2. PART EXPERIMENTAL I RESULTATS

La millora de les característiques que presenten els sorbents comercials per a l'extracció en fase sòlida ha estat durant els últims anys una via d'investigació en el nostre grup de recerca. Inicialment, els estudis realitzats estaven orientats principalment a l'obtenció de sorbents adients per a l'extracció de compostos polars. Posteriorment, es va proposar el desenvolupament de sorbents selectius per SPE i es van escollir els MIPs per la novetat que suposen aquests materials i pels avantatges que presenten front els ISs. Després d'un estudi inicial amb un MIP i degut als resultats tan satisfactoris que es van obtenir es va continuar i potenciar aquesta via de recerca, en la qual se centra la present Tesi Doctoral.

En aquest capítol s'inclou la part experimental i els resultats obtinguts en els estudis realitzats durant la present Tesi. Aquests resultats han estat publicats o estan pendents de publicació en diferents revistes científiques, de manera que es presenten en forma d'articles. Prèviament a cada estudi s'inclou una breu introducció en la qual s'indiquen els objectius de l'estudi, així com la innovació en el moment de la seva realització.

La part experimental es divideix en cinc apartats, corresponents cadascun d'ells al tipus d'analit emprat com a *template* per a la preparació dels diversos MIPs sintetitzats. Així doncs, en el primer apartat s'inclouen els dos estudis realitzats amb compostos fenòlics (4-nitrofenol i 4-clorofenol) com a *template*. El segon apartat descriu l'estudi d'un MIP sintetitzat amb l'àcid 1-naftalensulfònic. El tercer apartat inclou dos estudis realitzats amb dos antiinflamatoris com a *template*, l'ibuprofen i el naproxen. El següent apartat, el quart, descriu l'aplicació d'un MIP empremtat amb l'antibiòtic oxitetraciclina i el cinquè inclou dos estudis on dues fluoroquinolones, la enrofloxacina i la ciprofloxacina, han estat emprades com a *template.*

Tots aquests MIPs i els corresponents polímers de control (NIPs) emprats en els diferents estudis han estat sintetitzats al *Department of Pure and Applied Chemistry* de la *University of Strathclyde* a Glasgow (UK) sota la tutela del Professor David C. Sherrington i el Dr Peter A.G. Cormack durant dues estades que es van realitzar en aquesta universitat. En tots els estudis que es presenten

a continuació, els MIPs han estat preparats seguint el procés d'empremta molecular no covalent, a excepció de l'estudi realitzat amb el 4-nitrofenol com a *template*, on també es va preparar un altre MIP seguint el protocol semi-covalent. Pel que fa a la tècnica de polimerització emprada, en tots els casos va ser en solució convencional ja que permet obtenir MIPs d'una manera relativament senzilla. No obstant, les condicions de síntesi (porogen, monòmer funcional, temperatura etc.) van ser diferents en funció de les característiques de cada *template*.

El tipus de *template* escollit per tal de preparar els diversos MIPs utilitzats en cadascun dels estudis és molt diferent i ha estat escollit per l'interès en la seva determinació i a la vegada per explorar amb compostos de diferents característiques.

L'aplicació dels MIPs com a sorbents en processos d'extracció en fase sòlida (MISPE) és la que ha generat més interès fins al moment i ha estat objectiu de la present tesi. El procés de MISPE a l'igual que la SPE emprant sorbents convencionals pot ser desenvolupada tant en línia com fora de línia amb la posterior tècnica analítica. En els estudis que s'inclouen a continuació l'extracció fora de línia ha estat la més utilitzada i la tècnica analítica ha estat la cromatografia de líquids amb detector ultraviolat-visible (UV-vis).

Les mostres analitzades en els diversos estudis han estat mostres aquoses en alguns casos i biològiques en d'altres. En els primers treballs es va analitzar aigua de riu i degut als bons resultats obtinguts amb aquest tipus de mostra també es va portar a terme l'aplicació de la MISPE a l'extracció de compostos farmacològics (antibiòtics i antiinflamatoris) en mostres biològiques com orina humana i teixits d'animals (ronyó i fetge de porc) per tal d'ampliar el camp d'aplicació.

2.1 POLÍMERS AMB EMPREMTA MOLECULAR PER A L'EXTRACCIÓ SELECTIVA DE COMPOSTOS FENÒLICS MITJANÇANT L'ACOBLAMENT EN LÍNIA DE L'EXTRACCIÓ EN FASE SÒLIDA

El primer estudi que es va realitzar en el nostre grup de recerca per millorar la selectivitat dels processos d'extracció mitjançant un MIP, va ser desenvolupat per Masqué *et al*. [1]. En aquest estudi es va dur a terme la síntesi d'un MIP no covalent i es va aplicar a l'extracció del 4-NP (compost emprat com a *template*) en mostres d'aigua del riu Ebre. Com a resultat d'aquesta aplicació, es va veure que el MIP presentava una gran selectivitat per aquest compost, ja que era capaç d'extreure'l selectivament d'una mescla d'onze compostos nitro- i clorofenòlics. No obstant, la recuperació pel 4-NP no era molt elevada i per això es va plantejar la síntesi d'un altre polímer empremtat amb el mateix *template* però modificant algunes de les condicions de síntesi.

D'aquesta proposta sorgeix el primer estudi de la present Tesi Doctoral. Per tal d'intentar millorar els resultats obtinguts en el primer treball de Masqué *et al*. [1] pel que fa a les recuperacions obtingudes, es van preparar dos nous MIPs, on les condicions de síntesi emprades són les que es descriuen a la Taula 2.1.

Taula 2.1. Condicions de síntesi pels diversos MIPs empremtats amb 4-NP.

Polímer	Protocol de síntesi	Template	Monòmer
Masqué [1]	No covalent	4-NP	4-VP
P1	No covalent	4-NP	MAA
P2	Semi-covalent	4-nitrofenil metacrilat	Estirè

Porogen: ACN Entrecreuant: EGDMA Iniciador: AIBN MIP: monòlit

Com es pot observar en aquesta taula, P1 només difereix del polímer preparat per Masqué en el monòmer funcional emprat. En aquest cas el 4-NP estableix enllaços d'hidrogen amb el monòmer funcional (MAA), a diferència del MIP preparat per Masqué *et al*. on el MIP a més a més de les interaccions d'enllaç d'hidrogen podia formar també interaccions tipus π-π entre l'anell aromàtic del 4-NP i del monòmer funcional (4-VP).

En canvi, per la síntesi de P2 es va modificar el protocol de preparació (semi-covalent) el qual requereix partir inicialment d'un complex *template*-monòmer com a molècula *template*. En aquest cas el complex emprat va ser el 4-nitrofenil metacrilat, l'estructura del qual es mostra a la Figura 2.1. En aquesta figura també es pot veure l'estructura del monòmer funcional addicional (estirè) que es va afegir per tal de mantenir constant la relació entre el template: monòmer funcional: entrecreuant (T:M:X) emprada en les síntesis anteriors.

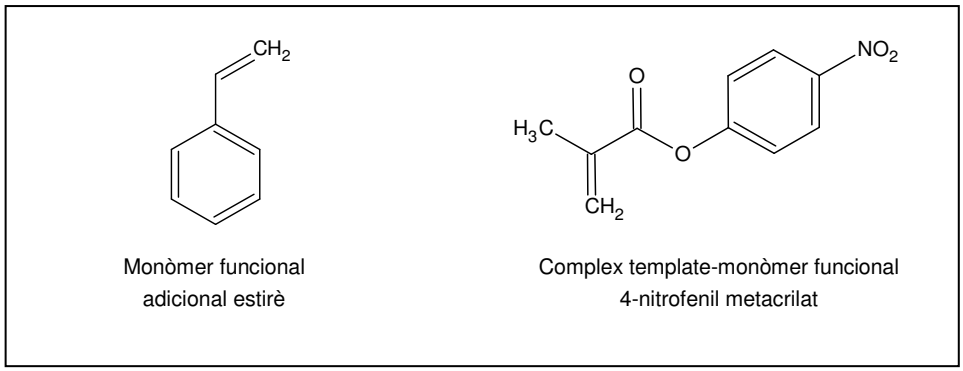


Figura 2.1 Estructura dels reactius emprats com a template i monòmer funcional per a la síntesi del MIP semi-covalent.

P1 i P2 es van aplicar primerament a l'extracció d'una mescla de compostos fenòlics (Figura 2.2) en aigua Milli-Q. El sistema d'extracció emprat es va acoblar en línia a la cromatografia de líquids amb columna de fase invertida i detecció ultraviolada. Per tal d'evitar l'eixamplament dels pics cromatogràfics degut a aquest acoblament, els analits retinguts al MIP es van eluir amb el solvent orgànic de la fase mòbil (ACN/àcid acètic (99:1)) i en sentit contrari al de preconcentració [2]. L'acoblament de la MISPE a la posterior tècnica analítica no és molt freqüent ja que normalment es treballa emprant sistemes fora de línia. En aquest cas però, va ser possible desenvolupar un sistema en línia degut a que el solvent d'elució òptim dels analits retinguts al MIP era perfectament compatible amb el subseqüent sistema analític.

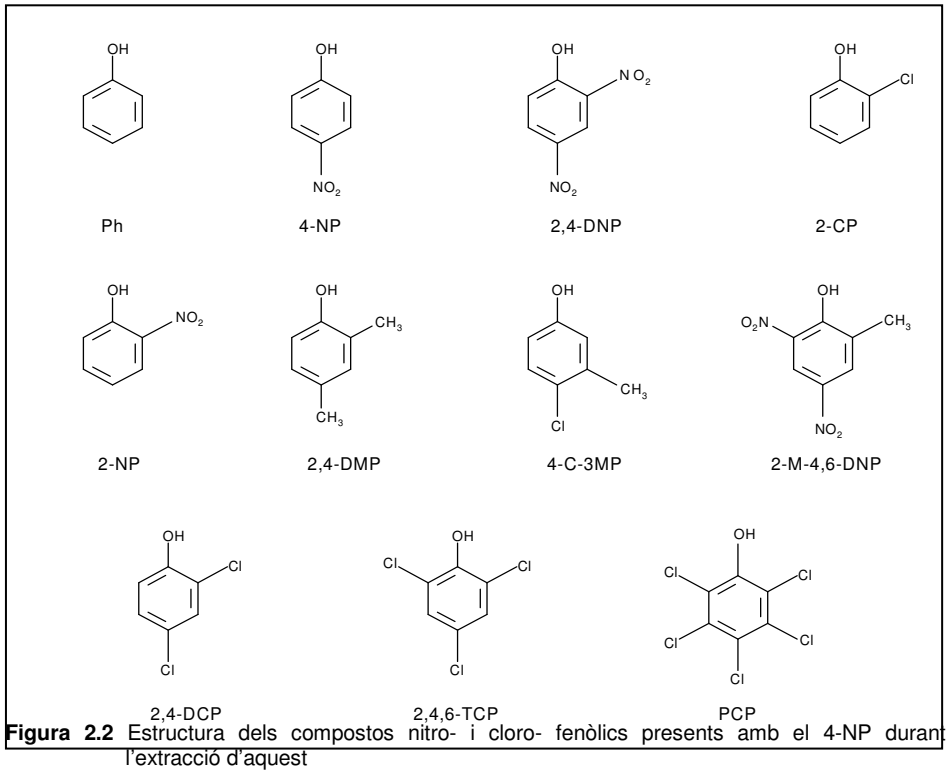
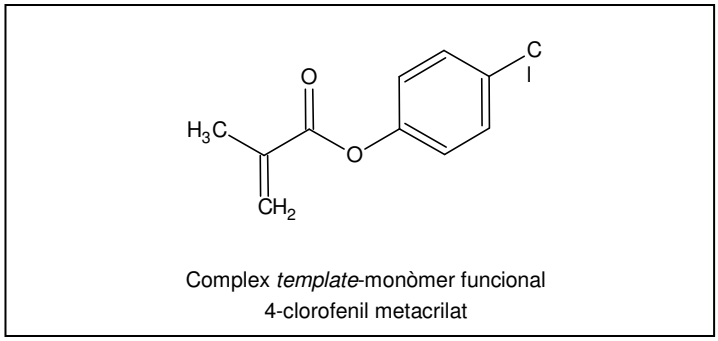


Figura 2.2 Estructura dels compostos nitro- i cloro- fenòlics presents amb el 4-NP durant l'extracció d'aquest

Un cop optimitzat el procés de MISPE, tots dos MIPs van ser aplicats a l'extracció d'aquests compostos en aigua del riu Ebre per avaluar tant la selectivitat com les recuperacions obtingudes quan els polímers eren aplicats en aquest tipus de matriu. Tot i que la selectivitat mostrada pels MIPs reduïa en gran part la càrrega orgànica, principalment àcids húmics i fúlvics que presenten aquestes mostres, es va afegir sulfit sòdic a la mostra [3] per acabar d'eliminar-la i així quantificar correctament el 4-NP. Una vegada establertes les condicions per a l'anàlisi de mostres reals, es van determinar diversos paràmetres analítics com la linealitat i la repetibilitat.

Emprant com a *template* un altre compost fenòlic es va realitzar un segon estudi. En aquest cas el *template* emprat va ser el 4-CP i la síntesi d'aquest polímer també es va dur a terme mitjançant dos protocols diferents, el no

covalent i el semi-covalent. Seguint el protocol no covalent, es van preparar dos MIPs, un amb la 4-VP (P1) com a monòmer funcional i un altre amb el MAA (P2). Mitjançant el protocol semi-covalent es va obtenir el tercer MIP pel qual va ser necessari partir del complex 4-clorofenil metacrilat (Figura 2.3) com a *template* i estirè com a monòmer funcional addicional pels mateixos motius que en el treball anterior.



Complex *template*-monòmer funcional
4-clorofenil metacrilat

Figura 2.3 Estructura dels reactius emprats com a template i monòmer funcional per a la síntesi del MIP semi-covalent

El sistema analític emprat va ser el mateix sistema en línia que el descrit en l'estudi anterior i els compostos estudiats també. Després del procés d'optimització de la MISPE, es va veure com els polímers P2 i P3 d'aquest estudi no mostraven un clar efecte d'empremta molecular. Sorprenentment, el que es va observar en el cas de P1 va ser una marcada reactivitat creuada pel 4-NP i per a tots els compostos que presentaven un àtom de clor a la posició 4 de la seva estructura, fet que no havia estat observat prèviament per a cap dels MIPs empremtats amb el 4-NP.

El polímer P1 va ser aplicat a l'extracció de la mescla de compostos fenòlics en aigua del riu Ebre. En aquest estudi, l'etapa de neteja amb el solvent orgànic va ser suficient per a eliminar completament la banda inicial del cromatograma corresponent als húmics i fúlvics i per tant no va ser necessària l'addició de sulfit a la mostra. La linealitat i repetibilitat també es van determinar per a aquest MIP.

Els dos treballs que s'adjunten a continuació inclouen els resultats obtinguts en aquests estudis. En el primer treball (apartat 2.1.1) s'inclou la síntesi i aplicació del MIP empremtat amb el 4-NP, i ha estat publicat en la revista *Journal of Chromatography A 963 (2002) 169*; la síntesi i aplicació corresponent al MIP empremtat amb el 4-CP s'inclou en un segon treball (apartat 2.1.2) i ha estat publicat a la revista *Journal of Chromatography A 995 (2003) 233.*

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

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: 4-nitrophenol; On-line solid-phase extraction; Semi-covalent molecularly imprinted polymer; Water sample

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 parti-cularly 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 (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 reformed. Another methodology is the self-assembly approach (non-covalent imprinting) where relatively weak non-covalent intermolecular interactions, such as electrostatic interactions, hydrogen bonding, π-π 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 interac-tions 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 mono-mer(s) in a suitable solvent. No che-mical 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 una-voidable 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 works 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 chromato-graphic 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 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 performed 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.

EXPERIMENTAL

Reagents and standards

The chemicals for the polymer synthesis were 4-NP, methacrylic acid (MAA) and ethylene glycol dime-thacrylate (EGDMA) from Aldrich (Steinheim, Germany), styrene from Fisher Chemicals (Loughborough, U.K.), 2,2'-azobisiso-butyronitrile (AIBN) from Acros Organics (Geel, Belgium) and acetonitrile from Rathburn Chemicals (Walkerburn, U.K.). 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 accor-ding to a protocol described in literature [27].

The HPLC-grade solvents were sourced from either Rathburn Chemicals or SDS (Peypin, France) and the water collec-ted 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-chloro-phenol (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-di-chlorophenol (2,4-DCP), 2,4,6-trichloro-phenol (2,4,6-TCP) and pentachloro-phenol (PCP), and were all supplied by Aldrich, except for PCP which was from Jansen Chemie (Geel, Belgium).

Instrumentation

In the MISPE study a Must column-switching device (Spark Holland, Emmen, Netherlands), a Waters (Milford, MA, USA) M45 pump and 10 x 3 mm i.d. stainless steel pre-columns, laboratory-packed with ~ 40 mg of the in-house 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 x 0.4 cm i.d. Spherisorb ODS2, 5 µm, supplied by Teknokroma (Barcelona, Spain).

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) 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 π-π 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 five minutes, sealed under nitrogen and then left to polymerise in a water bath at 60 ºC 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.

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 ºC and all compounds were detected at 280 nm, except for PCP which was detected at 302 nm.

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 (Figure 1).

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

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 poly-mers were evaluated subsequently via on-line MISPE.

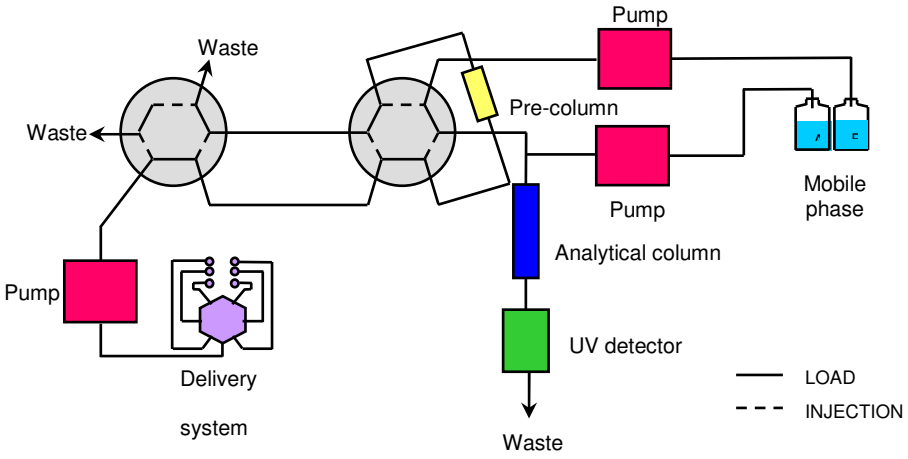

Figure 1. Set-up of the system used.

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 imprin-ted and to investigate the selectivity of polymers for 4-NP when this phenol was present with the other 10 priority EPA phenolic compounds in a water sample, an extraction step was develo-ped. 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 (Figures 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 dichloro-methane stripped all the phenols, in-cluding 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 dichloro-methane were tested (0.1, 0.2 and 0.3 ml). Figure 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 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.

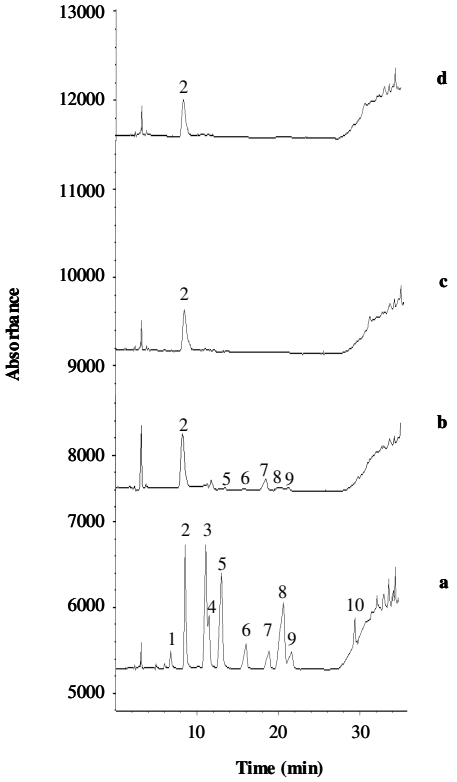

Figure 2. Chromatograms obtained by on-line MISPE with the non-covalent 4-NP imprinted polymer (P1) of 10 ml standard solution (pH 2.5) spiked at 10 µg/l with each phenolic compound. (a) Without washing step, and (b, c, d) With washing step using 0.1, 0.2 and 0.3 ml of dichloromethane, respectively: (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

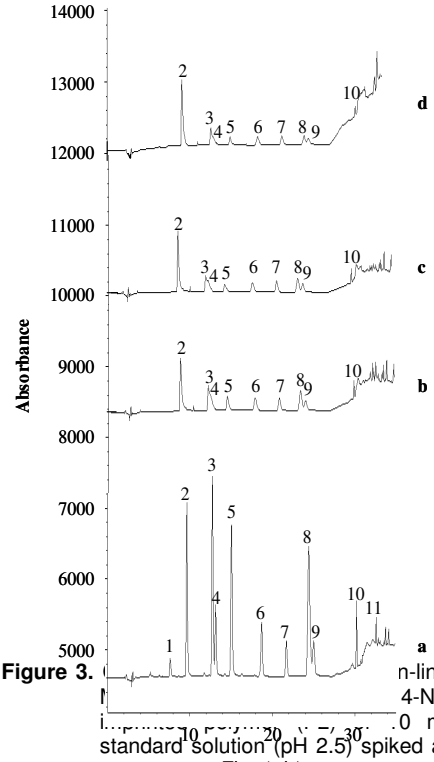

Figure 3. On-line MISPE with the non-covalent 4-NP imprinted polymer (P1) of 10 ml standard solution (pH 2.5) spiked at 10 µg/l with each phenolic compound. Without washing step (a), and with washing step using 0.2 (b), 0.3 (c) and 0.5 ml of dichloromethane (d). Peak designation, as per Figure 2.

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. Figure 3 shows the effect of changing the volume of dichloro-methane 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 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.

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 for each analyte[a]

Analyte	Volume of 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)

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 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 fact that the recovery of 4-NP did not decrease for the semi-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 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 dichloro-methane, 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.

If one compares the results obtained for the two MIPs described in this paper with the results obtained for the previously reported non-covalently im-printed 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 re-covery 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 dichlorome-thane 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-se-lective interactions between 4-NP and the polymer are totally eliminated with 0.2 ml of wash solvent.

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 in-terferences 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 dichloro-methane 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 accu-rately. These results are shown in Figures 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.

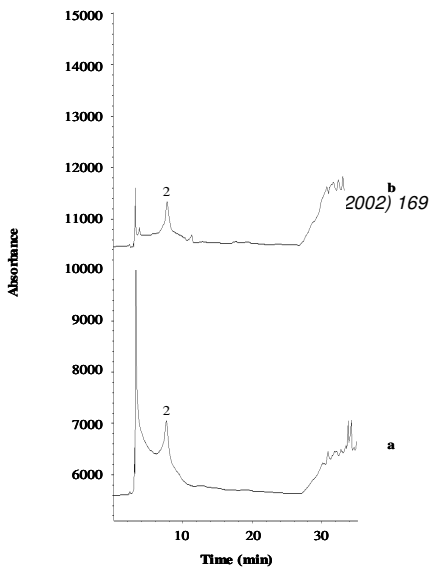

Figure 4. Chromatograms obtained by on-line MISPE with the non-covalent 4-NP imprinted polymer (P1) of 10 ml Ebro river water (pH 2.5) spiked at 10 µg/l with each phenolic compound. (a) With washing step using 0.2 ml of dichloromethane, and (b) With addition of Na_2SO_3 to the washing step. Peak designation,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

Different samples of 20 ml volume spiked with 4-NP at concentrations between 100 and 1 µg l^{-1} and con-taining 80 µl of Na_2SO_3 per 20 ml sample, were pre-concentrated and a washing step with 0.5 ml of dichloro-methane 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 (5µg 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.

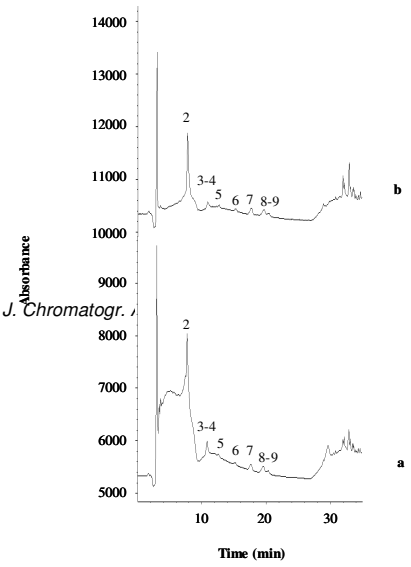

Figure 5. Chromatograms obtained by on-line MISPE with the semi-covalent 4-NP imprinted polymer (P2) of 20 ml Ebro river water (pH 2.5) spiked at 5 µg/l with each phenolic compound. (a) With washing step using 0.5 ml of dichloromethane, and (b) With addition of Na_2SO_3 to the washing step. Peak designation,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

CONCLUSIONS

The results demonstrated the prac-ticality 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.

Acknowledgements

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

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 eleven 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.

Keywords: 4-chlorophenols; 4-nitrophenol; Cross-reactivity; On-line solid-phase extraction; Molecularly imprinted polymer; Water sample

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 analytes [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 the 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 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.

EXPERIMENTAL

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 (Walkerburn, U.K.). 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 dichloro-methane (DCM) from SDS. The structurally related phenolic pollutants used to investigate the selectivity of the polymers were the eleven priority EPA phenolic compounds and 4-chlorophenol itself. Phenol (Ph), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chloro-phenol (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).

Instrumentation

The polymers were firstly evaluated in analytical columns to check the imprinting effect. The 15 x 0,46 cm i.d. stainless steel HPLC columns were slurry packed with the 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 x 0.4 cm i.d. Tracer Extrasil ODS2, 5 μm, supplied by Teknokroma (Barcelona, Spain).

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 poly-merisation 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 respec-tively, 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 poly-merisation step was the same as for P1 polymer.

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 micro-analysis demonstrated that the tem-plate had been successfully removed from the polymer.

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 ml min^{-1}. The injection volume was 20 μl, 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 ml min^{-1} and the gradient profile was 20-30% B from 0-30 min, 100% B at 32 min and then isocratic elution for 2 min. The oven temperature was set at 65 ºC and all compounds were detected at 280 nm, except for PCP which was detected at 302 nm.

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 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 μm filter and adjusted to pH 2.5 before MISPE.

RESULTS AND DISCUSSION

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 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, for this reason, the data obtained in these analyses was normalised by calculating the Normalised Retention Index (RI) [12]. Table 1 shows the K' values for the test compounds in the column and the corresponding RI values for P1. 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.

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.

Table 1. Capacity Factors (*K'*) and Normalised Retention Indices (RI) obtained from HPLC evaluation of polymer (P1)

Analyte	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

The P1 polymer was first evaluated and 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 twelve 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 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 (Figure 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.

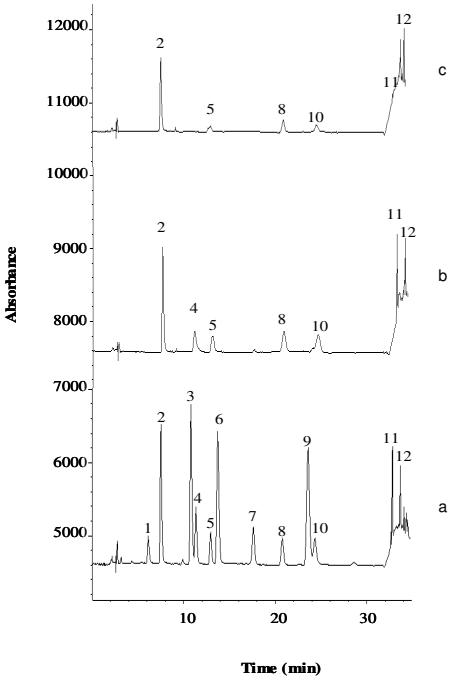

Figure 1. Chromatograms obtained by on-line MISPE with the 4-VP non-covalent 4-CP imprinted polymer (P1) of 10 ml standard solution (pH 2.5) spiked at 10 µg l^{-1} with each phenolic compound. (a) Without washing step, and (b, c) with washing step using 0.1 and 0.3 of dichloro-methane, respectively: (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.

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 μg l^{-1} for each analyte [a].

Analyte	Volume CH_2Cl_2 (ml)			
	0	0.1	0.2	0.3
Ph	59	-	-	-
4-NP	73	55	47	38
2,4-DNP	71	[b]	-	-
2-CP	82	[c]	-	-
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

[a]RSDs were lower than 9% in all instances (n= 3)

[b,c]Co-eluted compounds

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.

MISPE of Real Water Samples

An application of the imprinted polymer (P1) in the MISPE was developed with Ebro river water sample to show how selective the MIP can be with real water. Polar phenols can not 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 of the most polar compounds. However, when this MIP was used as selective sorbent in SPE the humic band was completely removed with a clean-up step with 0.1 ml of DCM (Figure 2) and without adding Na_2SO_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, were spiked with the eleven priority EPA phenolic compounds and 4-CP at concentrations between 100 and 1 μg l^{-1}, were 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.

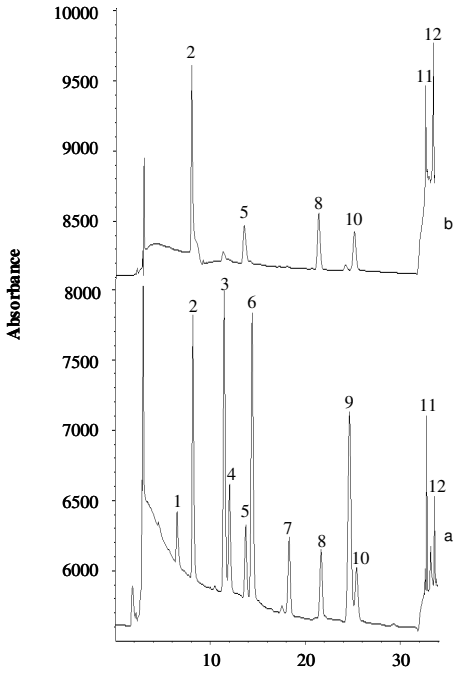

Figure 2. Chromatogram obtained by on-line MISPE with the 4-VP non-covalent 4-CP imprinted polymer (P1) of 10 ml Ebro river water (pH 2.5) spiked at 10 μg l^{-1} with each phenolic compound. (a) Without washing step and (b) with washing step using 0.1 ml of dichloromethane. Peak designa-tion, as per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

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 μg 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.

CONCLUSIONS

A polymer prepared using 4-VP as functional monomer and following 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 in real water samples was developed to demonstrate its applicability. All the retained compounds gave a linear response.

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