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Short communication

# On-line solid-phase extraction with molecularly imprinted polymers to selectively extract substituted 4-chlorophenols and 4-nitrophenol from water

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## Abstract

Three polymers have been synthesised using 4-chlorophenol (4-CP) as the template, following different protocols (non-covalent and semi-covalent) and using different functional co-monomers, 4-vinylpyridine (4-VP) and methacrylic acid (MAA). The polymers were evaluated to check their selectivity as molecularly imprinted polymers (MIPs) in solid-phase extraction (SPE) coupled on-line to liquid chromatography. The solid-phase extraction procedure using MIPs (MISPE), including the clean-up step to remove any interferences, was optimised. The 4-VP non-covalent polymer was the only one which showed a clear imprint effect. This MIP also showed cross-reactivity for the 4-chloro-substituted phenols and for 4-nitrophenol (4-NP) from a mixture containing the 11 priority EPA (Environmental Protection Agency) phenolic compounds and 4-chlorophenol. The MIP was applied to selectively extract the 4-chloro-substituted compounds and 4-NP from river water samples.

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**Keywords:** Water analysis; Solid-phase extraction; Molecular imprinting; 4-Chlorophenols; 4-Nitrophenol

## 1. Introduction

Molecularly imprinted polymers (MIPs), which can be prepared by three different protocols [1,2], are highly crosslinked polymers synthesised in the presence of a template molecule. However, in some cases, the MIP recognises not only the template in the rebinding step but also structurally related ana-

lytes [3–7]. This effect, known as cross-reactivity, is particularly interesting in environmental samples, since these may contain several structurally related compounds.

Nearly all the data published about MIPs as sorbents in solid-phase extraction (MISPE) has been generated in an off-line mode [4,8–10]. Few applications have been developed using an on-line mode with the MISPE coupled to liquid chromatography (LC) [2,3,11–14] and only some of them have used only one imprinted pre-column [2,12,13], which offers significant advantages in terms of the ease of

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instrumentation. Moreover, MISPE has been mainly applied to biosamples in which several drugs [15–18] have been determined and there are far fewer applications relating to the extraction of analytes in environmental samples [2,3,5,11,12,14].

The aim of this work was to synthesise and evaluate three different polymers potentially selective for 4-CP and to demonstrate how selective the MIP can be in the analysis of real water samples by on-line SPE-LC.

## 2. Experimental

### 2.1. Reagents and standards

The chemicals used for the polymer syntheses were 4-chlorophenol (4-CP), 4-vinylpyridine (4-VP), methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) from Aldrich (Steinheim, Germany), 2,2'-azobisisobutyronitrile (AIBN) from Acros Organics (Geel, Belgium) and HPLC-grade acetonitrile (ACN) from Rathburn Chemicals (Walkburn, UK). The monomers and the AIBN were purified prior to use via standard procedures in order to remove stabilisers. The monomer-derivatised template, 4-chlorophenyl methacrylate, was synthesised according to a protocol described in the literature [19].

The HPLC-grade solvents were provided by either Rathburn Chemicals or SDS (Peypin, France) and the water collected from a Millipore water purification system (Milli-Q water). The acetic and hydrochloric acids were from Probus (Badalona, Spain) and the dichloromethane (DCM) from SDS. The structurally related phenolic pollutants used to investigate the selectivity of the polymers were the 11 priority EPA phenolic compounds and 4-chlorophenol itself. Phenol (Ph), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2-nitrophenol (2-NP), 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3-MP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were all supplied by Aldrich, except for PCP which was from Jansen Chemie (Geel, Belgium).

### 2.2. Instrumentation

The polymers were firstly evaluated in analytical columns to check the imprinting effect. The 15×0.46 cm I.D. stainless-steel HPLC columns were slurry packed with ground polymer particles (25–38 μm) using an air-driven fluid pump (Haskel) with acetone as the solvent at 2500 p.s.i. An SP 8800 ternary HPLC pump and an SP 8450 UV detector (Spectra-Physics, Mountain View, CA, USA) were used in this pre-screening work.

The equipment used for the MISPE study, which has been described in some previous works [2,12], was on-line coupled to a LC system. This system has two pumps and enables the compounds retained on the pre-column to be eluted only by the organic solvent of the mobile phase [12]. The analytical column was a 25×0.4 cm I.D. Tracer Extrasil ODS2, 5 μm, supplied by Teknokroma (Barcelona, Spain).

### 2.3. Preparation of the imprinted polymers

Polymers P1 and P2 were prepared by the non-covalent approach. The pre-polymerisation mixture for P1 comprised 4-CP (2.02 mmol) as the template molecule, 4-VP (8.07 mmol) as the functional monomer, the cross-linking monomer EGDMA (40.36 mmol) and the initiator AIBN (1.13 mmol) dissolved in ACN (11.25 ml) in a 25 ml thick-walled glass tube. The polymerisation procedure followed and the subsequent treatment to obtain the small particles suitable for the SPE evaluation is described in a previous work [12].

The P2 pre-polymerisation mixture comprised 4-CP (2.14 mmol) and MAA (8.58 mmol) as the template molecule and the functional monomer, respectively, the cross-linker EGDMA (42.88 mmol) and the initiator AIBN (0.94 mmol) dissolved in ACN (11.75 ml) in a 25 ml thick-walled glass tube. The synthetic procedure followed during the polymerisation step was the same as for polymer P1.

Two reference, non-imprinted polymers, B1 and B2, which did not contain any template, were prepared analogously to P1 and P2 using the same protocols, respectively.

Polymer P3 was prepared by the semi-covalent approach using 4-chlorophenyl methacrylate (2 mmol) as a template, styrene (6 mmol) as an

additional functional co-monomer [2], the cross-linker EGDMA (40 mmol) and the initiator AIBN (0.88 mmol) dissolved in ACN (7.08 ml) in a 25 ml thick-walled glass tube. P3 was synthesised in the same way as P1 but, on this occasion, when the monolith obtained was dried, crushed and ground, the polymer was subjected to a different protocol which is described in a previous work [2] for a 4-NP MIP. A final elemental microanalysis demonstrated that the template had been successfully removed from the polymer.

#### 2.4. Chromatographic conditions

For the chromatographic evaluation of the polymers, ACN–acetic acid (99.7:0.3, v/v) was used as the mobile phase at  $0.5 \text{ ml min}^{-1}$ . The injection volume was  $20 \mu\text{l}$ , the UV detector wavelength was 280 nm and the analyses were performed at room temperature.

For the MISPE experiments the mobile phase consisted of Milli-Q-quality water, acidified to pH 2.5 with acetic acid, as solvent A and ACN [containing 1% (v/v) acetic acid] as solvent B. The flow-rate of the mobile phase was  $1 \text{ ml min}^{-1}$  and the gradient profile was 20–30% B from 0 to 30 min, 100% B at 32 min and then isocratic elution for 2 min. The oven temperature was set at  $65 \text{ }^\circ\text{C}$  and all compounds were detected at 280 nm, except for PCP which was detected at 302 nm.

#### 2.5. On-line MISPE procedure

The polymers were packed into stainless-steel pre-columns in order to be evaluated via on-line MISPE. Prior to any injections they were washed with solvent B to verify that there was no residual template (4-CP) present.

For on-line MISPE the polymers were conditioned with 5 ml ACN and 2 ml acidified Milli-Q water with HCl (pH 2.5) at  $3 \text{ ml min}^{-1}$ . The spiked water sample (adjusted to pH 2.5) was applied to the conditioned pre-column, and the polymer then washed with 0.1 ml (P1) of DCM and 4 ml Milli-Q water (pH 2.5). The retained analytes were desorbed using solvent B alone and in the back-flush mode [12].

Real samples were filtered through a  $0.45 \mu\text{m}$  filter and adjusted to pH 2.5 before MISPE.

### 3. Results and discussion

#### 3.1. Chromatographic evaluation of the polymers

The analytical columns packed with the polymers were first washed on-line with acetonitrile–acetic acid (99.7:0.3, v/v) to eliminate interfering compounds from the synthesis. For their evaluation,  $10 \text{ mg l}^{-1}$  solutions of Ph, 4-NP, and 4-CP were injected as test analytes onto the columns. The three compounds gave different retention times on the non-imprinted blank column and, for this reason, the data obtained in these analyses were normalised by calculating the normalised retention index (RI) [12]. Table 1 shows the  $K'$  values for the test phenolic compounds in the column and the corresponding RI values for P1. 4-NP and Ph give rise to smaller RI values than 4-CP, thus the imprinting effect seems to be verified.

The imprinting effect in P2 and P3 was evaluated by on-line MISPE since the RI values were not conclusive.

#### 3.2. On-line MISPE

To investigate the selectivity of the polymers for 4-CP when this phenol was present with the 11 priority EPA phenolic compounds in a water sample, an extraction step was developed. The P1 polymer was first evaluated and 10 ml of spiked ( $10 \mu\text{g l}^{-1}$  of each analyte) Milli-Q water, previously adjusted with HCl to pH 2.5, was passed through the sorbent. All 12 compounds were retained on the MIP when a clean-up step was not performed. However, to favour the selectivity of the polymer, a clean-up step with

Table 1  
Capacity factors ( $K'$ ) and normalised retention indices (RI) obtained from the HPLC evaluation of polymer P1

	$K'$ (MIP)	$K'$ (control)	RI
Ph	0.56	0.47	0.81
4-NP	1.57	1.17	0.93
4-CP	1.01	0.71	1

an organic solvent was included. DCM was selected as the washing solvent and different volumes were tested (0.1, 0.2 and 0.3 ml). When 0.1 ml of DCM was used, not only did the template (4-CP) remain strongly bound to the polymer in the imprinted sites, but also all the 4-chloro-substituted phenolic compounds (4-C-3MP, 2,4-DCP, TCP and PCP) as well as the 4-NP (Fig. 1). This behaviour can be explained by the fact that this polymer shows cross-reactivity. The other compounds were washed straight off the MIP. These results, which are shown in Table 2, prompted further experiments and the applicability to real water samples of this polymer. In the case of B1, when 0.1 ml of DCM was used, all the phenols were stripped off the polymer, including 4-CP, which indicated that there were no selective binding sites.

P2 and P3 polymers were evaluated in the same way as P1, but no clear imprinting effect was obvious, since even with 0.1 ml of DCM the 4-CP was almost completely eluted from P2 and P3. This behaviour was expected since, in the chromatographic evaluation, no clear imprinting effect was established.

MIPs selective for nitrophenols or chlorophenols synthesised using 4-VP as functional monomer show higher recoveries than those obtained using MAA [2], since there is much better interaction between the phenolic aromatic ring of the analytes and the 4-VP. Moreover, the basic pyridine group in the 4-VP functional monomer is able to form stronger non-covalent bonds with acidic compounds (nitro and chlorophenols) than the carboxylic acid derived from MAA.

The effect of the sample volume on the recovery was tested and 20 ml of sample was percolated. When the clean-up step was carried out with 0.1 ml of DCM, the recovery decreased considerably and larger sample volumes were therefore not tested. From these results, a volume of 10 ml was selected as the optimum value for further experiments.

### 3.3. MISPE of real water samples

An application of the imprinted polymer (P1) in the MISPE was developed with an Ebro river water sample to show how selective the MIP can be with

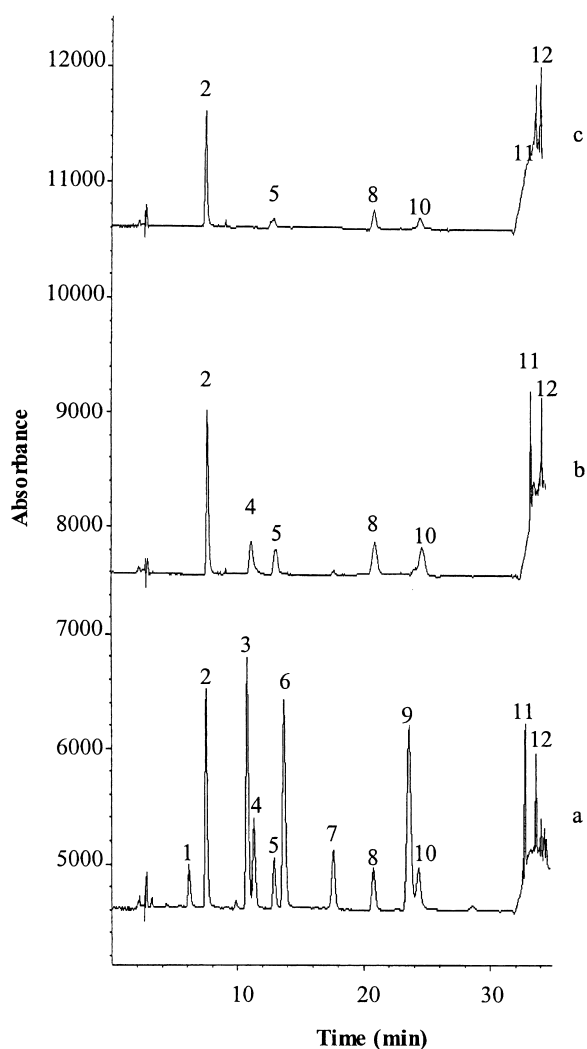


Fig. 1. Chromatograms obtained by on-line MISPE with the 4-VP non-covalent 4-CP imprinted polymer (P1) of 10 ml standard solution (pH 2.5) spiked at  $10 \mu\text{g l}^{-1}$  with each phenolic compound. (a) Without washing step, and (b, c) with washing step using 0.1 and 0.3 ml of dichloromethane, respectively: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 4-CP, (6) 2-NP, (7) 2,4-DMP, (8) 4-C-3-MP, (9) 2-M-4,6-DNP, (10) 2,4-DCP, (11) 2,4,6-TCP, (12) PCP.

real water. Polar phenols cannot be accurately quantified at low levels when they are in Ebro water because the complex matrix usually contains humic acids which appear as a broad band at the beginning of the chromatogram interfering in the quantification

Table 2

Recoveries (%) obtained by washing the non-covalent 4-CP imprinted polymer P1 with different volumes of dichloromethane following the pre-concentration of 10 ml of a standard solution spiked at  $10 \mu\text{g l}^{-1}$  for each analyte<sup>a</sup>

Analyte	Volume $\text{CH}_2\text{Cl}_2$ (ml)			
	0	0.1	0.2	0.3
Ph	59	–	–	–
4-NP	73	55	47	38
2,4-DNP	71	<sup>b</sup>	–	–
2-CP	82	<sup>b</sup>	–	–
4-CP	68	53	41	13
2-NP	74	–	–	–
2,4-DMP	71	–	–	–
4-C-3-MP	65	64	61	33
2-M-4,6-DNP	66	–	–	–
2,4-DCP	67	58	50	17
2,4,6-TCP	50	44	25	–
PCP	23	24	13	11

<sup>a</sup> RSDs were lower than 9% in all instances ( $n=3$ ).

<sup>b</sup> Co-eluted compounds.

of the most polar compounds. However, when this MIP was used as a selective sorbent in SPE the humic band was completely removed with a clean-up step with 0.1 ml of DCM (Fig. 2) and without adding  $\text{Na}_2\text{SO}_3$  as required with other sorbents [2,20]. The analytes selectively retained on the MIP were then accurately quantified. The recovery values for the retained compounds were nearly the same as in Milli-Q water when the clean-up step was not performed and when it was carried out under the optimum conditions (0.1 ml of DCM).

To check the linear range, 10 ml of river water, which did not contain any phenolic compounds, was spiked with the 11 priority EPA phenolic compounds and 4-CP at concentrations between 100 and  $1 \mu\text{g l}^{-1}$ , then pre-concentrated and a washing step with 0.1 ml of DCM was applied. Since the polymer showed cross-reactivity, linearity was checked for all the retained compounds in the MIP. Good linearity was obtained for all six phenols, with a determination coefficient ( $r^2$ ) higher than 0.999. The repeatability for 10 ml of spiked ( $10 \mu\text{g l}^{-1}$  of each component) river water, expressed as RSD ( $n=3$ ), was lower than 11%. The application of the imprinted polymers to on-line MISPE of real samples has therefore been demonstrated.

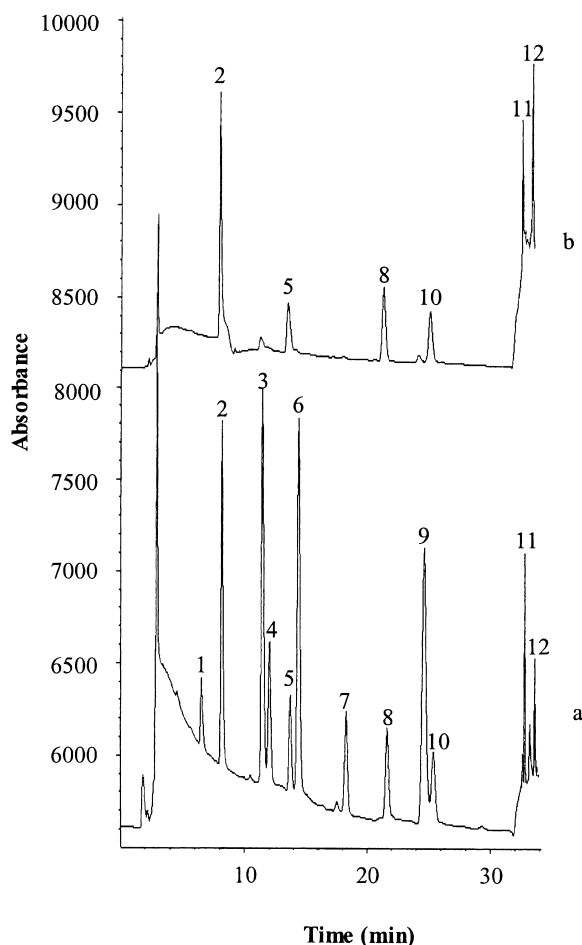


Fig. 2. Chromatogram obtained by on-line MISPE with the 4-VP non-covalent 4-CP imprinted polymer (P1) of 10 ml Ebro river water (pH 2.5) spiked at  $10 \mu\text{g l}^{-1}$  with each phenolic compound. (a) Without washing step and (b) with washing step using 0.1 ml of dichloromethane. Peak designation, as per Fig. 1.

#### 4. Conclusions

A polymer prepared using 4-VP as functional monomer and following a non-covalent imprinting protocol (P1) showed an imprinting effect for all 4-chloro-substituted phenolic compounds and 4-NP since this MIP showed cross-reactivity. The selectivity of the MIP, which was evaluated in SPE coupled on-line to liquid chromatography, and the cross-reactivity were evident when a clean-up step with DCM as washing solvent was performed. An

application of the MIPSE procedure to real water samples was developed to demonstrate its applicability. All the retained compounds gave a linear response.

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