Derivatization of 2-chlorophenol with 4-amino-anti-pyrine: A novel method for improving the selectivity of molecularly imprinted solid phase extraction of 2-chlorophenol from water

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Abstract

Molecularly imprinted polymer (MIP) may not selectively recognize small template of limited number of functional groups, such as 2-chlorophenol (2-CP). In this work, a novel method was proposed to improve the recognition ability of the molecularly imprinted solid phase extraction (MISPE) of 2-CP from environmental waters. This was achieved by derivatization of 2-CP with 4-amino-anti-pyrine (4-AAP) to enlarge its molecular size and add more binding sites. For that purpose, two MISPE methods of 2-CP were developed. In method 1, a polymer imprinted with 2-CP was used as the extracting sorbent but it suffered from low selectivity and high detection limit of 2-CP (7.10 ng L−1). In method 2, a polymer imprinted with 4-AAP derivatized 2-CP (2-CP-4-AAP) was used as the extracting sorbent. Prior to loading the water sample it was subjected to a simple derivatization procedure with 4-AAP. Method 2 showed high recognition ability/selectivity towards 2-CP-4-AAP with lower detection limit of 0.05 ng L−1 for 2-CP-4-AAP. Method 2 was able to detect the presence of 2-CP-4-AAP in unspiked real water samples and almost full spike recovery was achieved.

1. Introduction

Due to its bad impact on the environment, phenolic compounds are considered as priority pollutants. Since they may present in water resources at very low concentrations, their direct determination is not always possible. This necessitates development of selective solid phase extraction (SPE) methods for phenols prior to their instrumental determination [1]. SPE of phenols was reviewed by Cela et al. [1]. Various types of SPE sorbents have been used for preconcentration of phenols, such as C18 bonded silica [2], activated carbon [3], multi-walled carbon nanotubes [4], polymethacrylate [5], polymeric and functionalized sorbents [6], anion-exchange resins [7], and pyrrole-based polymer [8].

Molecularly imprinted polymers (MIPs) are synthetic polymeric materials with specific recognition sites complementary in shape, size and functional groups to a template molecule. It involves an interaction mechanism based on molecular recognition [9–14]. These recognition sites mimic the binding sites of biological entities such as antibodies and enzymes. Their stability, ease of preparation and low cost make them attractive for numerous applications.

The use of MIPs for determination of environmental pollutants was reviewed by many authors, such as Pichon and Chapuis-Hugon [15], He et al. [16], Haginaka [17], and Caro et al. [18].

Various phenolic compounds have been used as templates in the preparation of selective molecularly imprinted solid phase extraction (MISPE) sorbents. For example, pentachlorophenol [19], 4-chlorophenol [20], 2,4-dichlorophenol [21], 2,4,6-trichlorophenol [22,23], 4-nitrophenol [20,24–26], chloramphenicol [27], catechol [28], bisphenol A [29–37], 4-nonylphenol [38,39], p-acetaminophenol [40], caffeic acid and p-hydroxybenzoic acid [41] were used as templates. Some of these templates are rich in functional groups so that MIPs of high recognition abilities/selectivities are obtained. Contrary some templates have limited number of functional groups and thus MIPs of low recognition ability/selectivity are obtained.

Recognition ability of the MIP is the ability of the MIP to use its cavities to selectively capture the template molecule in the presence of competing compounds. It is believed that the recognition ability of MIP can be enhanced by improving the structure of the template. For example, derivatization of 2-chlorophenol (2-CP) with 4-amino-anti-pyrine (4-AAP) will produce a derivative (2-CP-4-AAP) of larger molecular size and more functional groups (compared to the original template 2-CP). Thus if 2-CP-4-AAP is used as the template in MIP preparation, then an MIP of higher selectivity/recognition ability towards 2-CP-4-AAP is expected.
the literature, there are many derivatization methods of phenols, but in our case we are interested in those that can be applied in aqueous medium such as derivatization using acetic anhydride, pentafluorobenzoyl anhydride [42] and 4-AAP [43]. In this work, 4-AAP was used to derivatize the phenolic compounds in aqueous medium due to its many advantages, such as: fast results, ease of optimization, the use of relatively stable regents and applicability in aqueous medium over a wide range of concentrations [44–47]. The derivatized phenols can be analyzed by chromatography [47].

In this work, the effect of derivatization of phenols with 4-AAP on the selectivity/recognition ability of the MIP towards the 4-AAP derivatized 2-chlorophenol (2-CP-4-AAP) is studied. This will add more binding sites to the template and enlarge its size. So that it is expected that the selectivity/recognition ability of the MIP towards 2-CP-4-AAP will be improved. 2-CP was selected as the template because it is a small molecule which has a limited number of functional groups.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals were purchased from the following sources: 2-chlorophenol (2-CP) and hydrochloric acid (HCl) from Scharlau (Spain); 3-chlorophenol (3-CP), 2-nitrophenol (2-NP), 2,4-dinitrophenol (2,4-DNP), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) from Acros (Belgium); phenol (Ph) from POCH (Poland); 4-chlorophenol (4-CP) from Fluka (Italy); 2-nitrophenol (2-NP) from Aldrich (Germany); potassium hexacyanoferrate (K₃Fe(CN)₆), 4-nitrophenol (4-NP) from Merck (Germany); acetonitrile (ACN) and 4-amino-anti-pyrine (4-AAP) from Janssen Chimica (Belgium); tetrahydrofuran (THF), glacial acetic acid (AA), ammonium hydroxide (NH₄OH) and methanol (MeOH) from Tedia (USA); 2,2′-azobisisobutyronitrile (AIBN) from BDH (England).

2.2. Instrumentation and equipment

The chromatographic determination of phenols and derivatized phenols was carried out using Shimadzu HPLC instrument. The instrument consisted of SPD-20A UV/Vis detector, LC-20AD pump, 20 µl sample loop, communication bus module CBM-20A communicator and LC solution software. Separation was performed using ODS hypersil column (150 x 4.6 mm, 5 µm) from Thermo Scientific. Molecularly imprinted solid phase extraction (MISPE) was performed using a visisprep-12-port vacuum manifold (Supelco, Germany) by connecting the outlet tip of the manifold to a vacuum pump (Heidolph, Germany). The MIP packed cartridge was prepared by placing a specific mass of the adsorbent in an empty 6 mL polypropylene SPE-tube “filtration tube”, Supelco. Polyethylene frits were used to hold the adsorbent packed in the cartridge. All pH measurements of water samples were conducted using a Weilheim (Germany) pH meter with a combined glass electrode (inoLab). In optimizing the derivatization procedure of 2-CP with 4-AAP in water samples, a Nicolet evolution 100 UV–vis spectrophotometer was used for determination of the absorbance of the derivative (2-CP-4-AAP) solution at 470 nm. Characterization of the synthesized solid derivative (2-CP-4-AAP) was conducted using the following instruments: melting point was measured on SMP1 Stuart apparatus. Infrared (IR) spectrum was recorded as KBr discs on nicolet-Magna-IR-560 spectrometer. ¹H and ¹³C NMR spectra were acquired with the aid of Bruker DPX-300 (¹H NMR: 300 MHz, ¹³C NMR: 75.5 MHz) spectrometer with CDCl₃ as solvents and TMS as an internal standard. Elemental analysis was performed on a Euro Vector elemental analyzer, model EA 3000 (CHNS).

2.3. Synthesis of 4-AAP derivatized 2-CP (abbreviated as 2-CP-4-AAP)

Synthesis of solid 2-CP-4-AAP was performed because it would be used as the template in preparation of 2-CP-4-AAP-MIP (see Section 2.4.2). Synthesis was performed as follows [44–47]: In 500 mL distilled water, the following regents were added successively: 1.0 mmol 2-CP, 1.50 mmol 4-AAP and 10.0 mmol of K₃[Fe(CN)₆]. The pH was adjusted to 10.0 using ammonium hydroxide. The mixture was stirred for 30 min. The derivative was extracted with chloroform. The chloroform was then evaporated, and the gummy-oily product was then washed with petroleum ether many times to get it as solid. The solid was collected and recrystallized from hexane to get a dark red crystals. The structure of 2-CP-4-AAP is shown in Fig. 1. The product was characterized by IR, NMR, and elemental analysis techniques (see Section 3.1).

2.4. Preparation of the molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers were prepared using bulk polymerization technique based on the non-covalent approach [13].

2.4.1. Preparation of 2-CP imprinted polymer (2-CP-MIP)

11.75 mL of acetonitrile and 2.14 mmol 2-CP were introduced into a 50-mL thick walled test tube. 8.08 mmol of MAA and 32.32 mmol of EGDMA were then added successively to the mixture, which was then purged with nitrogen gas. Next, 0.154 g of AIBN was added. The reaction mixture was sealed with a parafilm and left in a shaker at maximum speed at 60 °C for 24 h. The polymeric product was then ground and sieved for particle size ≤ 45 µm. The template (2-CP) was removed from the MIP using a (1:1) (methanol:acetic acid) as the extracting solvent for 18 h in Sauxhlet apparatus. The polymer was then washed with methanol and left to dry overnight in the oven at 65 °C.

2.4.2. Preparation of 2-CP-4-AAP imprinted MIP (2-CP-4-AAP-MIP)

Into a 50-mL thick walled test tube, 11.75 mL of acetonitrile and 0.6114 mmol of 2-CP-4-AAP were added. 2.244 mmol MAA and 8.976 mmol of EGDMA were then added to the mixture successively and the mixture was then purged with nitrogen gas. Next, 0.154 g of the initiator AIBN was added. The reaction mixture was sealed with a parafilm and left in a shaker at maximum speed at 60 °C for 24 h.
The polymer was then ground and sieved for particle size < 45 μm. The template (2-CP-4-AAP) was removed from the MIP by washing with 50 mL of THF:MeOH:HCl (60:20:20 %v/v/v), four times in an ultrasonic bath for half an hour each time. The polymer was then washed with methanol and left to dry overnight in the oven at 65 °C.

2.4.3. Preparation of non-imprinted polymer (NIP)
Preparation of the NIP was similar to the procedure described in Section 2.4.2 but without adding the template.

2.5. Method 1
Method 1 consists of two parts, first MISPE of 2-CP using 2-CP-MIP as the sorbent, second HPLC determination of the phenols in the eluate.

2.5.1. MISPE of 2-CP
As a general MISPE procedure of 2-CP (method 1), the cartridge was packed with 300 mg of the polymer (2-CP-MIP), then washed with 10 mL of ACN containing 1% AA, then 5 mL ACN followed by 2 mL H₂O (pH 3). 25 mL water sample (adjusted to pH 3.0) was loaded into the cartridge. The retained phenols were eluted with 5.0 mL methanol containing 1% acetic acid (AA). Eluates were then analyzed by HPLC as described below (Section 2.5.2).

2.5.2. HPLC determination of the phenolic compounds
The following parameters were set for quantitative determination of phenolic compounds in the eluates: the detector wavelength was 280 nm; 20 μL of the eluate was injected into the instrument; the mobile phase was (25% methanol:74% water:1% acetic acid %) at a flow rate 1.5 mL min⁻¹.

2.6. Method 2
In method 2, phenolic compounds that present in the water sample are first derivatized with 4-AAP in the water sample. MISPE of the derivatized phenols was then applied, which uses 2-CP-4-AAP-MIP as the sorbent. The eluates were then analyzed for determination of the derivatized phenols by HPLC.

2.6.1. Derivatization of phenols in the water sample
Into a 25 mL water sample containing the phenolic compounds: 1.5 mL of 3.0% 4-AAP and 8.0 mL of 3.0% K₃[Fe(CN₆)] were added successively. The pH was adjusted to 10.0 using dilute ammonium hydroxide. The mixture was stirred for 5 min then the produced derivative (2-CP-4-AAP) was subjected to MISPE (see Section 2.6.2).

2.6.2. MISPE of 2-CP-4-AAP
As a general MISPE procedure of 2-CP-4-AAP (method 2), the cartridge was packed with 100 mg of the polymer (2-CP-4-AAP-MIP), then washed with 10.0 mL of ACN containing 1% acetic acid, then with 5.0 mL acetonitrile followed by 2.0 mL H₂O (pH 10). The derivatized water sample (from Section 2.6.1) was loaded into the cartridge, and then washed with 4.0 mL of 2.0% methanol in water. Elution was done using 5.0 mL of methanol (containing 2.0% ammonium hydroxide). Eluates were then analyzed by HPLC as described below (see Section 2.6.3).

2.6.3. HPLC determination of the 4-AAP derivatized phenolic compounds
The following parameters were set for quantitative determination of 4-AAP derivatized phenols: the detector wavelength was 470 nm; 10 μL of the eluate was injected; the mobile phase was (50% methanol:45% water:10% ammonium hydroxide) at a flow rate 1.0 mL min⁻¹.

2.7. Environmental water samples
Two types of environmental waters were used for evaluation and validation of the proposed MISPE methods; reservoir water and stream water. Reservoir water composite sample was generated by collecting various samples from various household reservoirs in Amman. Stream water composite sample was generated by collecting several samples from Al-Zarqa stream. Before use, water samples were filtered through 0.45 μm pore membrane and stored in glass bottles at 4 °C.

3. Results and discussion
This work studied the effect of derivatization of 2-CP (as a model template of small molecular size and limited number of functional groups) with 4-AAP on the recognition ability/selectivity of the MIP towards the derivatized 2-CP in the presence of other competing phenols. Two MISPE methods were optimized: method 1, which is a MISPE procedure that uses polymer imprinted with 2-CP as the preconcentrating sorbent (sorbent 2-CP-MIP), Method 2, which is a MISPE procedure that uses polymer imprinted with 2-CP-4-AAP as the preconcentrating sorbent (sorbent 2-CP-4-AAP-MIP). The two MISPE methods were optimized in the presence of the competing phenolic compounds (3-CP, 4-CP, Ph, 2-NP, 4-NP and 2,4-DNP) to ensure selectivity of the MIP towards 2-CP.

3.1. Preparation and characterization of 2-CP-4-AAP
The reaction of phenols with 4-AAP in the presence of mild oxidizing regent (such as hexacyanoferrate (III)) produces colored dye [44-47]. Preliminary experiments showed that the derivatization of the phenols was only successful for Ph, 2-CP, 3-CP and 4-CP; while 2-NP, 4-NP and 2,4-DNP did not react. This could be explained by the rules reported by Emerson and Beegle [44]. The substituent present in the para position prevented the reaction except for the halogens; therefore 4-NP did not react while 4-CP reacted. The presence of nitro group in the ortho position prevented the reaction, explaining the unreactivity of 2-NP and 2,4-DNP. During the reaction of p-substituted phenols, 4-AAP expelled the p-subsituent or produce the meta or ortho transfer product, this was clear in 4-CP reaction with 4-AAP, which gave a product similar to that produced by the reaction of 3-CP with 4-AAP [44]. So that method 2 was limited to Ph, 2-CP and 3-CP. Therefore it was considered an advantage that derivatization would exclude number of phenolic compounds from the competition (2-NP, 4-NP, 2,4-DNP) due to their unreactivity towards 4-AAP.

2-CP-4-AAP was given special care because it was the template that would be used in imprinting the polymer in method 2. So that it was fully characterized to ensure the formation of the derivative. The formation of the derivative (2-CP-4-AAP) was initially confirmed by the infrared spectroscopy (KBr) data: (ν 1660 and 1625 cm⁻¹ (C=O), 1598 cm⁻¹ (C=N) and 1467 cm⁻¹ (C=C)). The results from mass spectrometry gave M⁺ = 328 g/mol. The results from elemental analysis for C₁₇H₁₄ClN₃O₂ (327.76 g/mol) were as follows: calculated: [C: 62.30%; H: 4.31%; N: 12.82%]; found: [C: 63.55%; H: 5.39%; N: 13.80%]. The results of ¹H NMR (300 MHz, CDCl₃) were as follows: δ 2.52 (s, 3H); 3.42 (s, 3H); 6.60 (dd, J=9.9 and 29.4 Hz, 1H); 7.14–7.58 (m, 6H); 8.81 (dd, J=2.5 and 10.2 Hz, 1H), while the results of ¹³C NMR (75 MHz, CDCl₃) were as follows: δ 10.68 (CH₃); 35.00 (CH₂); 121.91 (C); 126.72–133.47 (CH for C₆H₅ aromatic); 134.80 (C); 141.14 (C); 144.01 (C); 153.70 (C); 162.00 (C). The melting point was 76–80 °C.
Optimization of method 2 (see footnote for experimental conditions).

Table 1

<table>
<thead>
<tr>
<th>pH of water sample</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59.2 ± 7.3</td>
</tr>
<tr>
<td>3</td>
<td>100.9 ± 7.8</td>
</tr>
<tr>
<td>5</td>
<td>35.5 ± 2.2</td>
</tr>
<tr>
<td>7</td>
<td>24.3 ± 8.9</td>
</tr>
<tr>
<td>10</td>
<td>23.0 ± 3.1</td>
</tr>
</tbody>
</table>

Optimizing various variables was conducted to increase the method recovery, sensitivity and to ensure repeatability and complete derivatization of 2-CP in aqueous medium. This included: pH of water sample, amount of 4-AAP, amount of oxidizing reagent and reaction time. The results of optimization (not shown in details) showed that the derivatization was complete within 5 min and the derivative was stable for 200 min (at least) in basic medium (pH 9.0–10.0).

3.3. Preparation of the MIPs

The non-covalent approach was used for MIP preparation because it usually offers fast binding and release of the template [13]. The monomer used (MAA) contains hydrogen bond donor and acceptor site and hydrophobic part. The cross linker (EGDMA) can stabilize the network of the MIP and it can maintain the complementary properties of the cavities towards the template after its removal.

In preparation of 2-CP-MIP, a strong hydrogen bonding between the carboxylic acid group of the MAA and the phenolic group of 2-CP was first created. After cross-linking by EGDMA, a strong backbone of the polymer was formed where 2-CP molecules were entrapped. By leaching the 2-CP molecules from the polymer matrix, an MIP material was generated.

In preparation of 2-CP-4-AAP, the situation was totally different. Many functional groups in the template (2 ketonic groups, 2 amino groups, 1 imine group, 1 chlorine atom, 2 methyl groups, 1 phenyl group) were capable of interaction with MAA through H-bonding and hydrophobic interactions between 2-CP-4-AAP and MAA. After cross-linking with EGDMA, a backbone of the polymer was formed and 2-CP-4-AAP molecules were entrapped. After leaching 2-CP-4-AAP molecules from the polymer matrix, MIP (of larger cavities and more available binding sites) was generated.

Table 2

Optimization of method 2 (see footnote for experimental conditions).

<table>
<thead>
<tr>
<th>Type of washing solvent</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No washing</td>
<td>59.2 ± 1.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>59.2 ± 1.5</td>
</tr>
<tr>
<td>1.0% methanol aqueous solution</td>
<td>63.4 ± 2.3</td>
</tr>
<tr>
<td>2.0% methanol aqueous solution</td>
<td>29.2 ± 1.7</td>
</tr>
<tr>
<td>3.0% methanol aqueous solution</td>
<td>15.1 ± 3.4</td>
</tr>
</tbody>
</table>

3.2. Optimizing the derivatization procedure of phenols with 4-AAP in water

Derivatization of phenols in water samples was the most critical step, because it will ensure maximum recovery of the phenols in method 2. Optimizing various variables was conducted to increase the method recovery, sensitivity and to ensure repeatability and complete derivatization of 2-CP in aqueous medium. This included: pH of water sample, amount of 4-AAP, amount of oxidizing reagent and reaction time. The results of optimization (not shown in details) showed that the derivatization was complete within 5 min and the derivative was stable for 200 min (at least) in basic medium (pH 9.0–10.0).

Table 3

Analytical performance of the proposed MISPE method 1 and method 2 (n = 4).

<table>
<thead>
<tr>
<th>Method</th>
<th>R²</th>
<th>Cm (ng L⁻¹)</th>
<th>Recovery range (%)</th>
<th>RSD range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-CP</td>
<td>0.978</td>
<td>7.10</td>
<td>91.7–99.7</td>
<td>1.1–6.8</td>
</tr>
<tr>
<td>3-CP</td>
<td>0.987</td>
<td>3.54</td>
<td>70.2–86.2</td>
<td>0.8–5.3</td>
</tr>
<tr>
<td>4-CP</td>
<td>0.984</td>
<td>2.38</td>
<td>71.9–88.1</td>
<td>1.5–9.0</td>
</tr>
<tr>
<td>Ph</td>
<td>0.994</td>
<td>5.58</td>
<td>42.6–51.3</td>
<td>0.8–8.1</td>
</tr>
<tr>
<td>2-NP</td>
<td>0.991</td>
<td>2.90</td>
<td>81.8–97.0</td>
<td>1.4–13.4</td>
</tr>
<tr>
<td>4-NP</td>
<td>0.992</td>
<td>1.94</td>
<td>68.3–88.8</td>
<td>2.7–7.6</td>
</tr>
<tr>
<td>2,4-DNP</td>
<td>0.993</td>
<td>0.46</td>
<td>65.3–76.3</td>
<td>1.7–8.2</td>
</tr>
</tbody>
</table>

a Cm: detection limit.
ions in the solution. Thus pH 3.0 was selected as the pH adjustment value of the water sample in method 1.

Different masses of the adsorbent 2-CP-MIP were tested; the results are presented in Table 1. Full recovery of 2-CP was achieved when 300 mg of the sorbent was used. Other phenols also gave high recoveries, probably due to the increase in the number of available cavities when the mass of 2-CP-MIP increases. This indicated an increase in non-specific interaction as the mass of adsorbent increases.

Several volumes of dichloromethane were used as washing solvents of 2-CP-MIP; the results are presented in Table 1. The aim of the washing step was to remove the non-specific interaction without disrupting the interaction between 2-CP with 2-CP-MIP. It was noted that as the volume of dichloromethane increased, the recovery of all the phenols (including 2-CP) decreased. This indicated that 2-CP molecules were not strongly bound to the MIP cavities or this might indicate that 2-CP was not retained by the selective adsorption, but it was retained (as all other phenols) by physical adsorption. Thus 2-CP was not recognized by the 2-CP-MIP as a preferred adsorbate. Washing with other solvents gave similar results. So that washing step was excluded from method 1, due to its bad effect on the recovery of 2-CP. Consequently no recognition ability/selectivity of method 1 towards 2-CP could be observed.

3.5. MISPE of 2-CP-4-AAP using 2-CP-4-AAP-MIP as sorbent (method 2)

MISPE of 2-CP-4-AAP using 2-CP-4-AAP-MIP as the sorbent was tested in the presence of all other competing phenolic compounds to test the selectivity of the method. Various masses of the adsorbent (2-CP-4-AAP-MIP) and washing solvents were tested. Only water sample adjusted to pH 10.0 was used since the derivative was stable only at this pH in aqueous medium. The results are presented in Table 2, from which it is clear that 100 mg of 2-CP-4-AAP was enough to give almost full recovery for 2-CP-4-AAP. The other competing phenolic compounds gave moderate recovery, which was due to the presence of extra active sites available for non-specific interaction. The effect of washing solvent was then studied to remove non-specific interaction. Various washing solvents were tested. Results presented in Table 2 indicated that washing with pure methanol reduced the recovery of 2-CP-4-AAP and other derivatized phenolic compounds. Using lower percentages of methanol in aqueous solution maintained the recovery of 2-CP-4-AAP but significantly reduced the recovery of the competing derivatized phenols. The optimum washing solvent was 2.0% methanol aqueous solution. So that it was concluded that washing with 2.0% methanol in water was necessary to remove non-specific interactions from other competing phenolic compounds.

3.5.1. Discussion

Method 1 did not exhibit high selectivity towards 2-CP in the presence of other competing phenolic compounds. The lack of selectivity of method 1 towards 2-CP was probably due to small molecular size and limited number of functional groups in 2-CP. This directed the research to go into method 2, which was based on imprinting the MIP with the derivative 2-CP-4-AAP. It has many functional groups and larger molecular size than 2-CP.

Method 2 exhibited more selectivity/recognition ability towards 2-CP-4-AAP for many reasons. The number of competing compounds in method 1 was seven, while method 2 reduced the number of competing compounds to three by selective derivatization of some phenolic compounds with 4-AAP. Furthermore, 2-CP-4-AAP as template has larger molecular size and more functional groups than 2-CP. Thus the formed cavities have more available binding sites and the probability of specific interaction of 2-CP-4-AAP was higher. So that upon washing, non-specific interactions were removed while specific interaction of 2-CP-4-AAP was maintained.

3.6. Non-imprinted polymer (NIP)

To ensure that there was specific interaction and to test the selectivity of 2-CP-4-AAP-MIP towards 2-CP-4-AAP, similar procedure was followed as in method 2 but the NIP was used as the sorbent. It was noted that after application of method 2, the recovery of all the derivatized phenols was noticeably reduced, which indicated that all derivatized phenolic compounds (including 2-CP-4-AAP) were retained on the NIP by physical adsorption, and thus they were easily eluted in the washing step.

3.7. Analytical performance

The analytical performance of the two methods (method 1 and method 2) was primarily evaluated by determining the analytical figures of merits: linear range, detection limit, accuracy and precision. The proposed MISPE methods were applied on purified water samples spiked with the targeted phenols simultaneously (spiking levels: 10, 20, 40, 60, 80 and 100 μg L⁻¹). The linearity of the calibration curve of each phenol was determined by plotting the average peak area against the spike concentration of each phenol. The linearity was estimated based on R² value of the calibration curve. The detection limits of the analytes were estimated as three times the standard deviation of the average blank signal. Accuracies of the methods were estimated based on the recovery while precisions of the methods were estimated as the %RSD of replicate samples.

The analytical figures of merits of method 1 are presented in Table 3. Method 1 was linear within the studied linear range.

### Table 4

<table>
<thead>
<tr>
<th>Method</th>
<th>Recovery (%) “spiked with 10 μg L⁻¹”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream water</td>
<td>Reservoir water</td>
</tr>
<tr>
<td>Method 1</td>
<td></td>
</tr>
<tr>
<td>2-CP</td>
<td>ND</td>
</tr>
<tr>
<td>3-CP</td>
<td>66.0 ± 8.6</td>
</tr>
<tr>
<td>4-CP</td>
<td>ND</td>
</tr>
<tr>
<td>Ph</td>
<td>ND</td>
</tr>
<tr>
<td>2-NP</td>
<td>18.2 ± 0.8</td>
</tr>
<tr>
<td>4-NP</td>
<td>ND</td>
</tr>
<tr>
<td>2,4-DNP</td>
<td>20.2 ± 1.2</td>
</tr>
<tr>
<td>Method 2</td>
<td></td>
</tr>
<tr>
<td>2-CP-4-AAP</td>
<td>889.0 ± 6.2</td>
</tr>
<tr>
<td>3-CP-4-AAP</td>
<td>65.8 ± 5.1</td>
</tr>
<tr>
<td>Ph-4-AAP</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not detected.
Some chromatograms are shown in Figs. 2 and 3. The selectivity of 2-CP-4-AAP-MIP was evaluated by performing competitive adsorption of the derivatized phenols, in which 50 mL solution containing 5 mg L\(^{-1}\) of each derivatized phenol was mixed with 50 mg of the 2-CP-4-AAP-MIP in a 100 mL conical flask at pH 10. The mixture was agitated in a thermo-stated shaker for 24 h. The remaining concentrations were determined chromatographically. The distribution coefficient \(K_d\) was calculated as follows:

\[
K_d = \frac{Q_{ads}}{C_e}
\]

where \(C_e\) is the amount of derivatized phenolic compound left in the solution after extraction, while \(Q_{ads}\) is the sorbed amount of derivatized phenolic compound. Selectivity coefficients \(k\) of derivatized phenolic compounds were estimated as follows:

\[
k_{(2-\text{CP}-4\text{-AAP})/\text{derivatized\ phenolic\ compound}} = \frac{K_d(2-\text{CP}-4\text{-AAP})}{K_d(\text{derivatized\ phenolic\ compound})}.
\]

High values of \(k\) indicate that the adsorbent is very selective for 2-CP-4-AAP uptake. For the purpose of comparison, similar procedure was followed to study the selectivity of 2-CP-MIP towards 2-CP. The results indicate that the selectivity of 2-CP-4-AAP-MIP is 3–5 times more than that of 2-CP-MIP.

4. Conclusions

Although MISPE proved itself as a powerful technique capable of selective extraction of various analytes from water, however its application towards small templates is still limited due to cross selectivity. In this work, method 2 (which uses 2-CP-4-AAP-MIP as MISPE sorbent) offered higher selectivity and lower detection limit towards 2-CP-4-AAP than method 1 (which uses 2-CP-MIP as MISPE sorbent). The increase in the selectivity is due to more functional groups added to the analyte capable of specific interaction with the MIP functional groups. The non-specific interaction may be easily removed by proper washing solvent. This kind of selectivity was not observed in method 1, which showed cross-selectivity. Method 2 was able to selectively detect the presence of 2-CP-4-AAP in real water samples in the presence of other competing phenolic compounds. The derivatization procedure of the phenolic compounds in real waters was simple and straightforward. So that derivatization of small template (having small number of functional groups)
may be used as a new technique for improving the recognition ability/selectivity of the MIP towards small templates.

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**References**