2.2 EXTRACCIÓ EN FASE SÒLIDA DE NAFTALENS SULFONATS EN MOSTRES D'AIGUA DE RIU MITJANÇANT UN POLÍMER AMB EMPREMTA MOLECULAR

Arran dels resultats obtinguts en els estudis anteriors, on s'ha comprovat que és possible sintetitzar MIPs selectius per extreure compostos de característiques polars d'una mostra aquosa, es va proposar la síntesi d'un nou MIP que fos capaç d'extreure compostos iònics i de mida més gran com són els naftalens sulfonats.

En aquest apartat es presenta la síntesi i l'aplicació d'un MIP empremtat amb l'àcid 1-naftalensulfònic (1-NS) i amb la 4-VP com a monòmer funcional, ja que com es va comprovar en els dos treballs anteriors, aquest monòmer dóna lloc a interaccions més fortes quan el *template* presenta aromaticitat. No obstant, trobar el porogen apropiat per a dur a terme aquesta síntesi va ser força complicat, ja que en ser un compost tan polar era gairebé insoluble en la majoria de solvents orgànics emprats normalment per a la síntesi de MIPs (apolars i apròtics). Es va observar però que en acetonitril, aquest *template* sí que era soluble i per tant inicialment es va escollir aquest solvent com a porogen. No obstant, quan s'afegia el monòmer funcional a aquesta barreja, es formava immediatament un precipitat blanc, fet que indicava que hi havia una forta interacció entre l'àcid 1-NS i la 4-VP. La formació d'aquest precipitat no era adient per a poder continuar afegint els altres reactius necessaris per a la polimerització i per aquest motiu l'ACN va quedar descartat com a porogen. Un altre solvent de constant dielèctrica elevada i apròtic que es va provar va ser la dimetilformamida (DMF), però l'1-NS era poc soluble en aquest solvent.

Basant-nos en els resultats obtinguts en uns estudis previs desenvolupats per Haupt *et al*.[1] i Baggiani *et al*. [2], els quals havien preparat els respectius MIPs emprant una barreja de metanol/aigua (MeOH/H_2O) com a porogen, es va intentar sintetitzar el MIP en aquest medi. La quantitat d'aigua a emprar en el nostre cas venia determinada per la miscibilitat amb l'agent entrecreuant (EGDMA). Aquesta proporció va ser finalment de 4 parts de MeOH per cada part d'H_2O (4:1) i es va observar que l'ordre d'addició dels reactius era important per tal d'aconseguir una total solubilització de la molècula *template*. L'ordre òptim a seguir en aquest cas va ser afegir primer el template, després l'aigua, a continuació la 4-VP i per últim el MeOH.

El protocol d'empremta molecular va ser el no covalent. La polimerització en solució es va iniciar amb radiació UV (50 Hz) durant 24 hores a 5ºC. Després d'aquest període, el polímer obtingut es va introduir en un bany d'aigua a 60ºC durant 24 hores més per tal d'assegurar que s'obtenia un monòlit rígid i estable. El polímer de control es va sintetitzar simultàniament però en absència de la molècula *template*.

Per tal d'avaluar l'efecte d'empremta molecular del MIP abans de ser emprat com a sorbent en SPE, es va intentar realitzar una avaluació cromatogràfica, però no es va obtenir cap resultat ja que, degut a la forta interacció que presentava l'analit amb el MIP, resultava impossible eluir-lo.

Així doncs, per tal de poder optimitzar el procés de MISPE sense cap tipus de restricció pel que fa al solvent, es va optar per començar utilitzant el procés de MISPE fora de línia. En aquest cas es va empaquetar una xeringa d'extracció amb 200 mg del sorbent que es trobava contingut entre dos fritats. Després d'optimitzar l'etapa d'acondicionament i pas de la mostra, es va veure que per dur a terme l'elució era necessari emprar una barreja de MeOH/H_2O (4:1) amb un 10% d'hidròxid sòdic (NaOH) 1M. Per tal d'evitar problemes de degradació amb la columna analítica i degut a que aquesta composició de fase mòbil no era l'adient per dur a terme la separació cromatogràfica dels compostos, es va continuar treballant en aquest sistema fora de línia.

Tot i que el MIP havia estat sintetitzat en presència d'aigua, i que això podia suposar un inconvenient per la formació de les interaccions específiques durant la polimerització, es va comprovar com el MIP presentava un bon efecte d'empremta molecular i que a més a més també presentava una forta reactivitat creuada de manera que permetia extreure selectivament una barreja de vuit naftalens sulfonats i disulfonats (NSs) amb diversos grups funcionals (amino- i nitro-) d'una mostra de 1000 ml d'aigua Milli-Q amb bones recuperacions per a tots els compostos després d'una etapa de neteja amb un solvent orgànic

(MeOH/piridina (99:1)). Aquesta barreja de compostos es perdia gairebé per complet del polímer de control després de l'etapa de neteja.

Per tal de demostrar que aquest grup de NSs quedaven retinguts al MIP mitjançant interaccions selectives, es va preparar una mostra d'aigua Milli-Q on a més dels 8 NSs també es van afegir altres compostos polars com l'oxamil, metomil, el 4-NP, el 2,4-DNP, la bentazona i l'àcid 4-cloro-2-metil-fenoxi acètic i es va comprovar com després d'una etapa de neteja, en aquest cas amb MeOH, els NSs quedaven retinguts al 100% mentre que la resta d'analits s'eluien totalment. Aquest fet justifica que el grup sulfònic (SO_3^-) juga un paper molt important en el reconeixement molecular de l'analit a extreure i que per tant aquestes interaccions selectives estan basades en interaccions iòniques entre la 4-VP i el SO_3^-.

Finalment, aquest MIP es va utilitzar en l'extracció de vuit NSs en aigua de riu. En aquest cas, el volum de mostra analitzat van ser 500 ml.

Amb aquest MIP s'aconsegueix extreure una família de compostos d'elevada polaritat, els quals són difícils d'extreure amb sorbents convencionals. Aquests compostos normalment s'extreuen mitjançant SPE amb parell iònic amb l'inconvenient que sovint altres substàncies iòniques presents a la mostra interfereixen en l'extracció dels compostos d'interès [3]. Per tant, les recuperacions i el volum de ruptura disminueixen considerablement [4]. En aquest estudi queda demostrat, que també és possible extreure aquests compostos de grans volums de mostra amb bones recuperacions sense necessitat d'utilitzar un parell iònic quan es treballa amb aquest MIP com a sorbent.

Els resultats obtinguts en aquest estudi s'inclouen en el treball que s'adjunta a continuació i que ha estat publicat a la revista *Journal of Chromatography A 1047 (2004) 175.*

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2.2.1 *Molecularly imprinted solid-phase extraction of naphthalene sulfonates from water*

MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION OF NAPHTHALENE SULFONATES FROM WATER

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Abstract

A new polymeric sorbent synthesised by exploiting molecular imprinting technology has been used to selectively extract naphthalene sulfonates (NSs) directly from aqueous samples. In the non-covalent molecular imprinting approach used to prepare this polymer, 1-naphthalene sulfonic acid (1-NS) and 4-vinylpyridine (4-VP) were used as a template molecule and functional monomer, respectively, and both dissolved in a mixture of methanol/water (4:1) as porogen together with the cross-linker ethylene glycol dimethacrylate.

The new non-covalent molecularly imprinted polymer (MIP) prepared in aqueous environment was used as a sorbent in solid-phase extraction (SPE) to selectively extract a group of naphthalene mono- and disulfonates. When one litre of a standard aqueous solution, which contained a mixture of eight NSs, was percolated through the SPE cartridge, all the NSs were retained on the MIP because of the cross-reactivity of the polymer. Recoveries were higher than 80% for all the compounds even after a clean-up step with methanol (MeOH). The MIP was also used to analyse water from the Ebro river.

Keywords: Water analysis; Cross-reactivity; Molecularly imprinted polymers; 1-Naphthalene sulfonic acid

INTRODUCTION

Naphthalene sulfonates (NSs) are aromatic compounds that show low biodegradability, high polarity and high toxicity. All these properties make them potentially hazardous for the environment in particular surface and ground waters are of concern with regard to drinking water quality [1]. The presence of these pollutants in the environment can be attributed mainly to their use in the chemical industry [2]. For these reasons, NSs have been investigated for some time, and several analytical methods have been developed to determine them in environmental water [3-7]. Reversed-phase ion-pair liquid chromatography has been the most used method [1,2,6-10]. The characteristics of NSs make the interactions of these analytes with reversed-phase columns too weak to separate them effectively; consequent-ly, other retention mechanisms using an ion-pairing agent have to be exploited [1].

In environmental samples naphthalene sulfonates are normally present at low concentrations and an enrichment step is required for their quantification. Ion-pair solid-phase extraction (ion-pair SPE) is the most commonly used pre-concentration procedure for these analytes. However, ion-pair SPE suffers from some drawbacks in respect of aqueous samples since other ionic species can compete in the formation of the ion-pair, thus interfering with the SPE process [2]. Consequently the recoveries and therefore the break-through volumes decrease considerably [11]. Thus it would be very advantageous to work without using an ion-pairing reagent in the SPE procedure and this would be possible using selective SPE materials such as molecularly imprinted polymers (MIPs) [12-15].

The main advantage of this class of sorbents, compared to conventional sorbents, is the inherent molecular selectivity of the sorbent [16], which enables MIPs to extract one specified compound from a complex mixture of compounds, although in some cases the MIP shows cross-reactivity and is able to recognise also structurally related analytes [17-22].

In this study, a new non-covalently MIP has been prepared and evaluated for the extraction of a group of NSs from water samples. Non-covalently MIPs are usually synthesised in apolar organic solvents because polar solvents can disrupt hydrogen bonding interaction between the template and the functional monomer. Moreover, if ionic interactions are also established polar-protic solvents can also disrupt them. However, it has been previously reported [23-26] that some times is also possible to use a mixture of polar solvents as porogen (such as MeOH/water) when a polar compound is used as template in which a combination of the hydrophobic effect and ionic interactions will be the responsible in the molecular recognition step. To our knowledge this is the first time that an imprinted polymer has been synthesised using 1-naphthalene sulfonic acid as a template molecule using a mixture of methanol/water (4:1) as porogen. Thus, in the present paper a water compatible MIP (aqua-MIP), which is quite helpful when using aqueous samples, has been synthe-sised.

EXPERIMENTAL

Reagents and standards

The chemicals used for the polymer syntheses were 1-naphthalenesulfonic acid dihydrate (1-NS) from Avocado Research Chemicals (Lancarshire, England), 4-vinylpyridine (4-VP) and ethylene glycol dimethacrylate (EGDMA) from Aldrich (Steinheim, Germany) and 2,2'-azobisisobu-tyronitrile (AIBN) from Acros Organics (Geel, Belgium). The monomers were purified prior to use *via* standard procedures in order to remove stabilisers. The AIBN was recrystallised from acetone and the methanol dried over molecular sieves.

The HPLC-grade methanol was provided by SDS (Peypin, France) and the water collected from a Millipore water purification system (Milli-Q water). The phosphoric acid was from Probus (Badalona, Spain) and the disodium hydrogen phosphate, the sodium dihydrogen phosphate and the tetra-butylammonium bromide (TBA) were from Panreac (Barcelona, Spain), Probus and Fluka (Buchs, Switerland), respectively. The latter were to prepare the mobile phase.

Other reagents used to modify the pH of the sample were hydrochloric acid (37%) from Probus, sodium hydroxide (NaOH) from Prolabo (Fontenay, France), triethylamine (TEA) from Aldrich and pyridine, which was used in the washing step, from Fluka.

The structurally related naphthalene sulfonate pollutants (Figure 1) used to investigate the selectivity of the polymers were 2-naphthylamine-1-sulfonic acid (1-NS-2-NH_2), 1-naphthol-4-sulfonic acid sodium salt (1-NS-4-OH), naphthalene-2-sulfonic acid sodium salt (2-NS), naphthalene-1,5-disulfonic acid disodium salt (1,5-NDS), 2-naphthylamine-1,5-disulfonic acid disodium salt (1,5-NDS-2-NH_2), naphthalene-2,7-disulfonic acid diso-dium salt (2,7-NDS), 1-naphthol-3,6-disulfonic acid disodium salt (3,6-NDS-1-OH); all sourced from Fluka. Standard solutions of each compound at a concentration of 1000 mg l^{-1} were prepared in Milli-Q quality water. The 2-NS, was solubilised in Milli-Q water/methanol 70:30 (*v/v*).

Other compounds such as naphthalene, 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), oxamyl, methomyl, benta-zone and 4-chloro-2-methyl-phenoxy acetic acid (MCPA), supplied by Aldrich and Fluka, were used to check the selectivity of the MIP for other polar compounds.

Instrumentation

The liquid chromatographic system consisted of two LC-10AD pumps, a DGU-14A degasser, a CTO-10A oven and a SPD-10A UV spectrophotometric detector from Shimadzu (Tokyo, Japan)

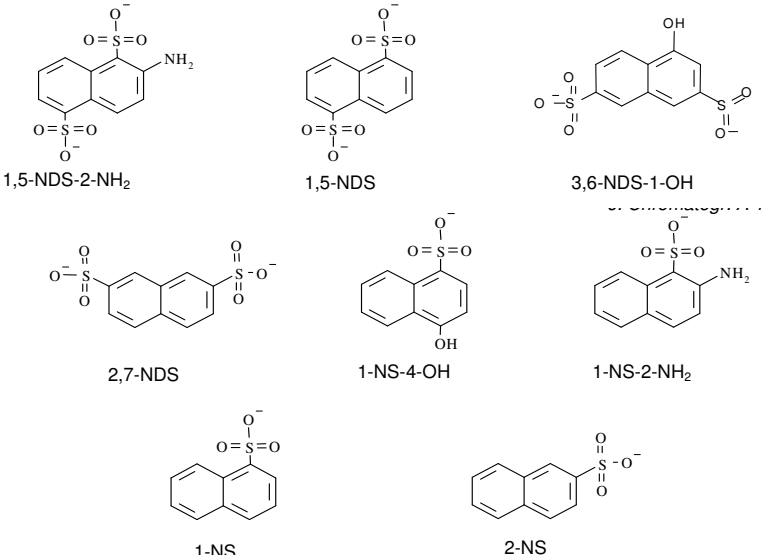

Figure 1. Chemical structures of the naphthalene sulfonates (NSs) used to probe the selectivity of the MIP.

The injection loop volume was 20 µl and 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 Polymer

The imprinted polymer was prepared by the non-covalent approach. The molar ratio template: functional monomer: cross-linker was (1:4:20) in which 1-NS (2.28 mmol) was the template molecule, 4-VP (9.12 mmol) was selected as the functional co-monomer, EGDMA (45.6 mmol) was the cross-linking monomer and AIBN (1.00 mmol) was the initiator. Due to the high polarity of the template, a mixture of

methanol/water (4:1 *v/v*) (13.33 ml) was used as porogen. Other solvents were also evaluated as possible porogens to avoid the presence of water during the polymerisation (note: water can disrupt hydrogen bonding or ionic interactions between templates and the functional monomers), but the template was not completely soluble in any of them. All the components were mixed in a 25 ml thick-walled glass tube fitted with a screw cap and added in the following order: template, water, 4-VP, methanol, EGDMA and AIBN. This solution was cooled in an ice bath, sparged with oxygen-free nitrogen for five minutes, sealed under nitrogen and then left in a cooled bath at 5 ºC for 24 hours while irradiated with a 50 Hz Black-Ray Non-UV Semi-conductor Inspection Lamp, Model B 100 AP. The polymer obtained was then left in a water bath at 60ºC for 24 hours to maximise the cure and to ensure formation of a rigid, stable monolith. The MIP was crushed mechanically, ground and wet-sieved using acetone to obtain regularly sized particles with diameters between 25 and 38 µm suitable for the MISPE evaluations.

A reference, non-imprinted polymer (NIP), which did not contain the template, was prepared in parallel with the imprinted polymer using the same synthetic protocol.

Chromatographic Conditions

The mobile phase used in the MISPE experiments was a mixture of two solvents [2]. Solvent A, the aqueous component, contained 8 mM of disodium hydrogen phosphate, 8 mM of sodium dihydrogen phosphate and 7 mM of TBA in Milli-Q water and the pH was adjusted to 6.5 with phosphoric acid. Solvent B was methanol (MeOH). The flow-rate of the mobile phase was 1 ml min^{-1} and the gradient profile was 20-50% B from 0-25 min, and then isocratic elution for 5 min. The oven temperature was set at 40 ºC and all compounds were detected at 230 nm.

MISPE Procedure

The MISPE study was developed in an off-line mode using a solid-phase extraction manifold supplied by Teknokroma (Barcelona, Spain) connected to a vacuum pump. 200 mg of polymer suspended in Milli-Q water was packed into a 6 ml SPE cartridge. Prior to any extraction the polymer was washed with an eluting mixture of MeOH/water (4:1) containing 10% NaOH (1M) until no more residual template (1-NS) was eluted from the polymer. For the MISPE experiments the polymer was conditioned with 15 ml of MeOH and 15 ml of acidified Milli-Q water (pH 2.3). The required sample volume (adjusted to pH 2.3 with HCl) was applied to the conditioned cartridge and the polymer then washed with 20 ml of MeOH. The retained analytes were desorbed using 5 ml of a mixture of MeOH/water (4:1) containing 10% NaOH (1 M) and 20 µl samples injected onto the analytical column.

River samples were filtered through a 0.45 µm filter prior to any experiment.

RESULTS AND DISCUSSION

To evaluate the imprinting effect of a MIP, a chromatographic evaluation is often performed. For this purpose, a chromatographic column (15 x 0.46 cm i.d.) was packed with the MIP by following a standard in-house procedure [12].

Several different mobile phases were investigated including MeOH, MeOH/water (95:5), MeOH/water (80:20), MeOH/water/pyridine (92.5:2.5:5), MeOH/pyridine (95:5), MeOH/acetic acid (80:20) and MeOH/TBA (80:20). However, no chromatographic peaks were obtained when a sample containing 10 mM of 1-NS was injected onto the column, which suggested that the analyte was still retained on the MIP and that the affinity was strong under these particular chromatographic conditions. Therefore, the imprinting effect was evaluated by SPE instead.

MISPE

Two hundred milligrams of the polymer was packed into a polyethylene cartridge. Off-line SPE was chosen as the preferred method of analysis because in this way the most effective solvents could be used to elute the analyte from the MIP. Thus, a mixture of naphthalene mono- and

disulfonic acid compounds, some of which are hydroxyl and/or amino substituted was percolated through the cartridge.

Several parameters were optimised in the SPE procedure. Firstly, the sample pH was investigated to optimise the interactions between the analytes and the MIP in the loading step. Accordingly, the samples were prepared in Milli-Q water and acidified Milli-Q water (pH 2.3). 25ml of these solutions spiked with 5 mg l^{-1} of the NSs mixture were percolated through the MIP. The recovery of each analyte in this loading step was determined by measuring the concentration of the analyte in the aqueous solution eluted from the cartridge. The compounds were strongly retained on the MIP when the sample was prepared in acidified Milli-Q water; however, when the sample was in pure Milli-Q water, very little of each analyte was retained (30%). This observation is presumably a reflection of the fact that electrostatic interactions between the analytes and the pyridyl group residues in the polymer would be expected to have higher affinity at the lower pH value.

The elution step was therefore optimised for samples delivered in acidic water. 5 ml volumes of different elution solvents such as MeOH/water (4:1), MeOH/water (4:1) containing 10% of TEA, and MeOH/water (4:1) containing 10% of NaOH (1M) were tested. The analytes were not eluted when MeOH/water was used as the eluting solvent. However, when 10% of TEA or 10% of NaOH (1M) were added to MeOH/water (4:1), the compounds were eluted with high recoveries although TEA gave rise to slightly distorted peaks for all analytes. To avoid this distortion MeOH/water (4:1) containing 10% of NaOH (1M) was chosen as the elution solvent. Thus, not only did the distortion disappear with this elution solvent but the recoveries of the analytes were also slightly higher than those obtained using TEA.

To evaluate the imprinting effect of the MIP a clean-up step was included. 5 ml volumes of dichloromethane (DCM), acetonitrile (ACN), methanol (MeOH), and a mixture of MeOH/water (4:1) were tested as possible washing solvents, but no clean-up effect was observed because the recoveries of 1-NS and indeed all the NSs present in the sample were the same as SPE without a clean-up. This suggests that the MIP shows cross-reactivity effect for the naphthalene sulfonates. When 1% and 5% of NaOH (1M) were each added to ACN, MeOH and Milli-Q water, the recovery values decreased slightly for all compounds.
Identical experiments were performed using the NIP, and it was found that the analytes were not washed straight off the polymer during the clean-up step with any of the organic wash solvents mentioned previously. Therefore, to demonstrate that the MIP was imprinted, a wash with the most effective solvent was performed. For this purpose, pyridine was added to the most polar solvent (MeOH) since pyridine will compete with the functional monomer (4-VP) for the analyte. Different amounts of pyridine were used (1%, 5% and 10%) and the best results obtained when the cartridge was washed with 2 ml of MeOH/pyridine (99:1) (Table 1).

Table 1. Recoveries (%) of naphthalene sulfonic acid compounds using the 1-NS imprinted polymer and the non-imprinted polymer, either without a clean-up step or with a clean-up step involving 2 ml of MeOH/pyridine (99:1) at different sample volumes of a standard solution.

Analyte	Sample Volume (ml)														
	1-NS						NIP								
	No clean-up			Clean-up			No Clean-up			Clean-up					
	5	500	1000	5	500	1000	5	500	1000	5	500	1000	5	500	1000
1,5-NDS-2-NH_2	107	106	104	100	80	82	88	52	14	17	14	10			
1,5-NDS	107	101	103	98	84	84	93	61	25	16	14	11			
3,6-NDS-1-OH	85	86	87	77	73	72	88	52	25	8	10	7			
2,7-NDS	119	112	110	98	87	86	83	58	27	14	16	10			
1-NS-4-OH	104	98	105	55	50	50	83	37	16	17	3	16			
1-NS-2-NH_2	105	100	82	70	59	60	84	29	9	13	6	5			
1-NS	117	90	85	51	50	54	80	44	14	14	16	6			
2-NS	115	110	102	51	62	65	88	54	17	18	15	8			

[a]RSDs were lower than 13% in all instances (n= 3)

The addition of pyridine to the MeOH disrupted the non-specific interactions established between the analytes and the NIP so that the NSs were almost completely eluted upon the introduction of the washing step. When the same washing step was applied to the MIP, the recoveries decreased slightly for the retained NSs because the non-specific interactions were also disrupted; however, all the NSs were still retained on the polymer because of the specific interactions between the analytes and the polymer. This also confirms that the MIP shows cross-reactivity effect.

The selectivity of the MIP was also evaluated in other ways. For this purpose, a second group of polar pollutants was also added to the sample. Thus 25 ml of acidified Milli-Q water (pH 2.3) containing 2 mg l^{-1} of the mixture of NSs and 2 mg l^{-1} of a mixture of several polar compounds (oxamyl, methomyl, Ph, 4-NP, 2,4-DNP, bentazone and MCPA) was percolated through the cartridge. All the com-pounds, except for oxamyl and methomyl, were retained on the MIP in the loading step.

The MIP was washed with an organic solvent to remove the non-selectively bound compounds. MeOH was chosen as the washing solvent because, as explained earlier, the NSs were retained on the MIP when this solvent was applied. However, it was expected that if the mixture of polar compounds were not selectively retained on the MIP, then MeOH would be able to elute them. A 10 ml volume of MeOH was sufficient to strip off the phenols from the polymer while the

NSs still remained strongly bound to the MIP (Figure 2).

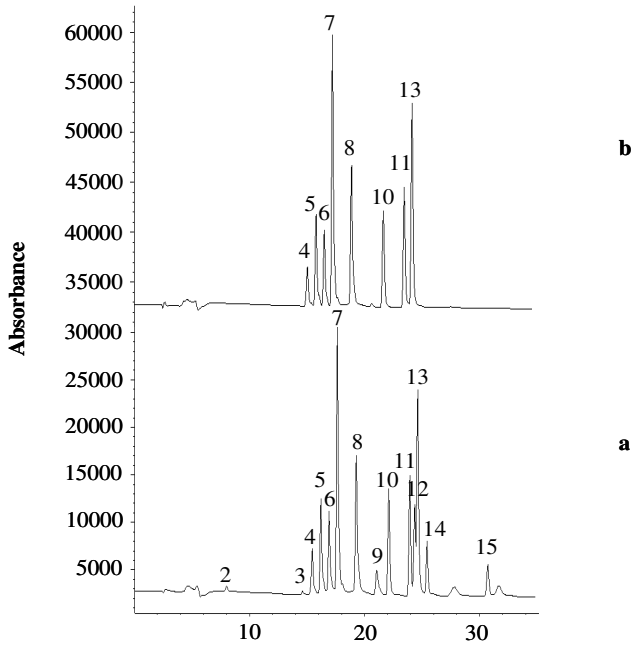

Figure 2. Chromatogram obtained by MISPE of 25 ml of a standard solution (pH 2.3) spiked at 2 mg l^{-1} with each polar and naphthalene sulfonic acid compound: (a) without washing step, and (b) with washing step using 10 ml of methanol: (1) oxamyl, (2) methomyl, (3) Ph, (4) 1,5-NDS-2-NH_2, (5) 1,5-NDS, (6) 3,6-NDS-1-OH, (7) 2,7-NDS, (8) 1-NS-4-OH, (9) 4-NP, (10) 1-NS-2-NH_2, (11) 1-NS, (12) 2,4-DNP, (13) 2-NS, (14) bentazone, (15) MCPA.

Table 2. Recoveries (%) of naphthalene sulfonic acid compounds using the 1-NS MIP when different sample volumes of a standard solution were extracted and a clean-up step with 20 ml of MeOH was applied.

Analyte	Sample volume (ml)			
100	1,5-NDS-2-NH_2	107	106	104	500
1000	1,5-NDS	107	105	103	
	3,6-NDS-1-OH	85	86	87	
	2,7-NDS	109	102	100	
	1-NS-4-OH	104	98	105	
	1-NS-2-NH_2	105	100	82	
	1-NS	107	90	85	
	2-NS	105	100	102	

[a]RSDs were lower than 5% in all instances (n= 3)

However, 20 ml of MeOH was chosen finally as the optimum volume because the recoveries for the NSs were the same as when 10 ml MeOH was used (Table 2) and a higher volume of MeOH would be expected to be more effective in removing a matrix of polar compounds when real river water is analysed.

Other polar compounds such as 2-CP, 4-CP, 2-NP and 2,4-DMP and an apolar aromatic compound such as naphthalene were also tested. As expected, when a 25 ml sample (spiked with 2 mg l^{-1} of polar compounds and naphthalene) was percolated, all the compounds were removed during the clean-up step with 10 ml of MeOH because they could not establish the required specific interactions with the MIP because they each lack the necessary SO_3^- group.

Thus it is clear that the SO_3^- group in the NSs plays an important role in the molecular recognition step by establishing ionic interactions with the 4-VP residues in the MIP. This also accounts for the cross-reactivity effects and this probably explains why after the washing step all the NSs are also retained on the MIP.

The recovery of the compounds from different sample volumes was studied also for both polymers (Table 1). The recoveries for the MIP and the NIP were nearly the same when a 5 ml sample as pre-concentrated and no clean-up performed. However, these values were completely different when a clean-up step was included and the polymers washed with 2 ml MeOH/pyridine (99:1). When 25 ml of sample was passed through the MIP, (data not shown) the recoveries for the NSs in

the MIP were still the same, while recoveries decreased in the NIP (the recovery of 1-NS was only 56%). At higher sample volumes (500 ml and 1000 ml) the differences between both polymers were yet higher, even in the absence of a clean-up step.

MISPE of Real Water Samples

The performance of the MIP in the extraction of NSs from real river water samples was investigated. For this purpose, Ebro river water was chosen. From this kind of water sample, a broad band due to the presence of humic acids appears in the middle of the chromatogram. However, the selectivity of the MIP for the NSs allows us to selectively extract and quantify them after a suitable clean-up procedure.

One thousand milliliters of an Ebro river water sample was percolated through the MIP. However, the recoveries decreased significantly for all the retained analytes even without the clean-up step due to the humic acids and other substances present in the sample matrix affect the retention of the compounds. Therefore 500 ml was chosen as the sample

volume. With this volume the recoveries were nearly the same as in Milli-Q water for all the compounds except for 1-NS-2-NH_2 where the recovery was 50%.

When the MIP was washed with 20 ml of MeOH (Figure 3) the band due to the humic acids was reduced and the recoveries of the NSs were nearly the same as those obtained without the washing step, except for the 1-NS-2-NH_2 (16%) (Table 3). This step was also performed using 2 ml of MeOH/pyridine (99:1) as the washing solvent. However, the recoveries were lower than those obtained using MeOH and the humic band did not decrease significantly with respect to the blank. For this reason, the experiments to test the useful linear range of the application in river water analysis were carried out using MeOH as the washing solvent.

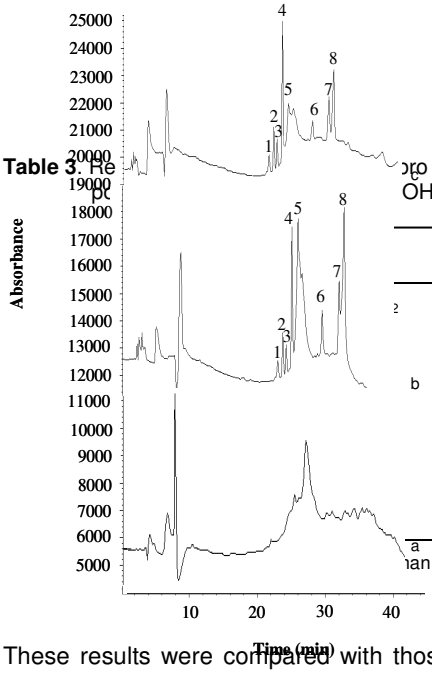

Figure 3. Chromatogram obtained by MISPE of 500 ml Ebro river water (pH 2.3). (a) Blank of Ebro river water, (b) Ebro river water spiked at 10 μg l^{-1} with each NS compound, and (c) Ebro river water spiked at 10 μg l^{-1} with each NS compound and a washing step using 20 ml of MeOH. Peak designation: (1) 1,5-NDS-2-NH_2, (2) 1,5-NDS, (3) 3,6-NDS-1-OH, (4) 2,7-NDS, (5) 1-NS-4-OH, (6) 1-NS-2-NH_2, (7) 1-NS, (8) 2-NS.

Table 3. Re pc ɔro river water spiked at 50 μg l^{-1} using the 1-NS imprinted OH.

	500 ml (50 μg l^{-1})
2	72
	94
	78
b	106
	81
	16
	72
	93

a
ıan 11% in all instances (n= 3)

These results were compared with those obtained by Alonso et al. [27] using a highly cross-linked sorbent (Isolute ENV+), in which an ion-pair reagent was neither added to the sample in the loading

step. The recoveries for the NSs with more than one SO_3^- with the Isolute ENV+ are better than those using other sorbents such as Lichrolut EN or graphitized carbon black, but are lower than those using the 1-NS MIP. Only 150 ml of groundwater sample was percolated and the recoveries for some of the NSs were still low (1,5-NDS-2-NH_2 in 27%, 1,5-NDS in 42%, and 3,6-NDS-1-OH in 71%).

To test the performance of the method in river water samples, the linear range under the optimum conditions was determined. 500 ml a sample, which did not contain any NSs, was spiked with the eight NSs at concentrations between 100 and 5 μg l^{-1} and their recovery examined. A washing step with 20 ml of MeOH was applied. Good linearity was obtained, with a

determination coefficient (r^2) higher than 0.9998 for all compounds. The repeatability for 500 ml of spiked (10 μg l^{-1} of each component) river water, expressed as RSD (n=3), was lower than 11%. Although the lowest level tested was 5 μg l^{-1}, this level could be easily decreased by evaporating the 5 ml of the elution solvent to 500 μl with a stream of nitrogen coupled with the use of a fluorescence detector.

The application of the MIP synthesised using 1-NS as template to selectively extract a group of NSs from river water samples has therefore been demonstrated.

CONCLUSIONS

A non-covalently molecularly imprinted polymer using 1-NS as a template has been synthesised in a mixture of methanol/water (4:1) and applied for the first time to the MISPE of eight NSs. This Aqua-MIP, which shows cross-reactivity, was able to extract selectively the NSs from a mixture of polar compounds, such as phenols, pesticides and naphthalene (apolar compound with a close structure to NSs), when a clean-up step with MeOH was used. 200 mg of the MIP used as SPE material was sufficient to extract 1000 ml of standard solution and 500 ml of river water with high recoveries for all the NS studied even after a clean-up step. This sorbent prepared in aqueous environment has better retention for NSs than most commercially available sorbents. Finally, the method was validated with river water and good linearity and repeatability demonstrated.

Acknowledgements

This work was financially supported by the “Direcció General de Recerca de la Generalitat de Catalunya” (2001 SGR00319). E. Caro would like to thank the Ministerio de Educación, Cultura y Deporte for a pre-doctoral grant AP (2001-2030).

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