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# Non-covalent and semi-covalent molecularly imprinted polymers for selective on-line solid-phase extraction of 4-nitrophenol from water samples

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**Abstract**

Two molecularly imprinted polymers (MIPs) have been synthesised for the selective extraction of 4-nitrophenol (4-NP) from water samples. One polymer was synthesised via a non-covalent approach and the other via a semi-covalent approach. The selectivity of the polymers for 4-NP was evaluated when these polymers were applied in on-line solid-phase extraction (MISPE) coupled to reversed-phase HPLC. The MISPE conditions for both MIPs were optimised and a clean-up step was included to eliminate non-specific interactions. Differences between the two MIPs were observed with the non-covalent MIP being the more selective of the two, whereas the recoveries were slightly higher for the semi-covalent MIP. The performance of the imprinted polymers in the MISPE of real water samples was also evaluated. 

*Keywords:* Solid-phase extraction; Water analysis; Molecularly imprinting; Nitrophenols

## 1. Introduction

Nowadays, one of the most interesting objectives for analytical chemistry researchers is to improve the selectivity of the sorbents used in solid-phase extraction (SPE), since many current SPE materials retain not only the target analytes but also other matrix components. This objective is particularly important when analysing complex matrices such as waste water or river water samples, whose humic acids may interfere in the determination of the analytes of interest. In the context of selective sorbents, two types are particularly important [1], the immunosorbents, which have been applied to various types of matrices, and the recently introduced molecularly imprinted polymers (MIPs), whose application in SPE is now being actively researched [2].

The selectivity of MIPs arises because the target analyte (template) is present in the polymerisation mixture during synthesis of the MIP. Once the highly crosslinked polymer has formed, the template molecules are removed from the polymer matrix revealing selective binding sites in the polymer matrix. As a consequence of these binding sites (i.e. molecular recognition sites), the molecularly imprinted polymer is able to selectively recognize the template molecule from other components in a complex sample [3].

Currently, a number of distinct approaches have been used to prepare molecularly imprinted materials. One of these is the pre-organized approach

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(covalent imprinting), which involves the formation of covalent bonds between the functional monomers and the template molecules prior to polymerisation. Thus the template molecules need to be chemically modified with the functional monomers, and after polymerisation the template molecule is removed from the imprinted polymer by cleavage of the covalent bonds via which it is attached to the polymer. Upon rebinding of the analyte (template) to the polymer, the covalent bonds are re-formed. Another methodology is the self-assembly approach (non-covalent imprinting) where relatively weak non-covalent intermolecular interactions, such as electrostatic interactions, hydrogen bonding, $\pi-\pi$ bonding and hydrophobic interactions, between the template and the functional monomers serve to form molecular assemblies. Hence the selection of functional monomers which interact strongly with the template is crucial to generate high affinity binding sites [4–6].

A third approach is the semi-covalent approach in which the template is covalently bound to a functional monomer during polymerisation, as in the covalent approach, whereas only non-covalent interactions are exploited during the rebinding [7,8]. The fact that the template is covalently bound to the functional monomer at the outset, can in principle yield imprinted polymers with higher binding capacities since there is much better binding site integrity during polymerisation.

It is generally believed that covalent imprinting gives better defined and more homogeneous binding sites than the non-covalent approach since the template-functional monomer interactions are far more stable and defined during the imprinting process than the template-functional monomer complex in the non-covalent approach. However, the general applicability of the pre-organized approach is limited because it can be difficult to design suitable binding sites for the target molecule in which covalent bond formation and cleavage are readily reversible under mild conditions. In contrast, non-covalent imprinting is much more flexible in terms of the binding sites that can be exploited and therefore the range of templates which can be targeted. Furthermore, the non-covalent approach is experimentally simpler to realize than covalent imprinting methods because the complexation step is achieved simply by mixing the template with the functional monomer(s) in a suitable solvent. No chemical derivatisation of the template is required, and template removal typically involves simply washing the polymer repeatedly with a suitable solvent or solvent mixture. A major drawback of non-covalent systems is the unavoidable heterogeneity of the binding sites obtained arising from the multitude of complexes formed between the template and the functional monomers which are apparently preserved to some extent during the polymerisation. The non-covalent bonding is generally not strong and thus an excess of functional monomer relative to the template is usually required to favor template-functional monomer complex formation and to maintain its integrity during the polymerisation. As a result, a fraction of the functional monomers are randomly incorporated in the polymer matrix resulting in the formation of non-selective binding sites [7–11].

The potential range of applications for MIPs is very extensive [12,13]. Although the application of MIPs as sorbents in molecularly imprinted SPE (MISPE) was firstly described in 1994 [14], few studies have been developed [15]. MISPE has been mainly applied in off-line mode to chromatographic systems, with few applications having been developed thus far in on-line mode [16–19]. In two of these on-line methods [17,18], two successive pre-columns packed with $C_{18}$-silica and MIP sorbents, respectively were on-line coupled to a liquid chromatographic system to extract selectively a group of triazines from environmental water. On the first pre-column, which contained the $C_{18}$-silica, all the compounds were retained. When they were eluted subsequently from this pre-column only the template and the related structural compounds were retained by the second pre-column which contained the MIP. In a similar way, Koeber et al. [20] also used two successive pre-columns, but in this case the first one was packed with a restricted access material (RAM) and the second one with a MIP to selectively extract triazines from water river samples. In contrast, in an on-line MISPE application developed by our group [16] only one pre-column, containing a MIP, was required for the selective extraction of 4-nitrophenol from environmental water. Haginaka and Sanbe [19] also used one pre-column, a combined RAM-MIP pre-column, to extract ibuprofen from plasma. The

use of only one pre-column as opposed to two in on-line MISPE clearly offers significant advantages in terms of the ease of method development.

Most MISPE research has been carried out with biological samples [18,19,21–23] with the use of MIPs for the analysis of complex matrices of environmental origin being in its infancy. There are a few such applications based on the determination of pesticides in water samples [17,18,20,24–26]. As mentioned above, in a recent paper from our group [16], a MIP for 4-nitrophenol (4-NP) was synthesised and evaluated for on-line MISPE. This non-covalently imprinted polymer used 4-vinylpyridine as the functional monomer and it enabled the selective extraction of 4-NP from river water samples even when other phenolic compounds were present. Joshi et al. [7] also synthesised a MIP for phenolic compounds but, in this case, it was a semi-covalent MIP useful in separating phenol from anisole.

In this paper, a detailed study is presented in which the performance of a non-covalently imprinted 4-NP polymer is compared with the performance of a semi-covalently imprinted 4-NP polymer in the on-line MISPE of 4-NP from environmental water. Both polymers exploit an identical, methacrylic acid-based, binding site. To our knowledge this is the first MISPE application of a semi-covalently imprinted sorbent.

## 2. Experimental

### 2.1. Reagents and standards

The chemicals for the polymer synthesis were 4-NP, methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) from Aldrich (Steinheim, Germany), styrene from Fisher (Loughborough, UK), 2,2′-azobisisobutyronitrile (AIBN) from Acros Organics (Geel, Belgium) and acetonitrile from Rathburn (Walkerburn, UK). The monomers were purified prior to use via standard procedures in order to remove stabilisers. The AIBN was recrystallised from acetone and the acetonitrile dried over molecular sieves. The monomer-derivatised template, 4-nitrophenyl methacrylate, was synthesised according to a protocol described in literature [27].

The HPLC-grade solvents were sourced from either Rathburn 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 dichloromethane from SDS (Peypin, France). The structurally related phenolic pollutants used to investigate the selectivity of the polymers were the 11 priority US Environmental Protection Agency (EPA) phenolic compounds: phenol (Ph), 4-NP, 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-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), and were all supplied by Aldrich, except for PCP which was from Jansen Chemie (Geel, Belgium).

### 2.2. Instrumentation

In the MISPE study, a Must column-switching device (Spark Holland, Emmen, Netherlands), a Waters (Milford, MA, USA) M45 pump and 10×3 mm I.D. stainless steel pre-columns, laboratory-packed with ~40 mg of the laboratory-synthesised polymers, were used. These pre-columns were on-line coupled to a liquid chromatographic system which consisted of two LC-10AD pumps, a DGU-4A degasser, a CTO-10A oven and a SPD-10A UV spectrophotometric detector from Shimadzu (Tokyo, Japan). Having two pumps enables the compounds retained on the pre-column to be eluted only by the organic solvent of the mobile phase. Upon elution, the organic solvent is mixed with the aqueous solvent to form the mobile phase that separates the analytes on the analytical column. The loop for direct injection was 20 μl and the analytical column was a 25×0.4 cm I.D. Spherisorb ODS2, 5 μm, supplied by Teknokroma (Barcelona, Spain).

### 2.3. Synthesis of the imprinted polymers

Polymer P1 was prepared by the non-covalent approach with 4-NP as the template molecule and MAA as the functional monomer. The pre-polymerisation mixture comprised 4-NP (2.15 mmol), MAA (8.58 mmol), the cross-linking monomer EGDMA (42.90 mmol) and the initiator AIBN (0.90

mmol) dissolved in the porogen acetonitrile (11 ml) in a 25-ml thick-walled glass tube.

A reference, non-imprinted polymer, B1, which did not contain any template, was prepared simultaneously using the same protocol as for P1.

Polymer P2 was prepared by the semi-covalent approach. The pre-polymerisation mixture comprised 4-nitrophenyl methacrylate (2 mmol), styrene (6 mmol), the crosslinker EGDMA (40 mmol) and the initiator AIBN (0.88 mmol) dissolved in the porogen acetonitrile (10.5 ml) in a 25-ml thick-walled glass tube. An additional functional monomer (styrene) was used in order to keep the template/functional monomer/crosslinker ratio nominally the same for the semi-covalent MIP as for the non-covalent MIP. Styrene was chosen because this gave the opportunity of potentially exploiting $\pi-\pi$ interactions in addition to the covalent interaction during the imprinting step.

All three polymerisation mixtures were cooled on an ice bath, sparged with oxygen-free nitrogen for 5 min, sealed under nitrogen and then left to polymerise in a water bath at 60 °C for 20 h. P1 and B1 polymer monoliths were crushed, ground and wet-sieved using acetone to obtain regularly sized particles with diameters between 25 and 38 μm suitable for the MISPE evaluations. The dry, crushed and ground, polymer P2 was refluxed initially with aqueous 2 *M* NaOH for 6 h in order to free it from template by breaking the covalent bonds linking the template to the polymer. The resultant polymer suspension was cooled and filtered under vacuum, and the polymer then washed successively with 0.1 *M* HCl (until the pH of the filtrate was $<7$), 200 ml of water and 200 ml of methanol. Finally, it was dried under vacuum and sieved to obtain regularly sized particles with diameters between 25 and 38 μm. Elemental microanalysis showed that there was no nitrogen present after NaOH treatment, which demonstrated that the template had been successfully removed.

### *2.4. Chromatographic conditions*

The mobile phase consisted of Milli-Q quality water, acidified to pH 2.5 with acetic acid, as solvent A and acetonitrile [containing 1% (v/v) acetic acid] as solvent B. The flow-rate of the mobile phase was 1 ml $min^{-1}$ and the gradient profile was 15–25% B from 0–10 min, 30% B at 25 min, 100% B at 34 min and then isocratic elution for 2 min. Afterwards, the mobile phase was returned to its initial composition over 2 min. The post-run time was 10 min. The oven temperature was set at 65 °C and all compounds were detected at 280 nm, except for PCP which was detected at 302 nm.

### *2.5. On-line MISPE procedure*

For on-line MISPE, the polymers were conditioned with 2 ml acetonitrile and 2 ml acidified Milli-Q water (pH 2.5). The spiked water sample (adjusted to pH 2.5) was applied to the conditioned pre-column, and the polymer then washed with 0.2 ml (P1) or 0.5 ml (P2) of dichloromethane and 2 ml Milli-Q water (pH 2.5). Flow-rate was 2 ml $min^{-1}$ in all these steps. The retained analytes were desorbed using solvent B alone and in the back-flush mode to reduce band-broadening, then transferred on-line to the analytical column. Both solvent A and solvent B were mixed prior to reaching the analytical column (Fig. 1).

When real samples were used they were filtered through a 0.45-μm filter and adjusted to pH 2.5 before MISPE.

## 3. Results and discussion

Three different polymers (B1, P1 and P2) were synthesised using methacrylic acid as a functional monomer. B1 (blank) was synthesised in the absence of template, P1 was synthesised via a non-covalent approach and P2 via a semi-covalent approach. All three polymers were evaluated subsequently via on-line MISPE.

### *3.1. On-line MISPE*

To evaluate the polymers via on-line MISPE they were packed into stainless-steel pre-columns, and before use they were washed with solvent B [acetonitrile containing 1% (v/v) acetic acid] to verify that there was no residual template (4-NP) present. To confirm that the polymers were imprinted and to investigate the selectivity of polymers for 4-NP when

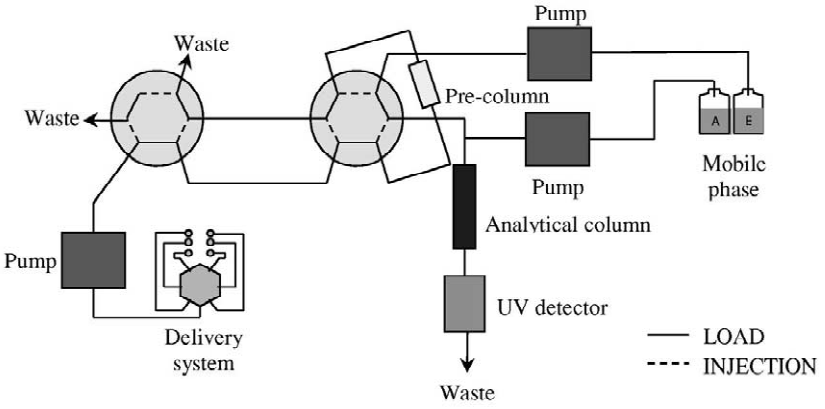


Fig. 1. Set-up of the system used.

this phenol was present with the other 10 priority EPA phenolic compounds in a water sample, an extraction step was developed. Initially, 10 ml of spiked (10 μg $l^{-1}$ of each analyte) Milli-Q water, previously adjusted with HCl to pH 2.5, was passed through the sorbent. All compounds, except for PCP, were retained on the MIPs (Figs. 2a and 3a) when a clean-up step was not carried out. This result can be explained by the fact that under such aqueous loading conditions the analytes interact with the sorbent primarily by hydrophobic interactions (non-specific interactions) which arise between all the analytes and the MIP. To increase the selectivity of the extraction, it was necessary to include a clean-up step with an organic solvent. In such a clean-up step the templated analyte (4-NP) remains strongly bound to the polymer in the imprinted sites whereas the non-templated analytes, which are non-selectively and therefore relatively weakly bound, are washed straight off the MIP. Dichloromethane was selected as the organic solvent because good results were obtained when applying this solvent in previous work [16]. In the case of the blank polymer, a clean-up step with 0.2 ml of dichloromethane stripped all the phenols, including 4-NP, from the pre-column, which indicated that there were no selective binding sites in the blank, as expected.

When the non-covalent MIP, P1, was studied, different volumes of dichloromethane were tested (0.1, 0.2 and 0.3 ml). Fig. 2 shows that when 0.1 ml of this organic solvent was applied in the clean-up step, not all of the non-selectively bound analytes had been washed off the pre-column. However, when the volume of the washing solvent was raised (0.2 and 0.3 ml), the imprinting effect was clearly evident, since only 4-NP was retained by the pre-column whereas the rest of the phenolic compounds were eluted by the dichloromethane. Therefore, 0.2 ml of dichloromethane was chosen as the optimum volume of washing solvent because with this volume the retention of 4-NP was already selective. These results are shown in Table 1. Here it can be seen that the recoveries for 2,4-DNP and 2-CP are not included because they co-eluted and thus their recoveries could not be calculated. PCP is not included since it was not retained by the pre-column in the loading step prior to the clean-up step.

With the semi-covalent polymer, P2, when no clean-up step was used, the recoveries were slightly higher than for the non-covalent MIP, presumably due to the higher hydrophobicity of styrene-containing P2. When 0.2 ml of dichloromethane was used in the clean-up step, the recovery of some of the phenolic compounds was still high, thus a larger volume of organic solvent was tested (0.3 and 0.5 ml). The results are shown in Table 2. Fig. 3 shows the effect of changing the volume of dichloromethane and it can be seen that even when 0.5 ml of this solvent was used some phenolic compounds were still retained. However, the recovery of 4-NP is similar for the different clean-up volumes tested, which can be explained because with 0.2 ml of washing solvent presumably all the non-specific interactions have already been eliminated. From the

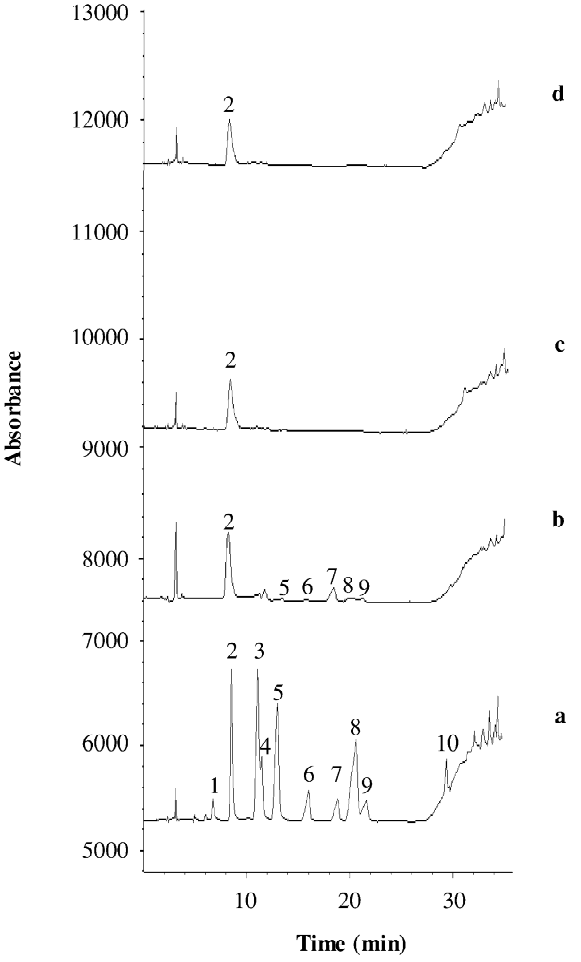


Fig. 2. Chromatograms obtained by on-line MISPE with the non-covalent 4-NP imprinted polymer (P1) of 10 ml standard solution (pH 2.5) spiked at 10 μg $l^{-1}$ with each phenolic compound. (a) Without washing step, and (b, c, d) with washing step using 0.1, 0.2 and 0.3 ml of dichloromethane, respectively: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 2-NP, (6) 2,4-DMP, (7) 4-C-3-MP, (8) 2-M-4,6-DNP, (9) 2,4-DCP, (10) 2,4,6-TCP.

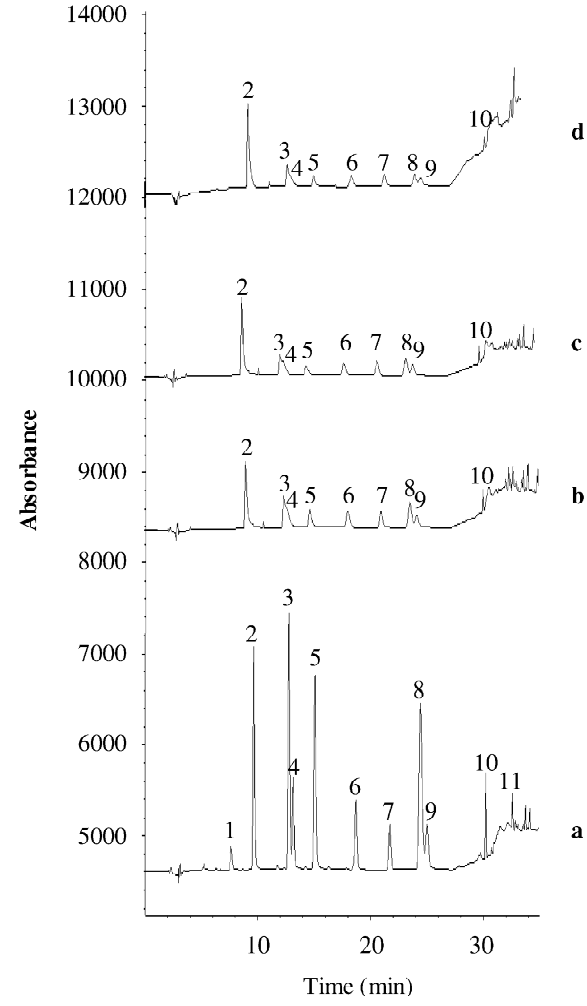


Fig. 3. Chromatograms obtained by on-line MISPE with the semi-covalent 4-NP imprinted polymer (P2) of 10 ml standard solution (pH 2.5) spiked at 10 μg $l^{-1}$ with each phenolic compound. Without washing step (a), and with washing step using 0.2 (b), 0.3 (c) and 0.5 ml of dichloromethane (d). Peak designation as in Fig. 2.

results obtained, a volume of 0.5 ml was selected as the optimum. Even larger volumes of this organic solvent were not tested because as the volume of the washing solvent was increased, the recovery of the other phenolic compounds decreased slowly.

If we compare the results obtained for the non-covalently imprinted polymer with those of the semi-covalently imprinted polymer, an important difference is seen between them in terms of the selectivity that they show for 4-NP. The non-covalent MIP (P1) is more selective than the semi-covalent MIP (P2) since with only 0.2 ml of dichloromethane, all the analytes, except for 4-NP, were eluted from the P1 pre-column. In contrast, when P2 was used, some analytes remained on the polymer even when 0.5 ml of dichloromethane was used. However, the recovery of 4-NP stayed constant for P2 even as the volume of the washing solvent was increased. The fact that the recovery of 4-NP did not decrease for the semi-

Table 1
Recoveries (%) obtained by washing the non-covalent 4-NP imprinted polymer (P1) with different volumes of dichloromethane following the pre-concentration of 10 ml of a standard solution spiked at 10 μg $l^{-1}$ for each analyte[a]

| Analyte | Volume $CH_2Cl_2$ (ml) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.3 |
| Ph | 38 | – | – | – |
| 4-NP | 69 | 68 | 52 | 46 |
| 2-NP | 66 | 3 | – | – |
| 2,4-DMP | 54 | 6 | – | – |
| 4-C-3-MP | 58 | 48 | – | – |
| 2-M-4,6-DNP | 56 | 6 | – | – |
| 2,4-DCP | 46 | 18 | – | – |
| 2,4,6-TCP | 38 | – | – | – |

[a] RSDs were lower than 10% in all instances ($n=3$).

covalent polymer may be attributed to the higher capacity of this polymer, derived from the fact that the template was covalently bound to the monomer during polymerisation, consequently with better binding site integrity as a result. Lower selectivity may be due to the fact that many of the binding sites offer only one point of attachment to the analyte, compounded by the fact that the sacrificial spacer approach was not employed.

The effect of the sample volume on the recovery was tested by passing different sample volumes through the pre-column (10, 20 and 50 ml). The concentration of analytes was different but the mass of each analyte was constant (0.1 μg). When P1 was tested, and the clean-up step was carried out with 0.2 ml of dichloromethane, the recovery decreased to 52, 40 and 20%, respectively for each of the sample volumes [%RSD ($n=3$) lower than 12% in all cases]. From these results, a volume of 10 ml was selected as the optimum value for further experiments. For P2, when 0.5 ml of dichloromethane was used, the recovery values were 50, 48 and 22% when 10-, 20- and 50-ml sample volumes, respectively, were pre-concentrated. Thus, a volume of 20 ml was selected for further experiments because recovery was similar to that with the 10-ml sample and higher sample volumes involve lower detection limits.

Table 2
Recoveries (%) obtained by washing the semi-covalent 4-NP imprinted polymer (P2) with different volumes of dichloromethane following the pre-concentration of 10 ml of a standard solution spiked at 10 μg $l^{-1}$ for each analyte[a]

| Analyte | Volume $CH_2Cl_2$ (ml) | | | |
|---|---|---|---|---|
| | 0 | 0.2 | 0.3 | 0.5 |
| Ph | 38 | – | – | – |
| 4-NP | 78 | 52 | 51 | 50 |
| 2-NP | 71 | 7 | 3 | 2 |
| 2,4-DMP | 75 | 20 | 13 | 5 |
| 4-C-3-MP | 68 | 30 | 25 | 12 |
| 2-M-4,6-DNP | 67 | 11 | 7 | 5 |
| 2,4-DCP | 62 | 21 | 16 | 9 |
| 2,4,6-TCP | 56 | 15 | 7 | 4 |

[a] RSDs were lower than 10% in all instances ($n=3$).

The cross-reactivity of the polymers was also studied. Two pesticides, atrazine and diuron, were used as test analytes since they are structurally very different from phenolic compounds. The test analytes were retained on P1 and P2 via non-selective (hydrophobic) interactions in the absence of a clean-up step. However, when a clean-up step with dichloromethane was included, while diuron was totally removed from P1 and P2 atrazine was partially retained on both polymers.

If one compares the results obtained for the two MIPs described in this paper with the results obtained for the previously reported non-covalently imprinted MIP [16] prepared with 4-vinylpyridine as the functional monomer and 4-NP as the template, it can be concluded that P1 and the non-covalent 4-vinylpyridine MIP [16] show similar recovery values for all the compounds for the same sample volume (10 ml) when the clean-up step was omitted. However, when the clean-up step is included, the recovery of 4-NP is lower for P1 under all conditions. The higher retention of 4-NP on the 4-vinylpyridine-based polymer can be attributed to ionic interactions between 4-NP (acidic) and 4-vinylpyridine (basic).

If P2 and the non-covalent MIP using 4-vinylpyridine as the functional monomer [16] are compared, it can be concluded that the recovery values for most compounds are higher in the case of P2 when the clean-up step is omitted. However, when the washing step is included in the comparison, the recovery for 4-NP is slightly lower for the semi-covalent MIP than for the non-covalent MIP. In spite of this, when the volume of the organic wash solvent is increased with the non-covalent MIP (0.4 and 0.6 ml), the recovery for 4-NP slightly decreases. So it appears that the non-specific interactions between 4-NP and the MIP are not totally eliminated when

0.4 ml of dichloromethane was used in the clean-up step. In contrast, the recovery of 4-NP is constant even though the volume of dichloromethane is varied from 0.2 to 0.5 ml when the semi-covalent polymer is used. This implies that the non-selective interactions between 4-NP and the polymer are totally eliminated with 0.2 ml of wash solvent.

### *3.2. MISPE of real water samples*

To evaluate the performance of the MIPs in the extraction of 4-NP from real samples, Ebro river water was chosen to demonstrate that the MIPs are able to selectively bind 4-NP from other interferences in complex matrices. Ebro river water is a complex sample due to the presence of high concentrations of humic acids and therefore represents an interesting test case. As expected, the clean-up step reduced the humic band considerably but the use of dichloromethane was insufficient to completely remove the humic acids and the analytes could not be quantified accurately. Hence it was decided to add $Na_2SO_3$ (10% w/v) to the sample (80 μl $Na_2SO_3$ per 20 ml of sample) since this gave cleaner chromatograms when Ebro river water was used in previous work [28]. Adding $Na_2SO_3$ did indeed decrease the humic band and enabled 4-NP to be quantified accurately. These results are shown in Figs. 4 and 5 for P1 and P2, respectively. The recovery of 4-NP is similar to the recovery obtained under the same conditions with Milli-Q water. Therefore, $Na_2SO_3$ plays an important role when real water samples are analysed.

P1 and P2 were compared in the extraction of 4-NP from real water samples. As in the model study, when river water was analysed, P1 showed a slightly higher selectivity since the interaction with humic acids was higher with the P2 polymer.

Linearity with river water samples under the optimum conditions was tested using P2 as an example. Different samples of 20-ml volume spiked with 4-NP at concentrations between 100 and 1 μg $l^{-1}$ and containing 80 μl of $Na_2SO_3$ per 20-ml sample, were pre-concentrated and a washing step with 0.5 ml of dichloromethane applied. The response was checked in the range described earlier and good linearity was obtained with a determination coefficient ($r^2$) higher than 0.999. The repeatability for 20 ml of spiked (5 μg $l^{-1}$ of each component) river water, expressed as RSD ($n=3$), was 7%. The application of the imprinted polymers to on-line MISPE of real samples was demonstrated.

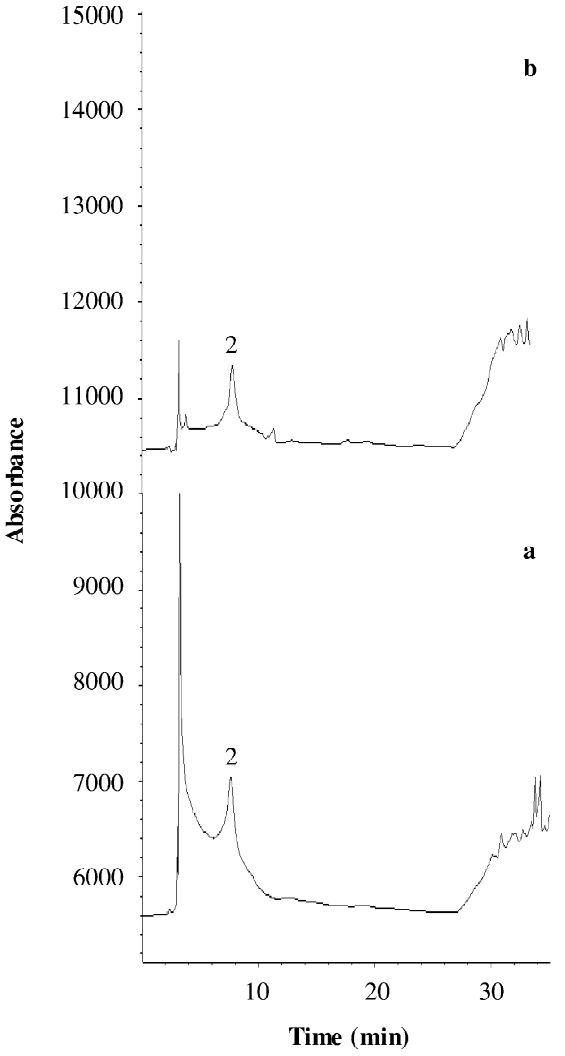


Fig. 4. Chromatograms obtained by on-line MISPE with the non-covalent 4-NP imprinted polymer (P1) of 10 ml Ebro river water (pH 2.5) spiked at 10 μg $l^{-1}$ with each phenolic compound. (a) With washing step using 0.2 ml of dichloromethane, and (b) with addition of $Na_2SO_3$ to the washing step. Peak designation as in Fig. 2.

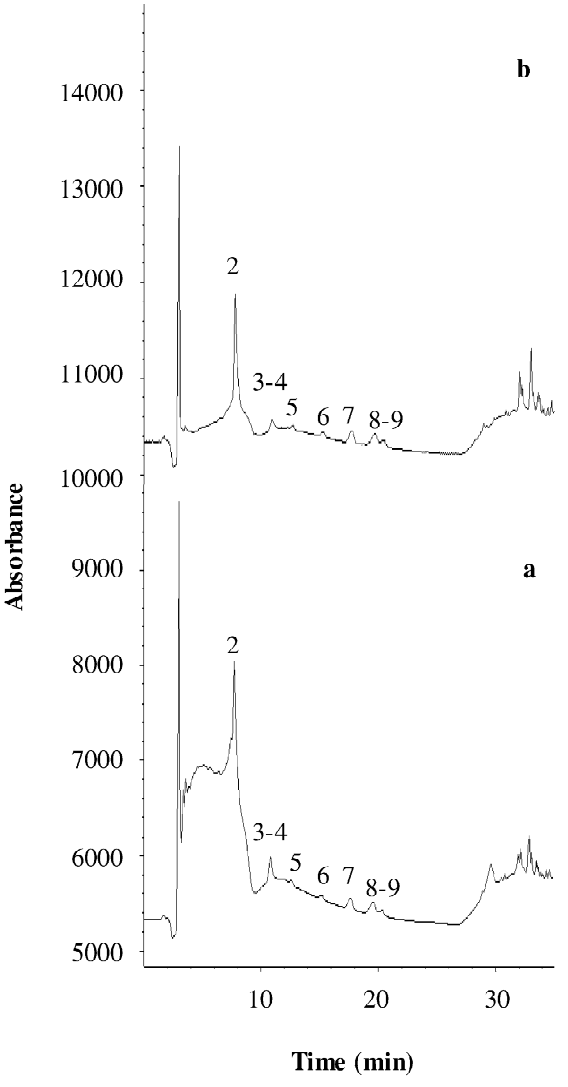


Fig. 5. Chromatograms obtained by on-line MISPE with the semi-covalent 4-NP imprinted polymer (P2) of 20 ml Ebro river water (pH 2.5) spiked at 5 μg $l^{-1}$ with each phenolic compound. (a) With washing step using 0.5 ml of dichloromethane, and (b) with addition of $Na_2SO_3$ to the washing step. Peak designation as in Fig. 2.

## 4. Conclusions

The results demonstrated the practicality of the on-line coupling of MIPSE to liquid chromatography. Two approaches (non-covalent and semi-covalent) were tested for the MIPSE of 4-NP from water samples and differences in selectivity and recovery were observed. Whereas the non-covalent MIP was more selective, the semi-covalent one showed slightly higher recoveries of 4-NP. The application of the MIPSE procedure to determine 4-NP in the presence of other compounds in real water samples was demonstrated.

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