Weak anion-exchange mixed-mode materials to selectively extract acidic compounds by stir bar sorptive extraction from environmental waters

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ABSTRACT

In this study, the first example of a polytetrafluoroethylene (PTFE)-based magnet coated with weak anion exchange (WAX) monolith as novel support for stir bar sorptive extraction (SBSE) is presented. Firstly, the PTFE magnets were properly modified and vinylized in order to immobilize polymer monoliths onto its surface. Then, a glycidyl methacrylate monolith was prepared and modified with ethylenediamine (EDA) to create weak anion exchanger via ring opening reaction of epoxy groups. The prepared covalently immobilized EDA-modified monoliths onto PTFE magnet exhibited good stability and reusability. Application of resulting material as stir bar for SBSE was investigated for a series of acidic compounds that includes acesulfame, saccharin, diclofenac or ibuprofen, among others as target compounds. Firstly, the SBSE conditions were optimized to promote the WAX interactions with the target compounds achieving recoveries from 37% to 75% and enable the selective extraction of these compounds as it provided values of % matrix effect from 17% to -13% when they were determined by SBSE followed by liquid chromatography - tandem mass spectrometry. The analytical methodology , was then validated and applied for the determination of the target solutes in environmental water samples, which were found at concentration up to 2500 ng L^{-1} in river waters.

1. Introduction

Sample preparation is a crucial step in any analytical determination, particularly when dealing with trace levels of analytes in complex matrices, because in most cases the direct analysis into the analytical instrument is not possible. Hence, sample preparation plays an important role in the analytical procedure since it cleans the matrix and enriches the target analytes increasing the sensitivity of the method. Sample pretreatment is in continuous growth and constantly novel trends of sample preparation are appearing. Some of the main trends in new extraction techniques involve miniaturization, automation, on-line coupling with analytical instruments and low-cost operation with low level of solvent consumption [1], being microextraction techniques a clear example [2,3]. Microextraction techniques are divided into two groups: liquid-based microextraction techniques, including liquid-phase microextraction (LPME), dispersive liquid-liquid microextraction (DLLME) and hollow fiber liquid-phase microextraction (HF-LPME), among others [2,3]; and sorptive-based microextraction techniques, including solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), fabric phase sorptive extraction (FPSE) or microextraction by packed sorbent (MEPS) [2–6].

Currently, SBSE has been broadly used owing to its versatility in both sampling and desorption modes and its great capacity for quantitative extraction [6]. Different materials are commercially available to be used as coatings for SBSE [6–8]. Polydimethylsiloxane (PDMS) is the most commonly used commercial coating film, but other commercial coatings such as ethylene glycol-silicone (EG-Silicone) and polyacrylate (PA) with a proportion of poly(ethyleneglycol) are discreetly employed. One reason is that they present mechanical and stability weakness, and for this, they are further coated with metal grid to prevent from the material to wear away during extraction. These coatings have been applied for the determination of different type of compounds in different kind of samples [6,9–13]. For instance, Galmiche et al. [10] compared EG-Silicone and PDMS coatings to determine nitroaromatic compounds in water, obtaining better results when using the PDMS coating.

Hence, current research demonstrates the concern of developing in-house synthesized novel materials for SBSE, especially those with improved stability and capable of extracting polar compounds. Various approaches, like sol-gel technology and one pot-polymerization have been used to attain SBSE coatings with high thermal or solvent stabilities [14–18]. For instance, in Wang's study [14], a hydroxyl-containing porous organic framework coating was prepared via sol-gel process and compared to the commercial PDMS and EG-Silicone coatings to retain triazole fungicides in grapes and cabbage samples. Most of the abovementioned SBSE publications use as typical substrate a thin glass jacket with an incorporated magnet core. The glass jacket surface is properly modified (through several stages) to immobilize sorbents onto the stir bar [7]. For instance, PDMS is immobilized by sol-gel process after hydrolysis of the glass surface, whereas for polymeric coatings, the glass surface is first silanized, immersed in the reaction mixture, and subsequently polymerized. In any case, the glass is fragile and can be easily broken during stirring and ultrasonication steps, which undoubtedly affects to the stability and reproducibility of sorbent. In this context, the investigation of other substrates with higher resistance such as commercial polytetrafluoroethylene (PTFE) magnetic stir bars is highly desirable. PTFE is a chemical resistant material with poor adhesion properties; which can be properly treated by plasma [19], electron beam [20] and chemical etching [21,22] to improve its adhesion and wettability surface. However, to the best of our knowledge, any work has been reported that attach the SBSE coatings onto PTFE.

In last years, a new generation of materials was introduced to achieve the selective extraction of ionic compounds: the mixed-mode ion-exchange materials. They were developed by introducing ionic moieties into a polymeric or silica backbone to specifically interact with ionizable compounds by ion-exchange groups (amines for anion exchange, and carboxylic acids and sulfonic acids for cation exchange) maintaining the reversed-phase interactions. Thus, four main groups exist: strong anion-exchange (SAX), strong cation-exchange (SCX), weak anion-exchange (WAX) and weak cation-exchange (WCX) [23–25]. So far, these mixed-mode ion-exchange materials have been mainly developed both commercially and in-house prepared as sorbents for solid-phase extraction (SPE). Nevertheless, recently, in-house materials for other sorptive techniques such as SPME or SBSE have been also introduced [26]. For instance, Yao et al. [25] achieved great retention for perfluoroalkyl acids using an in-house mixed-mode ion-exchange stir bar, a poly(1-vinylimidazole-ethyleneglycol dimethacrylate) (poly(VI-EDMA)) monolith material. Successful recoveries were achieved with the novel material when determining the acidic analytes in surface water samples. So far, most of the in-house ion-exchange mixed-mode materials for SBSE are strong exchangers with quaternary amines or sulfonic acids as functional groups [25–28].

In the present study, a novel SBSE device based on the use of PTFE as support containing mixed-mode WAX materials is developed. A chemical modification of PTFE support was first accomplished to guarantee a covalent bonding of the sorbent to the surface of PTFE coated magnet. Then, two WAX monolithic materials (EDA1 and EDA2), were prepared by thermal polymerization from glycidyl methacrylate (GMA)-based monolith (as starting material) and modified with ethylenediamine (EDA) to obtain weak anion-exchanger functionalities. A proof-of-concept application of this novel SBSE system is presented here for the extraction of a group of acidic compounds in environmental samples followed their determination by liquid chromatography with mass spectrometry in tandem (LC-MS/MS). To the best of our knowledge, this is the first time that anion exchange monoliths chemically bonded to PTFE-coated magnets have been developed for SBSE purposes.

2. Experimental

2.1 Reagents and standards

PTFE-coated stirring bar (15 mm length × 1.5 mm diameter) were obtained from VWR International Eurolab (Barcelona, Spain). Sodium naphthalene solution (FluoroEtch®) for the treatment of PTFE stir bar surface was provided by Acton Technologies (County Limerick, Ireland). Glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EDMA) were from Sigma-Aldrich (Steinheim, Germany). Triethylamine (TEA), lauroyl peroxide (LPO), cyclohexanol and 1-dodecanol were from Alfa-Aesar (Karlsrube, Germany), and N,N-dimethylformamide (DMF) and ethylenediamine (EDA) from VWR Chemicals (Fontenay Sous Bois, France). Fluorinated ethylene-propylene (FEP) tubing (3.60 × 6.00 mm) was provided by Bohlender GmbH (Grünsfeld, Germany).

For the SBSE evaluation, seven analytes with acidic properties were selected for this study as model compounds including artificial sweeteners, illicit drugs, pharmaceuticals and metabolites. Moreover, at initial stages, seven basic analytes were added to the previous acidic ones to evaluate the anion-exchange properties of the materials. Potassium acesulfame (ACE), atenolol (ATE), clofibric acid (CLO AC) (a metabolite of clofibrate), diclofenac (DICLO), fenoprofen (FEN), ibuprofen (IBP), methadone (MET), metoprolol tartrate salt (MTP), naproxen (NAP), propranolol (PROP), ranitidine (RAN), saccharin (SAC) and trimethoprim (TRI) were purchased as pure standards from Sigma-Aldrich. Mephedrone hydrochloride (MEP) was supplied by LGC Standards

(Luckenwalde, Germany). All standards were of a purity higher than 99%. **Table 1** shows the compounds selected and their pK_a values.

Stock solutions of individual standards at 1000 mg L^{-1} were prepared in MeOH and stored at -20°C. Working solutions of a mixture of all compounds were prepared weekly in ultrapure water: MeOH (50:50, v/v) and were stored at 4°C in the dark. Ultrapure water was provided by a Synergy UV water purification system (Merck Millipore, Burlington, MA, United States) and MeOH and ACN of HPLC-grade were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid (HCOOH) and ammonium hydroxide (NH_4OH) from Sigma-Aldrich and hydrochloric acid (HCl) from Scharlab (Barcelona, Spain) were used to adjust the mobile phase and the solutions for the SPE.

2.2 Preparation of monolithic coatings in PTFE magnets for SBSE

PTFE surface of the micro stirring bar was activated with FluoroEtch® following the operational procedure recommended by the supplier [29] with small modifications. Briefly, the stir bar was immersed in the FluoroEtch® solution and heated at 60°C under nitrogen stream for 30 min. Modified magnets were sequentially washed with MeOH, water and 1% acetic acid solution at 65ºC and finally dried in an oven for 2 h.

Surface of treated stir bars was vinylized in order to provide the appropriate anchorage of the polymer over the surface. Vinylization solution consisted of GMA 2 M in DMF, containing TEA 5 mM, and adjusted to pH 8 with HCl [30]. Next, stir bars were immersed in the vinylization solution for 2 h under stirring at 60°C. Vinylized magnets were then washed with acetone and dried in an oven for 2 h.

The selected polymerization mixture was composed of 32 wt% GMA as monomer, 8 wt% EDMA as cross-linker, 55.7 wt% cyclohexanol and 4.3 wt% 1-dodecanol as pore-forming solvents and 1 wt% (respect of total monomers amount) of LPO initiator. The mixture was sonicated for 3 min and next purged with nitrogen for 10 min.

A FEP tube was designed as a mold for the polymerization reaction, being one of its ends sealed. Then, the polymerization mixture was introduced into the FEP mold with the pretreated magnet vertically placed in the center of the mold with the aid of two plastic protectors (**Fig. S1**). The mold with the magnet inside was vertically placed in an oven, and polymerization was carried out at 70 ºC for 24 h. Afterwards, the FEP tube was cut in order to release the stir bar coated with the methacrylate polymer, which was sequentially washed with methanol and water. The thickness of the obtained polymer monolith coating was calculated to be about 1 mm. The resulting magnet was then closed its ends by two plastic protectors.

Functionalization of the GMA-based monoliths with EDA was carried out following two protocols reported in the literature. The first procedure was adapted from Gonçalves *et al.* [31,32] and resulted in EDA1. Briefly, the magnets coated with polymer were immersed in carbonate buffer 0.05 M at pH 9.5, stirred and then treated with 0.5 M EDA solution prepared in the same buffer. The mixture was stirred for 2 h, left at rest for 30 min, and stirred again 2 h. To prepare the second material (named as EDA2), the GMA-based monolith was chemically modified with EDA according to the procedure previously described [33]. The magnet with the polymeric matrix was treated with a 50 wt% aqueous EDA solution at 60ºC for 24 h. Boh materials (EDA1 and EDA2) were washed with distilled water until the washing solution reached neutral pH.

2.3. Material characterization

The morphology of the materials was characterized using a scattering electron microscope (S-4800, Hitachi, Ibaraki, Japan). Elemental analysis of the synthesized materials was done with an EA 1110 CHNS elemental analyzer (CE Instruments, Milan, Italy). Attenuated total reflection Fourier-transform infrared (FT-IR) spectra of materials were acquired with a DuraSamplIR II auxiliary from Smiths Detection Inc. (www.smithsdetection.com, Warrington, UK). The instrument was equipped with a nine reflection diamond/ZnSe DuraDisk plate, connected to a model Tensor 27 Bruker FT-IR spectrometer (Bremen, Germany).

2.4 SBSE conditions

The stir bars were first conditioned placing them in a 25 mL glass vial with 10 mL of MeOH for 5 min followed by 10 mL of ultrapure water adjusted to the same pH as the sample for another 5 min. The sample volume was set at 10 mL adjusted at pH 6 and it was extracted for 180 min at 600 rpm. Then, the samples were desorbed by liquid desorption (LD) in ultrasonic bath using 3 mL of 5% NH_4OH in ACN for 20 min. Afterwards, the elution solution was introduced to a centrifuge evaporator miVac Duo (Genevac, Ipswich, UK) to evaporate the extract to dryness and subsequently it was reconstituted with 1 mL of mobile phase (ultrapure water adjusted at pH 2.8 with HCOOH/ACN; 90/10; v/v). Before injecting to the chromatographic system, all fractions were filtered with 0.45 µm PTFE syringe filters (Scharlab). After each use, the stir bars were cleaned two times with the corresponding elution solution and two more times with MeOH in the ultrasonic bath for 10 min each, and kept in an Eppendorf tube with ultrapure water until the next experiment.

River water from Ebre River was the sample selected to evaluate the stir bars. Water samples were stored at -20 °C until analysis, when they were filtered through a 0.45 µm nylon membrane filter (Fisherbrand, Loughborough, UK).

2.5. Chromatographic conditions

The optimization of SBSE parameters was performed using an Agilent 1200 UHPLC equipped with a binary pump, an autosampler (Agilent, Waldbronn, Germany), an oven and a diode-array detector. The selected mobile phase was a mixture of ultrapure water adjusted at pH 2.8 with HCl (solvent A) and ACN (solvent B). A Tracer Excel 120 C_8 (150 mm × 4.6 mm i.d., 5 μm particle size) supplied by Teknokroma (Sant Cugat del Vallès, Spain) was used as the chromatographic column, and it was maintained at 30 °C. The mobile phase flow rate was 600 μL min^{-1} and the injection volume was 20 μL. The gradient profile started with 10% of B, which was raised to 40% B within 12 min, and then to 100% B within 16 min. Subsequently, it was held at 100% B for 3 min before returning to the initial conditions in 3 min. The signal was measured at 210 nm for all the compounds.

Once the SBSE procedure was optimized, the same chromatographic conditions except that the aqueous mobile phase was adjusted at pH 2.8 using HCOOH instead of HCl were used in LC-MS/MS for the validation and analysis of river water samples using an Agilent model 1200 series LC coupled with a 6460 QqQ mass spectrometer (MS/MS) detector. The LC system was equipped with an autosampler, a degasser, an oven and a quaternary pump. Electrospray ionization (ESI), working in negative mode, was the ionization selected in the mass spectrometer for the acidic compounds (ACE, SAC, CLO AC, FEN, DICLO, IBP, NAP). The optimal parameters for the acquisition of the acidic compounds were as follows: a fragmentor voltage of 75 V, a collision energy between 5 and 28 eV (**Table S1**), a source gas temperature of 350ºC, a nitrogen flow rate of 12 L min^{-1}, a nebulizer pressure of 25 psi and a capillary voltage of 3000 V. A precursor ion and two product ions were selected for each analyte. The most abundant transition was measured for quantification and the other transitions and its corresponding ion ratios were used for confirmation purposes in multiple reaction monitoring (MRM) mode (**Table S1**).

2.6 Validation

Instrumental linearity was plotted to interpolate concentration and calculate recoveries of the extraction procedure. The selected compounds offered good linearity (r^2=0.9983) in LC-MS/MS and the linear ranges were between 0.1 and 50 µg L^{-1} for ACE, CLO AC and IBP, between 0.1 and 100 µg L^{-1} for DICLO, 1 and 50 µg L^{-1} for NAP and FEN and between 5 and 100 µg L^{-1} for SAC. The LODs were 0.01 µg L^{-1} for CLO AC and DICLO, 0.075 µg L^{-1} for ACE, FEN and IBP, and 0.25 µg L^{-1} for SAC and NAP.

The apparent recovery (%R_{app}) was calculated as the concentration of each analyte when the sample was spiked at a concentration of 10 µg L^{-1} with the analyte mixture at the beginning of the analysis (before SPE) compared with the theoretical concentration. A non-spiked river sample was analyzed, and the signal obtained was subtracted from the signal of the spiked sample.

The %ME was calculated as the concentration of each analyte when the sample was spiked at a concentration of 10 µg L^{-1} with the analyte mixture after SPE, and it was obtained from the following formula $\%ME = (C_{exp}/C_{theo} \times 100\%) - 100\%$, where the C_{exp} is the concentration obtained from the calibration curve and the C_{theo} is the theoretical concentration. Depending on the result of %ME obtained, it can be signal enhancement or suppression, if it is an increment or a decrease on the signal, respectively. All the concentrations were calculated using the instrumental calibration curve.

Linearity was evaluated by matrix-matched calibration curve using 10 mL of river sample spiked with the analyte mixture at seven different concentration levels. Method detection limits (MDLs) correspond to the concentration whose signal-to-noise (S/N) ratio is greater than 3. Method quantification limits (MQLs) were defined as the lowest point of the matrix-matched calibration curve with $S/N \geq 10$.

The repeatability of the method on the same day and reproducibility between days was expressed as relative standard deviation (%RSD) of five replicates of river sample spiked at a concentration level of 10 µg L^{-1}.

3. Results and discussion

3.1. Modification of PTFE magnets, preparation of polymer monolith coated stir bar and characterization

As we mentioned in the Introduction, PTFE-based materials are well-known for their excellent thermal stability and chemical resistance. In this context, several treatments have been made to improve their adhesion to other molecules and materials. Wet chemical etching of PTFE by sodium naphthalene solution (Fluoroetch®) is a direct way to increase its ability to bond with other polymers or metals [21,22,34]. Indeed, our research group has successfully modified PTFE tubing [30] and disks [35] to prepare monolithic stationary phases for microbore HPLC and molecularly imprinted polymers, respectively. Inspired by these works, the PTFE stir bars were treated with this etchant solution in order to modify their surface through the conversion of C-F bonds into C-H, C-OH, and -COOH functional groups [30]. The introduced -OH groups into PTFE surface of magnet allowed its reaction with the epoxide groups of GMA, thus providing a vinylized surface. The presence of these vinyl moieties is essential since they allowed the later covalent binding of the polymer monolith to the PTFE surface during the polymerization step. **Figure S2** shows images of a bare and vinylized PTFE stir bar and their corresponding FTIR spectra. As it can be observed, the chemical treatment with Fluoroetch® turned the PTFE magnet from white to dark brown. Besides, the bare PTFE magnet (**Fig. S2A**) gave the characteristic absorption bands of C-F bonds (from 1100 to 1300 cm^{-1}), whereas the FT-IR spectra of vinylized PTFE surface (previously treated with Fluoroetch®) (**Fig. S2B**) showed a new broad absorption band at 3300 cm^{-1} (due to the OH group) jointly with the appearance of absorption bands at 1600-1700 cm^{-1} attributable to vinyl groups.

Once described the modification method of PTFE magnet to assure a chemical anchoring of the monolith to its surface, several polymerization mixtures were tested in order to obtain a polymer with suitable properties, that is, an adequate hardness and consistency without causing crushing after drying and good adhesion to the magnet surface. To reach this goal, initial polymerization conditions were as follows: 25 wt% monomers (20 wt% GMA and 5 wt% EDMA) and 75 wt% (70 wt% cyclohexanol and 5 wt% 1-dodecanol), in the presence of 1 wt% (respect monomers) of LPO, and polymerization was carried out at 70 ºC for 24 h. However, the resulting polymer turned out to be too soft and the adherence to the surface magnet was limited, detaching easily. In order to obtain a rigid and stable polymer monolith, the monomers/porogens ratio was investigated within the following proportions: 30/70% (wt/wt), 40/60% (wt/wt) and 50/50% (wt/wt). As a result of this study, a 40/60% (wt/wt) ratio was selected, since it provided the best compromise between permeability and mechanical resistance.

To obtain a weak anion-exchange material, the selected bare monolith (containing reactive epoxide groups) was modified with EDA to generate monoliths with amine groups (**Figure 1**). For this purpose, different procedures adapted from the literature were assayed (see Section 2.2). Thus, the amination process of GMA-based monolithic magnet was firstly carried out using mild conditions (0.5 M EDA in carbonate buffer (pH 9.5) at room temperature for 4 h) [31,32]. Elemental analysis of the resulting material (EDA1) gave a nitrogen content of 0.3 wt%. Alternatively, in the second protocol tested [33], larger amine concentration (8.3 M in water) and high reaction temperature (80ºC for 24 h) were used. However, the use of this temperature affected seriously the anchoring of the polymer coating to the magnet. In order to achieve a robust coating stability and a proper EDA amount onto the polymer, the influence of reaction temperature was evaluated. When temperature rose from 25°C to 60°C, the nitrogen content increased from 4.0 to 6.2 wt%, and the polymer coating on magnet showed good stability. Therefore, this latter temperature was selected to prepare the second weak anion-exchange material (EDA2) for SBSE purposes.

The resulting materials were also characterized by SEM to get information of their morphology. **Figure 2** shows a representative image of an EDA-modified monolith immobilized onto PTFE magnet and its corresponding SEM micrograph. The morphology of the EDA-modified monolith resembled the typical microglobular structure of polymethacrylate monoliths, with large-through pores, which is beneficial for permeability and favorable mass transfer in extraction applications. Similar morphological structure was observed for both EDA1 and EDA2 monoliths, although this latter material showed higher nitrogen content present in the polymeric matrix.

The successful preparation of GMA-based monoliths onto PTFE magnets and their functionalization with EDA was also confirmed by FT-IR (**Fig. 3**). As shown in **Fig. 3A**, the coating with GMA-based monolith presented the characteristic absorptions of C=O groups at ~1726 cm^{-1}, and the C–O–C vibrations in esters around 1240 and 1150 cm^{-1}, whereas the C-F characteristic peaks of PTFE surface were not evidenced. After further amino-functionalization, the characteristic peaks of –NH bending around 1500-1650 cm^{-1} and the bands associated with stretching of amines in the spectral region 3100-3450 cm^{-1} appeared (**Fig. 3B**). These data corroborated that the epoxy moieties of GMA-based polymer were successfully functionalized with the amino groups via ring-opening reaction.

3.2. Optimization of the SBSE procedure

To evaluate the performance of the stir bars, different steps of the SBSE protocol were optimized, such as the loading pH and volume, extraction time, desorption solvent, volume and time, to achieve a satisfactory retention of the model compounds selected. The materials contained amine moieties, so they behave as weak anion-exchanger, and are in ionic state at acidic or neutral pH. Regarding the model compounds, analytes with acidic character were selected. The basic compounds were firstly included to evaluate the selectivity of the SBSE towards the acidic compounds. Hence, careful attention was paid to the elucidation of the retention mechanisms present during the extraction for each acidic analyte, being the pK_a values of the analytes selected an important factor (**Table S1)**.

The starting conditions were selected based on previous studies [36,37], where similar analytes were determined in environmental waters and are the following: 10 mL of ultrapure water adjusted to pH 5 spiked at 750 µg L^{-1} with the mixture of analytes, stirred at 600 rpm for 180 min. The elution was carried out with two consecutive elution solutions of 3 mL of 5% HCOOH in MeOH in ultrasonic bath for 10 min. In order to enhance the sensitivity of the method, the elution extract was evaporated to dryness and redissolved with 1 mL of mobile phase. It should be mentioned that no losses of the analytes were observed during the evaporation step. Under these conditions, both stir-bars (EDA1 and EDA2) were evaluated. The results indicated that the basic analytes were completely lost during the loading step, while the acidic ones were recovered (recoveries between 5% and 26% in both materials). Therefore, for the further extraction optimization only the acidic analytes were determined. Under these conditions, the results of both sorbents were compared and EDA2 was the sorbent that showed the best results, since its EDA functionalization was higher and consequently contained more amine functional groups as mentioned in the previous section. Going forward, the EDA2 was further optimized. **Table 1** shows the recoveries attained with EDA2 for acidic compounds using these starting conditions.

3.2.1. Sample pH

The first parameter to optimize was the sample pH. The coating should be charged at low and neutral pH values since it presents a WAX character. All the acidic analytes should be in ionic state at a pH above their pK_a, thus pH 6 and 7 were also tested and compared to pH 5. As can be seen in **Fig. 4**, all compounds attained slightly better recoveries at pH 6, except NAP, whose recoveries kept constant. For instance, CLO AC and DICLO get recoveries of 24% and 21% at pH 6, respectively, while at pH 5 the recoveries were 20% and 14%, respectively. Moreover, at pH 7 the recoveries were slightly lower presumably because at this pH the amine functional groups of the coating might not be at ionic form. Therefore, pH 6 was selected as the optimal loading pH for the following experiments.

3.2.2. Extraction time

The next parameter studied was the extraction time. Different extraction times ranging from 20 min to 360 min were evaluated. As shown in **Figure 3S**, recoveries of most of the analytes kept increasing till 180 min, but the retention of all the compounds did not increase from 180 min to 360 min. Therefore, 180 min was chosen as the optimal extraction time for the following analysis.

Larger extraction times (up to 6 h) were reported in studies where similar drugs were SBSE using a polyurethane [38] and polydimethylsiloxane [13,38], EG-silicone [13] and PA [13] phases.

3.2.3 Desorption conditions

Desorption parameters such as type and volume of desorption solvent, and desorption time, were evaluated to get an effective desorption of the selected analytes. Firstly, different solvents, MeOH and ACN, with different amounts of acid and base (5% and 10%) of NH_4OH or HCOOH were tested. **Fig. 5** shows the recoveries obtained for the acidic analytes at different desorption conditions.

When using 5% NH_4OH in MeOH as elution solvent, the base neutralises the amine moieties present in the coating, causing the disruption of the interaction between analytes and the coating. When the elution is performed with 2 x 3 mL of 5% NH_4OH in MeOH, the recoveries of most of the compounds slightly dropped as compared to when using 2 x 3 mL of 5%HCOOH in MeOH, except for ACE, SAC and NAP, which kept constant (**Fig. 5**). Next, experiments using acidified and basified ACN instead of MeOH were performed, where the percentage of acidic or basic additive was also increased. When increasing from 5% to 10% of HCOOH in ACN, all the analytes showed an increase in their recoveries. For instance, DICLO and NAP attained recoveries of 27% and 28%, respectively, whereas with 5% of HCOOH the results for the same compounds were 17% and 22%, respectively. Nevertheless, the largest increase was for ACE and SAC, whose recoveries increased from 21% and 22%, respectively when using 5% HCOOH to 38% and 37%, when using 10% HCOOH. Using NH_4OH instead of HCOOH in ACN still caused a greater improvement in the recoveries of ACE and SAC, reaching recoveries of 71% and 67%, respectively (**Fig. 5**). It should be mentioned that these strong acidic compounds are challenging to extract as reported in previous studies [36,39,40]. An increase in the amount of NH_4OH in ACN to 10% did not lead to a rise in the recoveries of all the compounds. Thus, 2 consecutive elution solutions of 3 mL ACN containing 5% NH_4OH were selected as desorption solutions. It should be mentioned that similar elution conditions were used in studes [25,36] where coatings with strong anionic-exchange character were evaluated to retain a group of acidic compounds.

In addition to the starting elution volume of 2x3 mL, the elution volume was raised to 2x5 mL with the purpose of eluting all the acidic compounds with only one elution instead of two. The recoveries for all the compounds did not increase when raising the desorption volume from 2x3 mL to 2x5 mL and one single elution of 5 mL was not enough to completely elute them. In addition, volumes lower than 3 mL were not tested since they did not properly cover the stir bar. Therefore, 2x3 mL was chosen as the optimal desorption volume since the posterior evaporation time to dryness decreases when using less desorption volume.

The desorption time was increased, and 20 min as desorption time was tested. When rising from 10 min to 20 min of desorption time in the ultrasonic bath, the results significantly improved specially for the less polar compounds and recoveries between 37% and 75% were attained, as can be seen in **Table 1**. In addition, only one fraction of elution solvent was enough to achieve a complete elution of all analytes in 20 min. Hence, one elution solution of 20 min was selected as the optimal extraction time.

3.2.4. Sample volume

The following parameter tested was the sample volume. To extract a higher volume, 25 mL of sample was tested. Using 25 mL as the loading volume, the extraction recoveries decreased from 37-75% to 16-32%. Consequently, 10 mL was selected as the loading volume for the further experiments.

Comparable results were obtained to other studies that determine the same analytes. In a previous study [36], the capsule-phase microextraction (CPME) technique was employed to determine model acidic and basic compounds in environmental samples. The materials used include two magnetic bars, one with C_{18} and SAX character and the other with C_{18} and SCX character to selectively retain acidic and basic compounds, respectively. For instance, when using the SAX magnetic bar with the optimal conditions (25 mL of sample volume) in ultrapure water, recoveries of 46% and 50% were reported for ACE and SAC in the previous study, while in this study higher recoveries were obtained for these analytes, 74% and 75%, respectively. For CLO AC, FEN, DICLO, NAP and IBP, lower recoveries were obtained in this study, between 37% and 63%, compared to the previous one, between 60% and 80%. In addition, slightly higher recovery (%R 45.3 – 90.6) results were attained in a study where the polyurethane phase was used to SBSE similar acidic drugs; however, the extraction time required was longer [38]. It should take into account that SBSE is a non-exhaustive technique, and typically, not high recoveries are expected. Nevertheless, we consider to maintain this acceptable recoveries with improved selectivity in the present study.

3.2.5. Stability/reusability of the stir bar coating

Once the best extraction conditions were found, the stability of the stir bar was also investigated in the aspect of consecutive use. That is, the extraction device was treated with stirring, ultrasonication and solvents, respectively. The results of this study showed no obvious losses of the monolith layer were observed for all of the solvents and conditions tested. Besides, covalent bonding of polymer monolith on PTFE surface was stable enough to be used in SBSE.

Moreover, the reusability of the PTFE-based magnet coated with WAX monolith was carried out using the recommended SBSE protocol (see Section 2.3). It was checked that the developed sorbent could be reused at least 25 times without significant decrease of extraction efficiency. This reusability is within the reported for similar monolithic stir-bars [7].

Other additional strength of the developed sorbent is the possibility of manufacturing several stirring units simultaneously (ca. 42 in 36 h) at the low cost (0.6 €/device), which undoubtedly makes this protocol economically attractive and potentially feasible to be commercialized.

Once the SBSE procedure has been optimized, the optimal conditions for the SBSE protocol were fixed as follows: 10 mL of ultrapure water adjusted to pH 6 stirred at 600 rpm for 180 min; elution with 3 mL of 5% NH_4OH in ACN in the ultrasonic bath for 20 min. The elution extract was evaporated to dryness and reconstituted with 1 mL of mobile phase. Although the preconcentration effect is not high, great selectivity is displayed from the coating towards the selected acidic compounds. Going forward, the method was further validated and applied to environmental samples.

3.3. Method validation

The optimized method was validated with the parameters described in section 2.6 by SBSE and LC-MS/MS using river water after the extraction parameters were optimized. The apparent recovery (%R_{app}), and the matrix effect (%ME), as well as linearity, MDLs and MQLs and the repeatability and reproducibility were evaluated. A non-spiked river sample was analyzed, and the signal obtained was subtracted from the signal of the spiked sample. The %R_{app}, %ME, MDLs and MQLs obtained for river samples are shown in **Table 2**. **Figure S4** shows chromatograms of when 10 mL of river water samples spiked at 10 µg L^{-1} with the analyte mixture were analyzed using the developed method. The %R_{app} of the analytes in river water ranged between 30% and 47%, except for NAP, whose %R_{app} was 20%. The %R_{app} obtained in the sample decreased from the values obtained during optimization due to the complexity of the matrix analyzed. Moreover, as pointed, SBSE is a non-exhaustive technique and it is not usual to attain complete recoveries. It should be highlighted, however, that the %ME in the river water analyzed was below 20%, which denoted the high selectivity exploited in the developed SBSE phase. In detail, the % ME is in form of ion suppression in most of the compounds, ranging from -3% to -15%, except SAC and DICLO, which presented ion enhancement with %ME values of 3% and 17%, respectively, and IBP, with no %ME. Since the %ME of all the analytes was low, a washing step was not introduced and demonstrated the selectivity of the material. Larger %ME were attained in previous studies which determine the same analytes in river water from the same Ebre River, using mixed-mode ion-exchange materials by other techniques, such as SPE [41] and CPME [36], and materials based on hypercrosslinked magnetic particles by d-SPE [39]. For instance, Salas et al. [41] reported %ME between -21% and -41% when determining some of the same compounds determined in this study in 100 mL Ebre River water using a combination of a SCX/SAX commercial sorbent by SPE, whereas %ME between 17% and -13% were reported in this study.

Curves were plotted and the linear range of matrix-matched calibration was from MQLs to 35 µg L^{-1} for all the compounds, except CLO AC, FEN and DICLO, whose linear range went from MQLs to 10 µg L^{-1}. Linearity was good for all the compounds ($R^2 \geq 0.9902$), except FEN (R^2=0.9629). In river sample, the MDLs ranged between 5 ng L^{-1} and 25 ng L^{-1}, except SAC and IBP, whose MDLs were 1000 ng L^{-1} and 250 ng L^{-1}; and the MQLs ranged from 25 ng L^{-1} to 2000 ng L^{-1}. The repeatability and reproducibility (%RSD, n=5) were lower than 9% and 12%, respectively.

3.4. Analysis of samples

The optimized SBSE method was applied to determine the selected acidic analytes in Ebre River water, and three different samples of river water were analyzed in triplicate. To confirm the presence of the analytes, apart from the confirmation transition, the retention time and the ion ratio were considered. The concentration levels of the compounds found in the sample are presented in **Table 3**. In the Ebre River sample, most of the acidic compounds were detected, except ACE, SAC and NAP in two of the three samples analyzed. CLO AC and IBP were the analytes found at higher concentration levels, precisely from 575 ng L^{-1} to 1719 ng L^{-1} for CLO AC and from 737 ng L^{-1} to 2446 ng L^{-1} for IBP. DICLO was found below the MQLs in all the samples analyzed. The concentration levels found for the acidic analytes selected in this study are similar to those reported in other studies [42–46]. Oliveira et al. [42] reported concentrations of 0.1-3110 ng L^{-1} for IBP in different river waters, while 737-2446 ng L^{-1} were the concentration levels found in this study. Nevertheless, for DICLO and NAP low concentration levels were found in river water in other studies [43–46]. For instance, in Guadalquivir river DICLO was reported below the MQLs (22 ng L^{-1}) [44] and NAP was also found at low concentration levels in the same Ebre river as in this study [43].

4. Conclusions

In this work, the covalent attachment of polymer monoliths onto commercial PTFE-based magnets for SBSE was successfully achieved to further functionalize the epoxy-based monoliths with EDA and create two different WAX materials, EDA1 and EDA2. Additionally, the covalent immobilization of polymer monoliths on PTFE-based magnets is simple and reproducible, and the immobilized phases could be used as excellent platforms to generate novel desired surface functionalities on demand, which undoubtedly would expand the application field of SBSE.

Both materials were applied as coatings in the SBSE after the optimization of the extraction conditions. From both coatings, the EDA2 with larger nitrogen content (i.e. ion-exchange capacity) performed better during extraction since it provided higher extraction efficiency and selectivity for the acidic compounds. The selection of the optimal SBSE parameters was critical in the extraction recoveries of the selected model compounds.

The developed SBSE method is simple and very selective, with encouraging application in trace analysis in environmental samples, such as river water. In fact, during analysis of river water samples the matrix effect found was very low which was attributed to the selectivity of the coating material. The novel stir bars using the suggested SBSE method could be extended to extract other acidic compounds in different kinds of samples in the future.

Acknowledgments

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Table 1. %R obtained with the EDA2 material for the acidic compounds when using the starting and optimal conditions. For the starting conditions, the %R were taken as the sum of the recoveries of the 2 fractions of the elution step.

				Starting conditions	Optimal conditions
			pK_a	%R	%R
Acidic	Strong	ACE	-0.3	8	74
		SAC	1.6	9	75
	Weak	CLO AC	3.4	20	50
		FEN	4.0	18	44
		DICLO	4.0	14	63
		NAP	4.2	22	37
		IBP	4.9	26	45

% RSD (n=5) <10% for %R>20%.

Table 2. $\%R_{app}$, %ME, MDLs and MQLs of the selected compounds in river water analyzed with the EDA2 material by SBSE.

	River water				
	pK_a	$\%R_{app}$[a, b]	%ME[a]	MDLs (ng L^{-1})	MQLs (ng L^{-1})
ACE	-0.3	43	-7	25	200
SAC	1.6	47	3	1000	2000
CLO AC	3.37	37	-3	25	500
FEN	3.96	31	-13	10	25
DICLO	4.00	47	17	5	25
NAP	4.19	20	-15	5	25
IBP	4.85	30	0	250	500

[a] spiked at 10 μg L^{-1}.

[b] % RSD (n=5) <9%.

Table 3. Concentration levels in ng L^{-1} of the model analytes selected in river water.

	Concentration (ng L^{-1})
	River water
ACE	n.d.
SAC	n.d.
CLO AC	575-1719
FEN	228-407
DICLO	<MQL
NAP	<MQL
IBP	737-2446

Figure captions

Fig. 1.Scheme of preparation of GMA-based monoliths in PTFE magnets and their functionalization with EDA.

Fig. 2. Photograph of PTFE magnet coated with EDA-modified monolith and its corresponding SEM micrograph (at 5000 × magnification).

Fig. 3. FTIR spectra of: (A) GMA-based monolith and (B) EDA-modified monolith immobilized onto treated PTFE magnets.

Fig. 4. Effect of the pH on the acidic analytes when using the EDA2 material for SBSE.

Fig. 5. %R obtained with the EDA2 material when 2x3 mL elution solutions were tested.

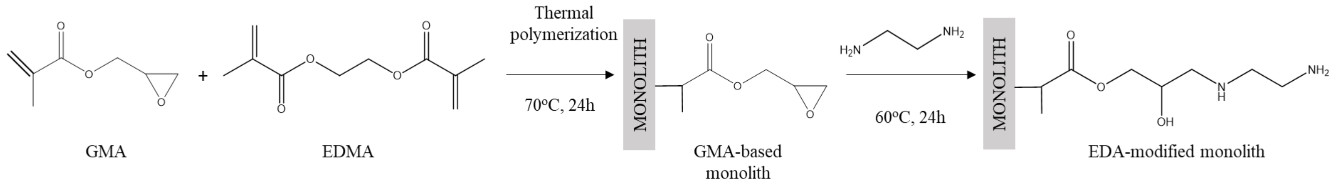
GMA
EDMA
Thermal polymerization
70ºC, 24h
MONOLITH
GMA-based monolith
H₂N
NH₂
60ºC, 24h
MONOLITH
OH
N H
NH₂
EDA-modified monolith

Fig. 1

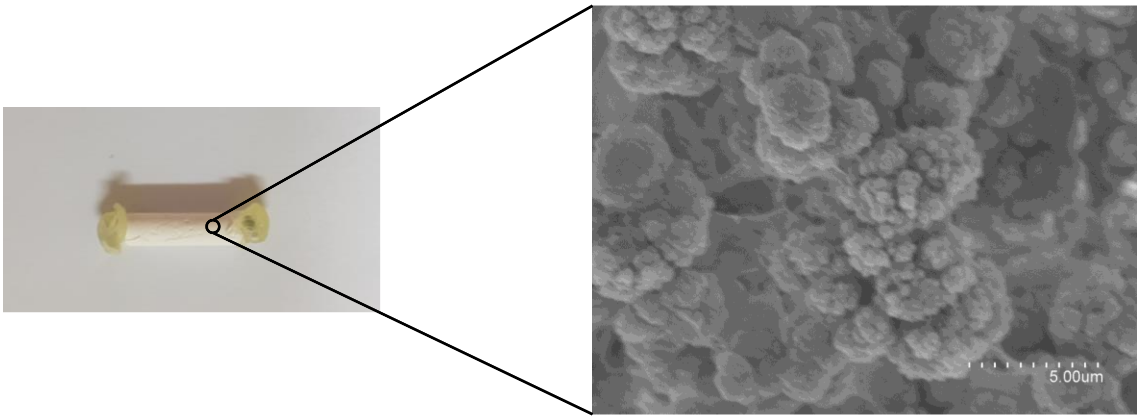

Fig. 2

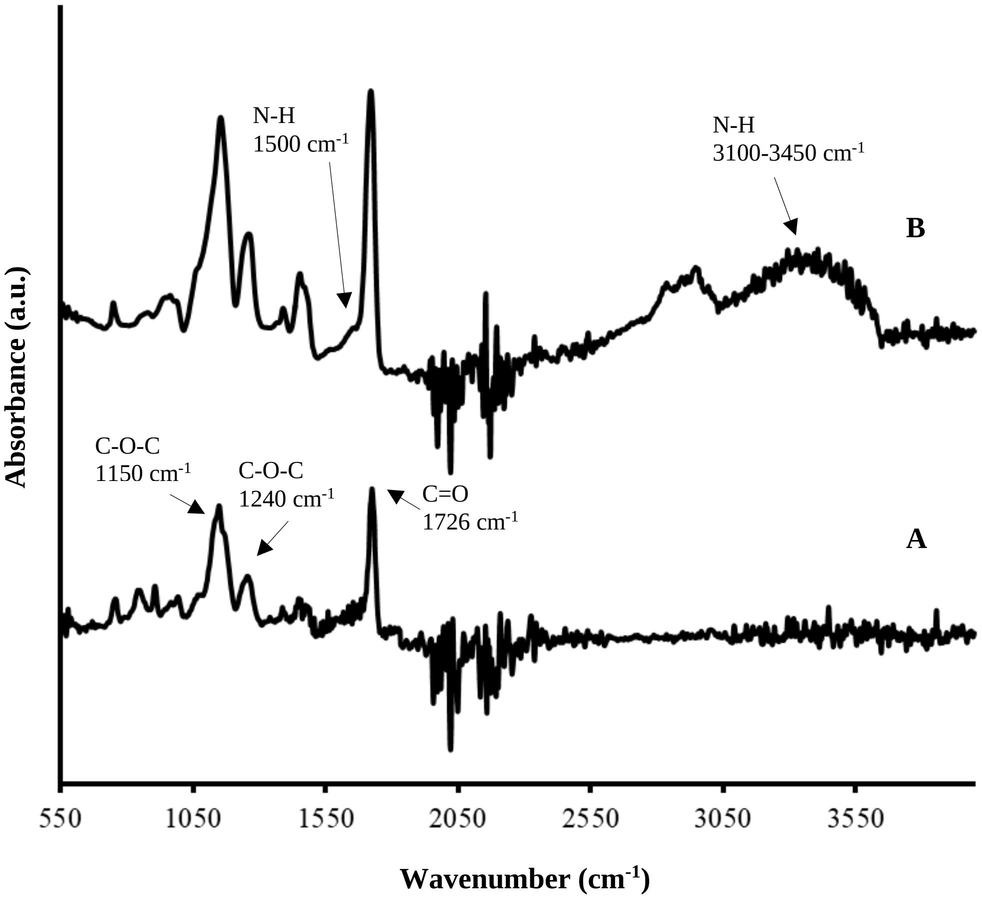
Absorbance (a.u.)
N-H
1500 cm-1
N-H
3100-3450 cm-1
B
C-O-C
1150 cm-1
C-O-C
1240 cm-1
C=O
1726 cm-1
A
550
1050
1550
2050
2550
3050
3550
Wavenumber (cm-1)

Fig. 3

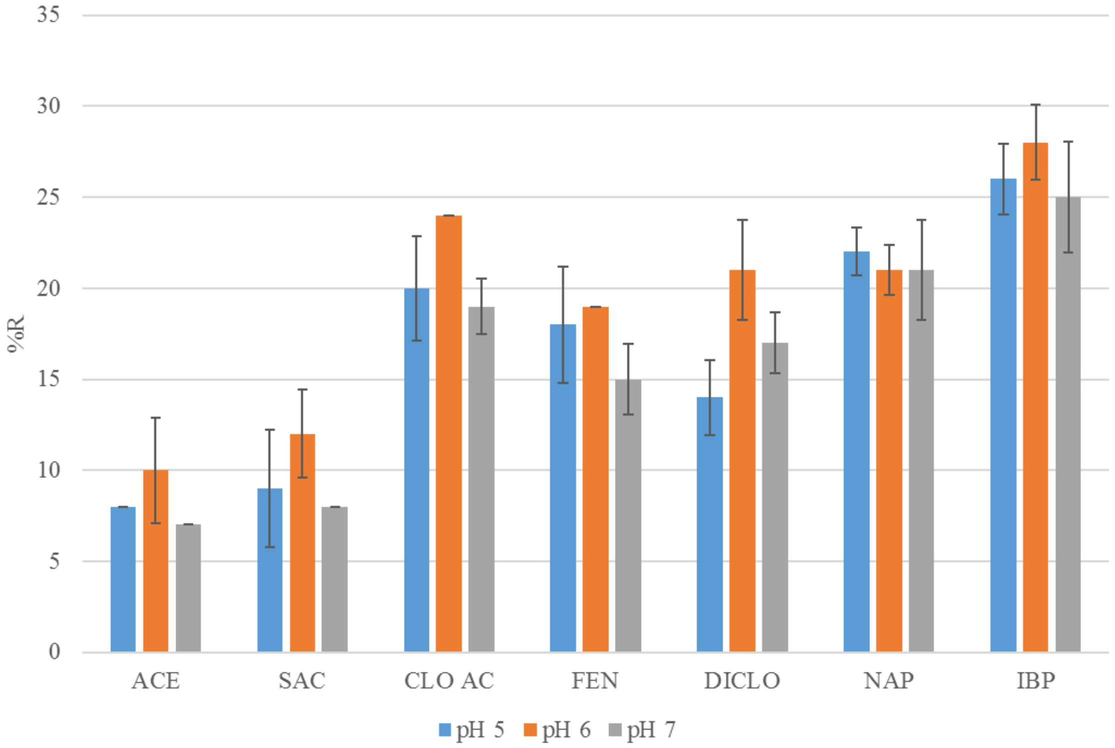

Fig. 4

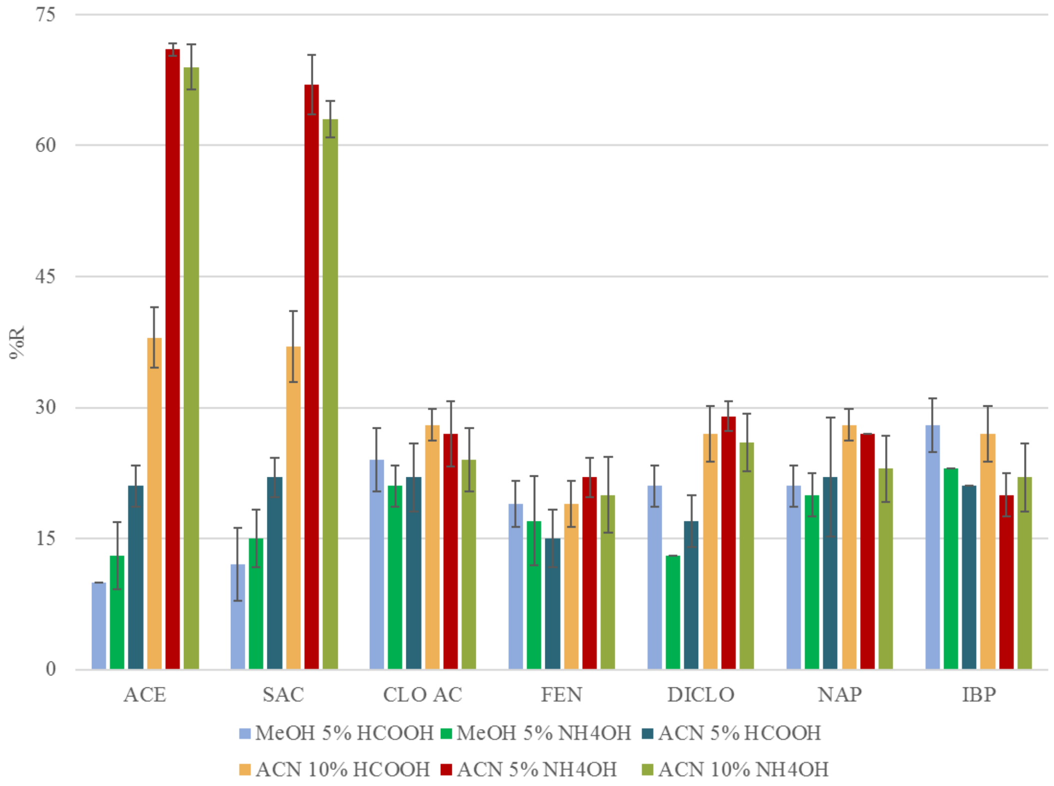
75
60
45
30
15
0
%R
ACE
SAC
CLO AC
FEN
DICLO
NAP
IBP
MeOH 5% HCOOH
MeOH 5% NH4OH
ACN 5% HCOOH
ACN 10% HCOOH
ACN 5% NH4OH
ACN 10% NH4OH

Fig. 5