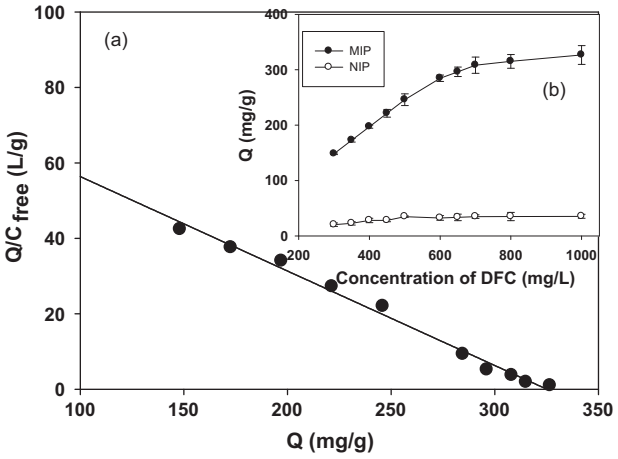

Fig. 1. (a) Binding isotherms of MIP and NIP for DFC in dionized water; (b) Scatchard plot of MIP.

distribution of functional monomers in the NIP resulted in a lower affinity binding than that of the MIP, which is also a proof of the successful creation of specific binding sites through imprinting. The high affinity of MIP toward DFC in water was also demonstrated by dynamic adsorption tests. MIP adsorbed more than 90% of DFC within 120 min and the binding equilibrium between MIP and DFC was almost established within 15 min. In comparison, NIP adsorbed less than 20% of the DFC in the same time period. The Scatchard plot for MIP shown in Fig. 1b is straight line, indicating that the affinities of the binding sites in MIP is homogeneous among the concentrations tested. Similar Scatchard plots were obtained with the case of cinchonidine-imprinted polymers prepared by a precipitation polymerization method [30]. From the Scatchard plot, the Q_{max} value of MIP was calculated to be 324.8 mg/g (1.09 mmol/g), while that of NIP was 45.2 mg/g. In addition to the good binding capability, a dissociation constant K_d of 3.99 mg/L demonstrated the strong affinity between DFC-MIP and DFC. The dissociation constant K_d of NIP was 434 mg/L. Compared to MIP for DFC reported in the literature, MIP in this study has 10-fold higher binding capacity as opposed to the DFC-MIP prepared by a bulk polymerization method [28]. The factors contributing to this phenomenon are that the MIP obtained by our method was porous structure providing excellent specific surface areas for binding and the increasing number of effective recognition cavities. The microscopic characteristic of the imprinted polymer was shown in Fig. 2, and a porous surface could be clearly observed. The specific surface area, pore volume and pore size obtained from nitrogen adsorption experiments were 57.18 m^2/g, 0.33 cm^3/g and 24.13 nm for DFC-MIP. The short contact time needed to reach the binding equilibrium as well as the high binding site capacity suggests that the MIP prepared by precipitation polymerization possess highly potential applications for extracting pollutants from water.

3.2. Optimization of the MISPE procedures

It is well-known that the molecular recognition principle of most of MIPs are based on the hydrogen binding between the target and the polymer functional groups, which often occurs in apolar solvents. In such system, specific hydrogen bonds are stabilized, and nonspecific hydrophobic interactions are suppressed [31]. However, nonspecific hydrophobic interactions always exist in the adsorption process of MIP in the water samples as discussed above. Many compounds present in the water sample are normally retained on the MIP. Therefore, to avoid interference of these compounds in the analysis of the target molecule, a washing step is

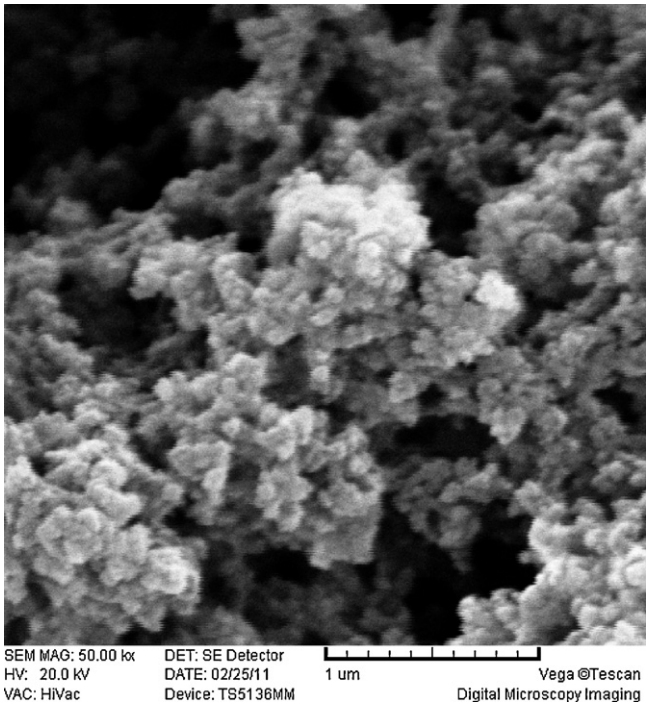

Fig. 2. Scanning electron microscopy of the MIP.

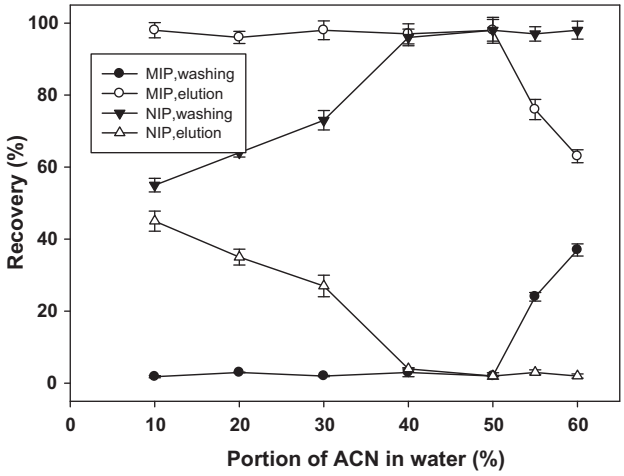

Fig. 3. The recoveries of DFC in the washing and elution fractions after preconcentration on the MIP and NIP cartridges in dependence on the ACN concentration in water.

normally included in the analytical protocol. The washing step was the most crucial procedure to maximize the specific interactions between the analytes and binding sites, and to simultaneously decrease non-specific interactions to discard matrix components in the polymer [32]. The washing solvent must wash off the non-specifically adsorbed meanwhile it should still keep the selectively retained fraction on the MIPs. To evaluate the usefulness of this clean-up step, and to demonstrate that the polymer synthesized was indeed imprinted, a comparative analysis between NIP and MIP was carried out. Several nonpolar solvents (toluene and chloroform), polar aprotic solvents (dichloromethane and ACN), and polar protic solvent (methanol) were tested in terms of washing efficiency. During the washing process, DFC non-specifically bound to the polymer will be eluted, whereas part of the DFC specifically bound remains trapped in the polymer due to the specific interactions. ACN was proved the most effective washing solvent, though DFC could not be eluted from NIP completely. On the contrary, methanol could efficiently remove most non-specifically bound DFC from the NIP cartridge, however, the specific interaction between the template and the MIP was also disrupted. Thus, ACN was selected as the washing solvent. When it was used at a volume of 2 mL, about 45% of DFC loaded on NIP cartridge was washed off while DFC bound on MIP was still retained. With increase of the volume of ACN to 5 mL, the amount of DFC eluted from the NIP cartridge increased along but did not exceed 65%. Therefore, ACN was mixed with different volumes of water to increase the polarity stepwise. ACN/water (10:90, 20:80, 30:70, 40:60 and 50:50, v:v) were investigated in washing step. 3 mL of DFC standard solution (0.5 mg/L) in water was applied to the MIP and NIP cartridges. After loading DFC solution, both the MIP and NIP cartridges were submitted to a washing step, and then the cartridges were eluted with 2 mL of methanol/acetic acid (9:1, v:v). Both the washing and elution fractions of the solvent were collected and analyzed. In the washing process, DFC non-specifically bound to the polymer will be eluted, whereas part of the DFC specifically bound remains trapped in the polymer due to the specific interactions. In the NIP, quantitative elution of the template was expected in order to eliminate the disturbance of the non-specific interactions. Fig. 3 shows the recoveries of DFC in the washing and elution fractions after preconcentration on the MIP and NIP cartridges by using 2 mL of each of the washing solvents. According to the results, the amount of DFC removed from the NIP cartridge increased with the increasing portion of ACN in water, and it was totally washed off the NIP cartridge when the portion of ACN in water is up to 40% (v/v). On the contrary, DFC could be selectively and efficiently retained on the MIP cartridge when the portion of ACN in water is lower than 50%. However, when the portion of ACN is higher than 50%, a large decrease of DFC retention on the MIP cartridge was observed due to the disruption of specific interactions between the DFC and binding sites [32]. The effect of the washing solvent volume on MISPE extraction was investigated. The results showed that the optimum volume of the washing solvent was 1.5–2 mL. Therefore, 2 mL of ACN/water (40:60, v:v) was selected as the washing solvent for the further experiments.

For the elution, five aliquots of methanol/acetic acid (9:1, v:v), each of 1 mL in volume, were used to elute DFC from the MIP cartridge after washing step. The recovery for every 1 mL aliquot of methanol/acetic acid (9:1, v:v) was calculated separately. The results showed that 2 mL of methanol/acetic acid (9:1, v:v) was sufficient to elute DFC from MIP cartridge completely (data not shown).

The breakthrough volume is one of the parameters characterizing the MIP adsorbent bed and determined the maximum volume of water sample which can be introduced into the adsorbent bed under given hydraulic conditions [33]. Five different sample volumes (100, 300, 500, 700 and 1000 mL) spiked with 5 μg/L of DFC were used to evaluate the breakthrough volume of the MISPE cartridge. After loading DFC solution, the MISPE cartridges were washed by 2 mL of ACN/water (40:60, v:v), and then were eluted with 2 mL of methanol/acetic acid (9:1, v:v). High recoveries (>95%) of DFC were maintained when the sample volume was up to 1000 mL (Fig. 4). The results demonstrated that the MIP, prepared by precipitation polymerization, gave high recoveries of analyte at sample volumes considerably greater than the volumes which could be tolerated by cartridges packed with DFC-MIP derived from crushed and ground polymer monoliths (96% recovery of DFC using a sample volume of 200 mL on the monolith-derived particles) [28].

3.3. Matrix effect

Environmental samples are always complicate matrix, which may reduce the performance and lifetime of the MISPE cartridge

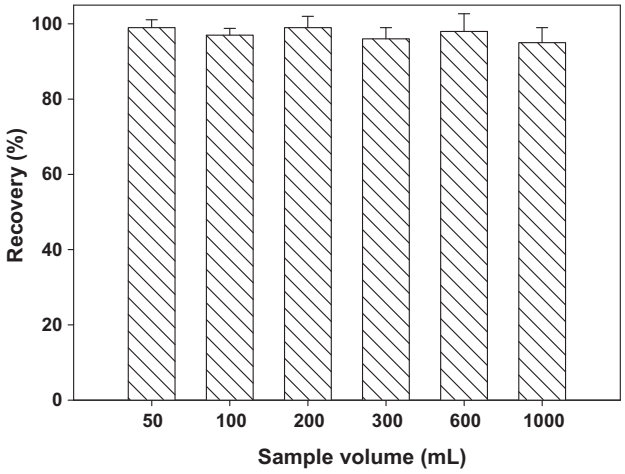

Fig. 4. Recoveries of DFC on MISPE cartridge with different sample volumes.

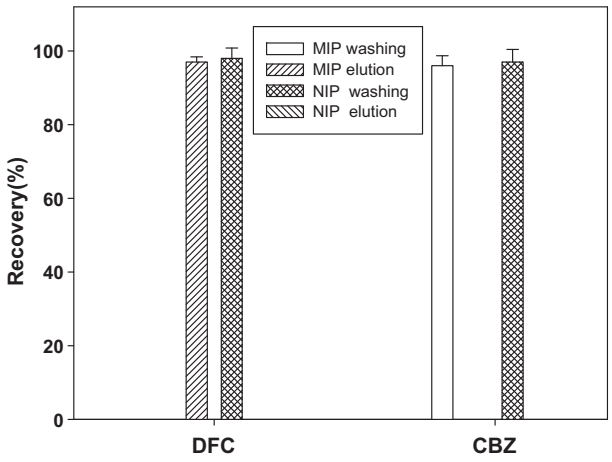

Fig. 6. The recoveries of DFC and CBZ on MISPE and NIP cartridges in washing and elution fractions.

when the MISPE cartridge was directly applied to extract the target analytes from the environmental water samples [31]. Therefore, it is essential to evaluate the matrix effect on the MISPE cartridge for recovering target analytes. The evaluation was performed comparing the recovery of DFC spiked in pure water with that in tap water, river water and wastewater. Meanwhile a comparison of MISPE and commercial SPE C18 cartridge (ENVI-18) cartridges with regard to DFC recovery was also carried out. 3 mL of DFC standard solution (0.5 mg/L) spiked in different water samples were applied to the MISPE and commercial SPE (ENVI-18) cartridges. The ENVI-18 cartridge was preconditioned with 5 mL of methanol followed by 5 mL of Millipore water. After loading sample, the ENVI-18 cartridge was washed with 2 mL 5% methanol in water and dried under a gentle nitrogen stream. The analytes were then eluted with 6 mL of methanol [34]. Solvent removal and residue reconstitution were the same as in the MISPE procedure. The results are shown in Fig. 5. No significant DFC recovery differences were obtained among the different water matrix, but DFC recovery from ENVI-18 varied from 89% in pure water to 74% in wastewater. This suggests that MISPE provides an effective extract even when working with complex water samples as sewage.

3.4. Specificity of the MISPE cartridge

The specificity of MISPE cartridge for DFC was evaluated by using CBZ as interfering compound because its chemical molecular

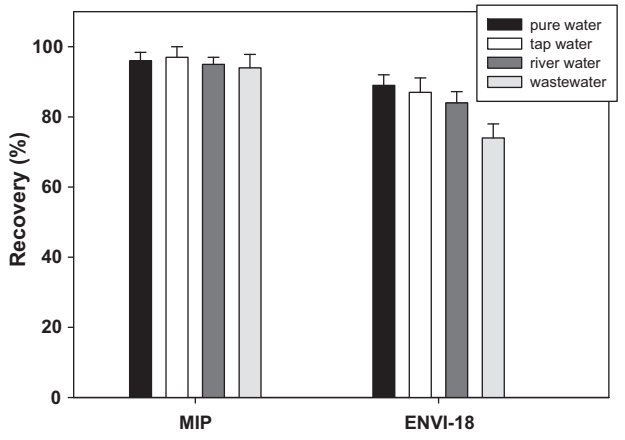

Fig. 5. Recoveries of DFC on MISPE and ENVI-18 cartridges with different water matrix.

structure is similar to DFC at a certain extent and it also widely coexists with DFC in water bodies. 3 mL of a mixture of 0.5 mg/L of each compound was percolated through MISPE and NIP cartridges, and then the compounds in both the washing and elution fractions were analyzed by HPLC. Fig. 6 shows the recoveries of DFC and CBZ in washing fraction and elution fractions. It can be seen that DFC and CBZ were completely washed off from the NIP cartridge after the washing step (recoveries of DFC and CBZ in elution fractions were almost zero, and recoveries of DFC and CBZ in washing fraction are 98% and 97%, respectively). DFC was selectively retained on the MIP cartridge and the recovery in the elution fractions was 96.1%. In addition, CBZ was completely washed off from the MIP cartridge (recoveries of CBZ in washing fractions and elution fractions were 95.7% and 0, respectively). The result showed that the MIP exhibited high selectivity for the template DFC. The selectivity characterization provides an efficient way for removing interferences from compounds with similar structures to that of DFC. MIPs can recognize their template molecules due to the existence of memory cavities with fixed size, shape, binding sites, and specific binding interaction between target molecule and sites. CBZ cannot bind as strongly as DFC and was washed off from MIP by washing step, because its size cannot match the cavities or its functional group position does not correspond to functional groups in cavities and thus cannot bring about specific binding in the same way as DFC [35,36]. As pointed out by Turner et al. [37], the shape of the binding cavity is likely to be as important in terms of rebinding as the distribution of the functional groups themselves. Moreover, the strength of the interaction between target molecules and binding sites also determines the selectivity of MIP [38].

3.5. Stability of the MISPE cartridge

One of the major advantages of MIPs compared to other adsorption materials is their good physical stability (mechanical resistance to high pressures and temperatures) and high chemical robustness, providing the opportunity to clean and reactivate them under relatively harsh conditions for multiple uses in adsorbent applications [39,40]. The recovery of DFC from MIP cartridge indicated that the DFC-MIP can be reused after elution with methanol/acetic acid (9:1, v:v), and is stable for up to 30 binding/regeneration cycles (Fig. 7), which shows that the MIP is sufficiently stable for being applied as an efficient SPE sorbent.

The imprinted molecules are highly embedded in the polymer networks, and it is difficult to completely remove the template prior to enrichment of the analyte. Consequently, a common problem

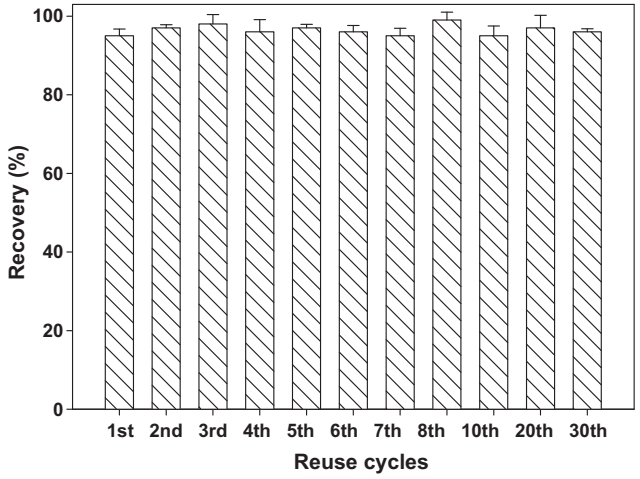

Fig. 7. Stability of the MISPE.

associated with MISPE when using the target analyte as a template is the template bleeding from the polymer during desorption process and its interference with the quantification [31]. In the present study, this phenomenon was only observed when the polymer was used for the first time. A washing step with 6 mL of methanol prior to loading sample was enough to eliminate this problem. During the continuous use of MISPE cartridge, no any significant bleeding effect was observed. Possibly the amount of released DFC was so low that it could not be detected in our system.

3.6. Application of MISPE to real water samples

To demonstrate the applicability and reliability of DFC-MIP for environmental application, real environmental samples were selected and analyzed. The feasibility of applying DFC-MIP to extract DFC from real water samples was evaluated by comparing the concentrations of DFC measured using MIP–HPLC/DAD to those measured on LC/MS/MS. The recoveries of DFC in the surface and effluent samples were higher than 94%. The DFC concentrations in the influent and effluent samples were 0.69 ± 0.002 μg/L and 0.31 ± 0.004 μg/L, respectively. Analysis of the two corresponding influent and effluent water samples using LC/MS/MS revealed DFC concentrations of 0.63 μg/L and 0.29 μg/L. These values were in reasonable agreement with the MISPE–HPLC/DAD discussed above. Therefore, the result indicates that MIP is applicable to the extraction of low concentrations of DFC in environment water samples.

4. Conclusions

In this work, the applicability of the DFC-MIP prepared by precipitation polymerization for the selective enrichment of DFC in water samples was confirmed. The easy handling and high stability of DFC-MIP allowed reliable, rapid analysis of the analytes within complex matrix at trace level. The optimized method is based on an SPE trace-enrichment step using MIPs cartridges followed by HPLC–UV analysis. Different water matrices did not affect the selective performance of DFC-MIP, which is another advantage of MISPE cartridge compared with commercial C18 cartridge. The results showed that MISPE is a potentially competitive technique with traditional SPE for its selectivity and stability. The DFC-MIP was successfully applied to the analysis of DFC in influent and effluent wastewater followed by HPLC–UV detection. The results compared well with a highly selective and sensitive LC–MS/MS analysis that was performed in parallel. Therefore, our results suggested that the MIP provided a reliable and effective solution to enrich low concentrations of DFC in water.

Acknowledgements

This study is financed by the Natural Science Foundation of China (41101480, 41070641), and the National Key Technology R&D Program (006BAJ04A09, 2008BAJ08B13, 2009BAC62B02).

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