UNIVERSITAT ROVIRA I VIRGILI

EVALUATION OF NOVEL MIXED-MODE ION-EXCHANGE MATERIALS TO ADDRESS ENVIRONMENTAL ANALYTICAL PROBLEMS

Joan Carles Nadal Lozano

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Evaluation of novel mixed-mode ion-exchange materials to address environmental analytical problems

JOAN CARLES NADAL LOZANO

DOCTORAL THESIS
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DOCTORAL THESIS

Supervised by

Prof. Rosa Maria Marcé and Dr. Núria Fontanals

Department of Analytical Chemistry and Organic Chemistry

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2023

UNIVERSITAT ROVIRA i VIRGILI
Dept. de Química Analítica
i Química Orgànica

Prof. ROSA MARIA MARCÉ RECASENS, Professor at the Department of Analytical Chemistry and Organic Chemistry of the Faculty of Chemistry of the Universitat Rovira i Virgili, and

Dr. NÚRIA FONTANALS TORROJA, Researcher at the Department of Analytical Chemistry and Organic Chemistry of the Faculty of Chemistry of the Universitat Rovira i Virgili,

WE STATE that the present study, entitled "EVALUATION OF NOVEL MIXED-MODE ION-EXCHANGE MATERIALS TO ADDRESS ENVIRONMENTAL ANALYTICAL PROBLEMS", presented by JOAN CARLES NADAL LOZANO for the award of the degree of Doctor, has been carried out under our supervision at the Department of Analytical Chemistry and Organic Chemistry of this university, and it fulfils all the requirements to be eligible for the distinction of International Doctor.

Tarragona, 30th January 2023

Doctoral Thesis Supervisors

Prof. Rosa Maria Marcé Recasens

Dr. Núria Fontanals Torroja

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ABSTRACT

Contamination is still a current problem around the world to be solved despite the efforts focused on reducing the environmental impact of industrialization. The presence of various types of emerging organic contaminants in environmental samples postures a big issue. The necessity of determining them arise from their widespread use and presence, and their contamination problems they cause in the environment.

Environmental samples are complex matrices that contain many compounds with different properties and make almost impossible to perform a direct analysis to determine them. Furthermore, target analytes are usually present at low concentration in the sample, being difficult to determine.

To determine these contaminants present in the environment, selective and sensitive analytical methods have to be developed. Analytical methods involving sample preparation, detection, and quantification of analytes in complex matrices are essential in modern-day analysis. Sample pretreatment is typically required before any instrumental analysis to ensure reliable results. Therefore, sample preparation is a critical factor that determines both the accuracy and the time consumption of a sample analysis process, since more than 80 % of analysis time is spent on sample collection and preparation. The sample preparation is usually conducted to concentrate the analytes as well as to remove interferent compounds for subsequent chromatographic separation. Several extraction techniques can be used depending on the type of sample, being solid-phase extraction (SPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE), among others recently developed, the most used for sample treatment of liquid samples.

In recent years, the preparation of materials used as sorbents with specific characteristics, high sorption capacity and unique interactions or selectivity toward target analytes has been the focus of interest in the development of sample preparation on sorptive extraction approaches. Therefore, this Doctoral Thesis focuses on the development and evaluation of in-house mixed-mode ion-exchange materials for sorptive extraction techniques to address environmental analytical problems.

The first section focuses on the preparation of in-house hypercrosslinked mixed-mode ion-exchange materials and their evaluation by SPE to extract ionizable analytes as model compounds from three different families: pharmaceuticals, artificial sweeteners and illicit drugs in environmental waters. The novelty of these studies are based on the introduction of the zwitterionic character to the sorbent which enable acidic and basic analytes to be selectively extracted from the sample. A weak anion- and weak cation-exchange material (HXLPP-WAX/WCX) was the first material evaluated. Two other hypercrosslinked mixed-mode ion-exchange polymeric sorbents, one with weak anion- and strong cation-exchange properties (HXLPP-WAX/SCX) and the other with strong anion and weak cation-exchange properties (HXLPP-SAX/WCX), were synthesized and evaluated by SPE followed by liquid chromatography mass spectrometry in tandem (LC-MS/MS) to extract ionizable analytes in environmental waters. The different parameters affecting the extraction of the target compounds, such as the sample pH, elution solvent or sample loading volume, were evaluated for each material and successful results were obtained for the selective extraction of the acidic and basic compounds

In the section devoted to the stir bar sorptive extraction (SBSE) and capsule phase microextraction (CPME) techniques, in-house mixed-mode ion-exchange materials were synthesized to be used as coatings for the SBSE and CPME of a group of ionizable compounds determined by LC-MS/MS. In the first study, two different polytetrafluoroethylene (PTFE)-based magnets (EDA1 and EDA2) coated with monolithic weak anionic-exchange properties were designed as stir bars for SBSE. The magnets exhibited good stability and reusability. The simple and selective method was successfully applied to determine and quantify acidic analytes in environmental waters. The second study focuses on the preparation of two microextraction capsules (MECs) for the CPME of ionizable analytes. A sulfonic acid was used as the cation-exchanger group in MEC-C18/SCX, and a quaternary amine as the anion-exchanger group in MEC-C18/SAX. Both approaches were thoroughly investigated and compared, focusing on their retention mechanisms.

CHAPTER 1. INTRODUCTION

Complex samples, such as biological, environmental (e.g., air, water, soil), foods and beverages among others, are characterized by a large number of components, which may exhibit a wide range of physical and chemical properties. Moreover, the effects of a complex matrix generally include sample tortuosity, influence of pH and salts, as well as matrix binding and competition. Therefore, the analysis of complex samples is a challenging task that requires deep understanding of the principles and processes involved in the entire analytical method. Analysis procedure normally includes sampling, sample preparation, detection, and data handling. For instance, an inappropriate sample treatment will produce unreliable results, even when using powerful analytical techniques, due to the lack of a representativeness of the extract and pronounced matrix effect in the instrumental technique. Thus, the present scenario focusses on the selection of a suitable sample preparation to achieve successful results. Likewise, the selection of the instrumental technique is also important and depends on the property of the analytes to be measured [1,2].

Even though chromatographic techniques are usually chosen when handling with complex matrices, chromatographic instruments cannot deal with complex matrices directly. The identification and quantitative determination of target analytes in complex samples, such as environmental waters, is a challenging task, requiring selective and sensitive analytical techniques. Thus, chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC), mainly coupled to mass spectrometry (MS), are normally used to analyse the samples. Although various detectors are commonly used in LC, such as an ultraviolet detector (UV), diode array detector (DAD) or fluorescence detector, when analysing complex samples, a mass spectrometry-based detector has become the most preferred option because of the high levels of sensitivity, that enable low concentration levels (ng/L) to be quantifies of confirmation of the selected analytes and identification power of unknown compounds [3,4]. In the case of MS detectors in LC, three different types of ionization source or interfaces are mainly used: electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). They are all atmospheric pressure ionization interfaces included in the group of soft ionization sources, since the molecule does not undergo excessive fragmentation while working in positive and negative mode. Among latest new trend chromatographic instruments, stands out the use of tandem mass spectrometry to detect and quantify compounds at trace levels. The LC-MS/MS method has proved to be one of the most effective tools with high specificity, sensitivity, particularly for the determination of analytes at trace levels in complex samples, which can provide a high confirmation mode. Nowadays, LC-MS/MS is becoming the mainstream LC-MS analysis because of the improved detection specificity of multiple reaction monitoring (MRM) mode in MS/MS that improve sensitivity. Methods based on LC-MS/MS applied in complex samples have broadly been reported owing to its excellent versatility [5].

Recent advances in liquid chromatography-mass spectrometry have led to ultra high performance liquid chromatography (UHPLC) and high-resolution mass spectrometry (HRMS), which takes advantage of technological advances made in particle chemistry performance and mass accuracy measurements, respectively. When smaller particle diameter analytical columns and instrumentation are used and operated at higher pressures than those used in LC, high efficiency, sensitivity and speed of analysis can be obtained.

Sample preparation plays an important role in any analytical determination, mainly when working with trace concentrations of analytes in complex matrices, due to matrix interferences, the low concentration levels in which target analytes are present, or incompatibility of the matrix with the instrument. The removal of potential interferents is one of its main aims and advantages. By including a sample pretreatment after the sampling step, the complexity of the extract is simplified to make easier the separation of the chosen analytes from other components in the analytical column. As a result, fewer compounds are introduced into the chromatographic detector thus making their identification and quantification straightforward. The physical state of the sample and the chemical properties of the analytes, such as polarity and ionisation state, determines the sample pretreatment technique chosen. Some of the compounds present in environmental and biological samples are polar compounds, that can present an ionizable form depending on the pH. Moreover, in this step, it is necessary to ensure the correct isolation of the interested analytes in the complex matrix, to develop reliable methods and consequently obtain higher sensitivity, precision, accuracy, and recovery in the results [6,7].

Another important advantage of including a sample pretreatment is the analyte preconcentration. Sometimes the concentration levels in which the analytes are present in environmental samples like water, air, soil, and living organisms are too low and they need to be concentrated to be detectable. Additionally, analytical techniques are not sensitive enough for direct determination. In this way, preconcentration is a denoting procedure that could help to concentrate the target organic contaminants and lower the limit of detection. Sample preparation can enrich the target analytes increasing the sensitivity of the method.

Nevertheless, sample preparation is considered as the most critical and time-consuming step in any analytical procedure, typically taking 60-80% of the total analysis time, and it is likely to cause problems and difficulties (e.g., cost, contamination, poor reproducibility and low extraction yields). Sample preparation is the first and often the main step in analytical methods. As a result, there is an increasing demand for sample preparation techniques that are simple, inexpensive and environmentally friendly, and that allow acceptable recovery and selectivity. In addition, sample preparation consumes large amounts of solvent volumes. In terms of an environmentally friendly concept, reducing the volumes of non-hazardous solvents is a way to implement the green chemistry in novel and forthcoming analytical methodologies.

Several pretreatment techniques have been used in the past decades to extract organic contaminants from different types of liquid samples. Traditional liquid-liquid extraction (LLE) technique has played a major role in sample pretreatment and concentration over the past years due to its high selectivity of separation, high production capacity, low energy consumption and short method development time. Nevertheless, the disadvantages of this technique, which include the use of large volumes of organic solvents, difficult automation, laborious and time-consuming steps, use of large volume of toxic organics, emulsion formation, poor reproducibility and loss of sensitivity among others, gave way to the use of sorptive extraction techniques.

Sorptive extraction techniques are the preferred extraction techniques for liquid sample analysis. Sorptive extraction techniques are those whose sorbent is a solid material and the analytes are trapped in this suitable sorbent and subsequently desorbed in elution steps. The most commonly used sorptive extraction technique for liquid samples is the solid-phase extraction (SPE). SPE was introduced as a substitute for LLE and shortly became one of the preferred extraction techniques, for its simplicity, high preconcentration factors and availability of different sorbents. SPE is an exhaustive sample preparation technique based on batch and flowthrough equilibrium, whereas LLE is an equilibrium technique. SPE represented a considerable advance by lowering the amount of organic solvent.

In the recent years, miniaturization of extraction techniques have gained attention allowing to increase recoveries of target analytes even when analysing very complex sample matrices. Miniaturized sample preparation procedures are known as microextraction techniques and are based on the same fundamental principles as conventional extraction approaches and use less organic solvent [8,9]. Microextraction techniques are divided in two groups depending on the nature of the extraction/acceptor phase: those based on liquid extraction phases, such as liquid-phase microextraction (LPME), dispersive liquid-liquid microextraction (DLLME) and single drop microextraction (SDME) [10]; and those based solid extraction phases (sorbents), such as solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE) and dispersive solid-phase extraction (dSPE), among others [9,11,12]. A classification of the different miniaturized formats of liquid- and solid-based techniques is shown in Figure 1.

LPME is a type of sample preparation technique in which the target analytes are extracted from an aqueous solution with a small volume of extraction solvent. In LPME the extraction phase is based on the extractive properties of different solvents which play the most important role in the entire process. There are three main types of LPME based on how the extraction solvent contacts the sample to extract the target analytes: a single drop microextraction (SDME), hollow fiber liquid-phase microextraction (HF-LPME), and dispersive liquid-liquid microextraction (DLLME) [9–11,13], among others. Figure 2 shows examples of miniaturized LLE techniques. The most used ones are HF-LPME and DLLME. HF-LPME typically employs disposable propylene membranes that contain the extraction solvent. The hollow fiber is immersed in the aqueous sample solution that contains the target analytes for the extraction [14]. With HF-LPME, it is possible to extract target analytes from aqueous samples, across a supported liquid membrane (SLM) and into an acceptor solution, so it requires specific material. HF-LPME is divided in two groups: two-phase HF-LPME and three-phase HF-LPME. In the first approach, the hollow fibers contain the organic solvent (acceptor phase), and extraction takes place via passive diffusion of the analytes from the aqueous sample (donor phase) to the acceptor phase. This method is used for the extraction of analytes with low polarity, and the technique is compatible with GC, and the extraction and preconcentration of the analytes are performed by an organic solvent. In the second group, after the extraction of the analytes to the organic solvent that is immobilized in the pores of the fiber, the analytes are further extracted into a new aqueous phase in the lumen of the fiber. This second method is more suitable for ionisable compounds, such as acids and bases. HF-LPME presents some advantages such as high efficiency regarding sample clean up, as well as the provision of high enrichment factors, and low cost [9,10,15].

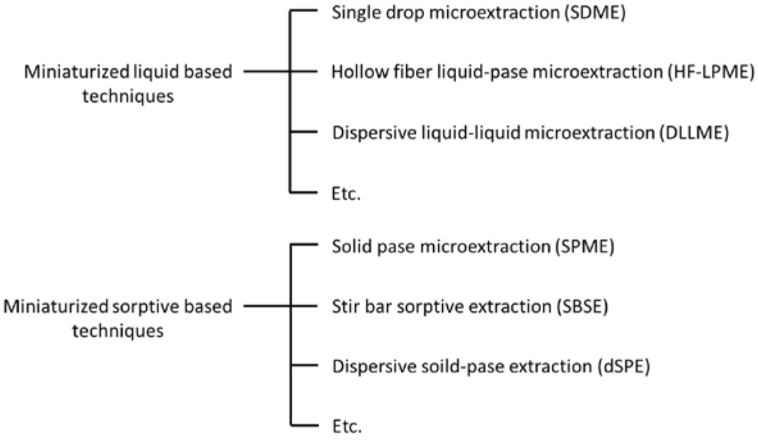

Figure 1. Classification of the most representative liquid and sorptive-based techniques.

DLLME is another form of LPME based on a solvent system involving an appropriate mixture of water-immiscible organic solvent as the extraction solvent, and water-miscible organic solvent as the disperser solvent, which is injected rapidly into an aqueous sample solution with the use of a syringe. When extraction is performed, the acceptor phase is usually separated by centrifugation and directly analysed. The main purpose of dispersion is to accomplish a higher surface contact with the sample, resulting in more favourable extraction kinetics and lower extraction times. Despite the great advantages that this sample treatment offers such as simplicity, short extraction periods, high preconcentration factors for target analytes, low cost, and the possibility to combine with almost every analytical technique, it is limited by the poor selectivity caused by the intrinsic properties of the organic solvents.

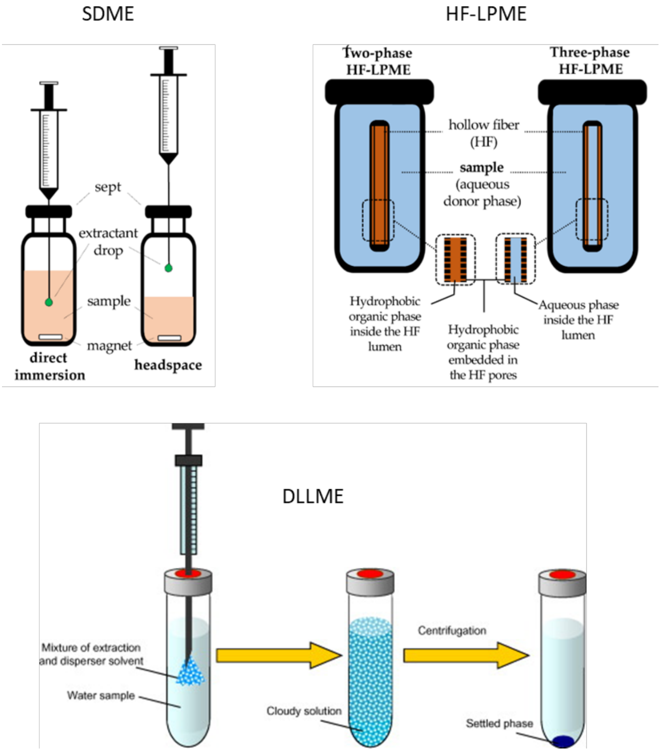

Figure 2. Representation of different miniaturised LLE techniques [16,17].

Apart from the liquid phase microextraction techniques above explained, other miniaturized approaches of LLE have been developed with the same purpose of addressing the shortcomings of classical sample preparation techniques and simplification of the sample preparation workflow. Nevertheless, this Thesis is focused on the sorptive extraction techniques, explained in more detail in the following section.

1.1. Sorptive phase extraction techniques

For liquid samples, although solvent extraction techniques have been miniaturised to achieve high enrichment factors, minimise solvent consumption and reduce waste, sorptive extraction techniques are the preferred extraction techniques. Sorptive extraction techniques can achieve high degrees of preconcentration and selectivity by decreasing matrix effects (ME%) and thereby improve sensitivity and reproducibility. Some of the most commonly used sorptive extraction techniques, such as solid-phase extraction (SPE), solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) are described in this section.

1.1.1. Solid-phase extraction

It was first used in the 1970's for the extraction of analytes with different chemical properties from liquid samples. The principle of SPE involves partitioning between a liquid sample or solvent with analytes and a solid sorbent phase. SPE is recognized as beneficial alternative to LLE, because it has many advantages over the LLE, such as less consumption of organic solvents, reduction of processing time, simplicity, many sorbent choices, ability to interface with different instrumentations and low intrinsic costs. Moreover, SPE can extract a wide range of organic analytes, being non-polar or very polar, from a large variety of samples. In fact, SPE can extract a large variety of organic compounds from biological, food and environmental samples thanks to the different available materials [18].

The common SPE protocol involves 4 main steps: conditioning, loading, washing and elution (Figure 3) [3,19]. Initially, the sorbent is conditioned with a solvent similar in composition to the sample matrix, before passing the sample through the sorbent to extract the analyte from the liquid sample. In some cases, matrix interferences can be removed from the sorbent using a washing solvent. Washing solutions are usually introduced to increase selectivity. Lastly, the analytes of interest are eluted using a minimal amount of organic solvent. The liquid phase can be passed through the column by gravitational force or by the use of positive pressure using syringes, air or nitrogen lines, a vacuum flask or a centrifuge. The SPE technique can be coupled off-line or on-line to a liquid chromatograph and a suitable detector. The current trends are focused on developing methods aimed to reduce labour and time-consuming operations, as well as reducing energy consume per one analytical cycle [20].

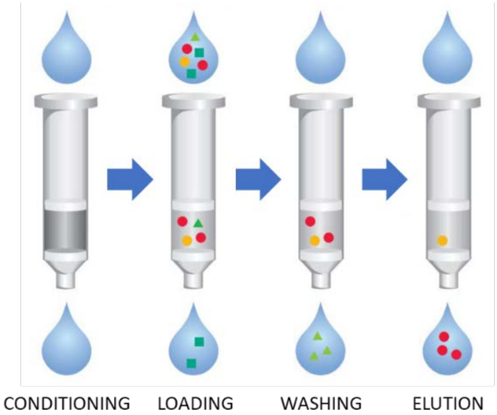

Figure 3. Schematic representation of the common SPE protocol.

1.1.1.1. Sorbents

Initially, solid particles such as silica-based, activated carbon and porous polymeric resin were used as sorbents for the extraction of traces of organic contaminants in water samples. Important development has been achieved in both the sorption process and the versatility of sorbent materials able to trap different analytes. Over the years, efforts have been made to develop and characterize new advanced sorbent materials that have improved target analytes' selectivity or specificity. These materials have also been shown to have a high sorptive capacity and enhance physicochemical and mechanical stability [21–25]. These developments have made SPE an attractive sample preparation alternative now designed with micro-gram levels of sorption material that could achieve high recovery rates and enrichment with a significant reduction in the total organic solvents used. The applicability of SPE is primarily dependent on the sorbent used in the extraction column [20,22,24]. Recently, these media have aroused sample preparation researchers to develop materials with high selectivity and efficiency for dealing with trace analysis in complex matrices, explained in more detail in chapter 1.2.

Different types of commercial sorbents are available for SPE with good extraction efficiencies for retaining many organic contaminants. The selection of the SPE sorbent depends on understanding the mechanism of interaction between the sorbent and the target analyte. The most common retention mechanisms in SPE are based on Van der Waals interactions, hydrogen bonding, dipole-dipole interactions and ionic interactions. Therefore, the development of different sorbents for SPE has become an extensively important objective. The SPE sorbents are divided into two groups: inorganic sorbents, such as magnesium silicate (Florisil), alumina, silica and chemically bonded silica sorbents; and organic sorbents, such as polymer-based ones. For instance, magnesium silicate (Florisil) is used for cleaning up extracts from fatty foods because it retains specifically some lipids [19]. Normal-phase sorbents, such as those that incorporate cyanopropyl (CN), aminopropyl (NH_2) or diol functional groups; and reversed-phase sorbents, such as silica-based materials and those modified with long alkyl chains, including octadecyl (C_{18}), octacyl (C_8), ethyl (C_2), phenyl (Ph) and cyclohexyl (CH) were the first sorbents to be developed and has been the most common material for SPE for many years. The interaction mechanisms of the reversed-phase sorbents are mainly based on hydrophobic interactions (Van der Waals forces) between the analytes and the sorbent. First, silica modified with C_{18} cartridges were packed with the same stationary phases as those in LC columns except that the granulometry was larger. Their evolution followed those of LC with the objective of having more hydrophobic phases containing a minimum amount of residual silanol groups. Almost every SPE silica modified with C_{18} are made from silica with high specific surface areas in the range of 500 to 600 m^2/g. Some are available with a high percentage of carbon loading corresponding to a maximum surface coverage by the alkyl moieties [26,27]. But their properties can differ depending whether mono- or trifunctional alkylsilanes have been used for the bonding and whether the bonding was followed by endcapping or not. However, SPE silica-based sorbents have some disadvantages, such as a narrow pH stability range, low recovery in extracting polar compounds and the presence of some residual silanol groups that can have a negative effect on analyte retention. Although silica sorbents are extensively used to extract lots of different compounds, providing high recoveries for apolar analytes, their characteristics make them not suitable for the extraction of polar and/or ionisable analytes such as pharmaceuticals, sweeteners or drugs [28].

Over the later years, there has been growing interest in the development of polymer-based materials. Polymeric sorbents combine outstanding morphological properties (high surface area and well-defined porosity) that promote capacity and retention, that allow suitable interactions with many types of compounds and show enhanced stability under several SPE conditions, since they are stable throughout the pH range and the compounds sorbed are easily eluted. The diversity of synthetic routes to provide polymeric sorbents facilitates incorporation of various chemical functionalities into the porous framework. All these features overcome the previously mentioned disadvantages of silica-based materials. The most widely used polymeric sorbent is macroporous styrene-divinylbenzene (St-DVB), which presents a hydrophobic structure with specific surface area up to 500 m^2/g. Their interactions with the analytes, due to the hydrophobic character of the sorbent, are basically by the π–π interactions of active aromatic sites that form the sorbent structure and Van der Waals forces.

Since the main retention mechanism of the conventional macroporous styrene-divinylbenzene (St-DVB) polymeric sorbents involve π-π interactions and most polymeric sorbents have a lower than desirable sorption capacity towards many organic compounds, higher surface area resins were exploited and gradually led to introduce the hypercrosslinked sorbents.

Hypercrosslinked materials, first introduced by Davankov and Tsyurupa [29], are a new generation of permanently porous, polymeric resins that conform an expanded, rigid, and three-dimensional network with enhanced analyte retention characteristics arising from their high micropore contents and exceptional specific surface area (>1000 m^2/g), which intensifies the interactions between the sorbent and the analyte. Hypercrosslinked polystyrene-type materials thus represented a fundamentally new class of polymeric networks and form the base for the third generation of polystyrene-type adsorbing materials. Davankov-type hypercrosslinked sorbents differ from conventional macroporous polymeric sorbents since they are obtained by an extensive post-crosslinking of a preformed linear (or very lightly crosslinked) polystyrene using a Friedel-Crafts catalyst. Alternatively, vinylbenzene chloride-divinylbenzene (VBC-DVB) copolymers can be used as starting material in the presence of a solvent and a Friedel-Crafts catalyst. This converts the chloromethyl groups into methylene bridges, creating new crosslinks additional to those included in the original resin by DVB [30–32].

This approach has been extensively used by the research group of Cormack and Sherrington group at the Strathclyde University of Glasgow [33–35].

Nevertheless, despite the large specific surface areas they present, the hydrophobic character of the polymeric resins is not ideally suitable for the sorption of most polar compounds. To overcome the hydrophobicity of the polymeric sorbents and increase the retention of polar compounds, two mainly strategies are adopted: by introducing a polar functional group to the hydrophobic polymers or copolymerizing monomers containing suitable functional groups. Functionalisation with polar groups in the St-DVB polymeric network by a Friedel-Craft reaction allowed the development of hydrophilic polymeric sorbents, providing enhanced polarity in the polymer material and better mass transfer, and improving the retention of polar compounds. The copolymerization with a hydrophilic monomer consists of copolymerizing a hydrophilic monomer with a crosslinking monomer. The hydrophilic monomer favors hydrophilic interactions, whereas the crosslinking monomer, usually DVB, increases the specific surface area (SSA) and favors hydrophobic interactions. A suitable sorbent for extracting polar compounds should have a proper balance between specific surface area and polar group content [20,21,36,37].

Nowadays, there are several polar sorbents commercially available and one hydrophilic sorbent recognized world-wide is Oasis HLB, manufactured by Waters. Oasis is a macroporous resin based on a poly(N-vinylpyrrolidone-DVB) (PVP-DVB) copolymer obtained by copolymerisation that has a specific surface area of 800 m^2/g. Since its commercialisation, Oasis HLB has become more and more popular for sample preparation due to the advantages it offers, such as its ability to extract both polar and apolar compounds, its high capacity and effectiveness in terms of removing interferences. The analytical methods developed using Oasis HLB in environmental analysis have enabled high sample volumes to be loaded (from 50 to 1000 mL), providing high enrichment factors. Combining the high capacity of the Oasis HLB sorbent and the sensitivity of MS/MS detection technique, method detection limits (MDLs) have been achieved at levels of ng/L. It is used to extract different types of compounds, such as pharmaceuticals, proteins, pesticides, sweeteners, high volume production chemicals and illicit drugs, from matrices, such as food, environmental waters, and biological fluids and tissues [38–42]. For instance, Franklin et al. [42]

developed a method for the determination of four antibiotics (sulfamethoxazole, trimethoprim, lincomycin, and ofloxacin) in water and soil matrices using an Oasis HLB SPE cartridge to clean up and concentrate the samples. Samples were loaded onto cartridges followed by one wash with deionized water (3 mL) and then the cartridges were dried under vacuum for approximately 5 min. Antibiotic residues were finally eluted with a 50:50 mixture of ACN and MeOH (2x 3 mL). Recoveries from 75% to 118% were obtained for all the target compounds analysed. In another example of Oasis HLB application, Łukaszewicz et al. [5] used Oasis HLB to extract four new generation neonicotinoids (acetamiprid, sulfoxaflor flupyradifurone, and 6-chloronicotinoic acid) from surface waters. For the extraction process, a sample volume of 200 mL at pH 4.6 was used as loading; and a mixture of 0.3% HCOOH in ACN was used as elution solution. The absolute recoveries obtained for the optimal SPE conditions were above 80% for all the analytes.

Other hydrophilic macroporous polymeric sorbents have been also developed, such as Bond Elut Plexa, from Agilent Technologies with 700 m^2/g, or Strata-X, from Phenomenex, which are the most commonly used chemically modified sorbents with polar functionalities. Strata-X, is a commercially available sorbent based on PS-DVB functionalized with pyrrolidone moieties with a specific surface area of (800 m^2/g) that confer hydrophilic properties [40,42–45]. Several SPE commercially available polymeric sorbents are listed in Table 1.

Regarding hypercrosslinked sorbents, the same two strategies were used to acquire hydrophobic character in styrene-based hypercrosslinked resins. When using the copolymerization approach, different precursor resins can be employed, such as gel-type VBC-DVB, prepared using either p-VBC, or a mixture of p-and m-isomers, attaining thus different degrees of hydrophilicity and specific surface areas [39].

Currently, some types of hypercrosslinked SPE sorbents are commercially available. Purosep (Purolite, Wales, UK), Isolut-ENV+ (International Sorbent Technology, Cambridge, UK) and Lichrolut EN (Merck, Germany), with a pore diameter of 1-3 nm and high specific surface area of 1100-1200 m^2/g, are the widespread used commercially available hypercrosslinked SPE sorbents. They have been tested in SPE and the recovery levels proved to be acceptable when moderately polar compounds were analyzed. Others such as Chromabond HR-P (Macherey-Nagel), Hysphere SH (Spark Holland) are also used.

Table 1. Commercially available SPE polymeric sorbents.

Sorbent	Supplier
Oasis HLB	Waters Corporation
Porapak	Waters Corporation
Bond Elut Plexa	Agilent Technologies
Bond Elut Focus	Agilent Technologies
Bond Elut ENV	Agilent Technologies
PLRP-S-10	Agilent Technologies
Strata-X	Phenomenex
Strata SDB-L	Phenomenex
Abselut Nexus	Varian
Super select HLB	Supelco
Speed Advanta	Applied Separation
AttractSPE	Affinisep
Isolute 101	Biotage

In our group various in-house hypercrosslinked sorbents were synthesized and afterwards applied in SPE [39,46]. For instance, Fontanals et al. [39] prepared and tested a hydrophilic hypercrosslinked polymer with hydroxyl moieties by SPE to determine several polar compounds, including oxamyl, desethylatrazine, 4-

nitrophenol, among others, from water samples. The in-house prepared sorbent presented a specific surface area of 908 m^2/g; and was compared to hydrophilic commercially available sorbents, such as Oasis HLB and other previous in-house prepared sorbent based on N-vinylimidazole-divinylbenzene. The recoveries obtained were consistently better with the new synthesized sorbent, probably due to the higher level of polarity in the hydroxyl-based sorbent. In another study [46], hydrophilic hypercrosslinked polymers in the form of small particles with hydroxyl moieties (poly(Hydroxyethylmethacrylate (HEMA)-co-VBC-co-DVB)) with different molar ratios were prepared and evaluated by SPE-LC-UV to determine polar pollutants in mineral water, tap water and Ebre river water. The sorbent with optimal performance characteristics, the one with the molar ratios: HEMA:VBC:DVB (20:40:40) was selected for further sample analysis, obtaining high recoveries of analytes. In comparative SPE studies with the commercial sorbents LiChrolut EN, Oasis HLB and Isolute ENV+, the sorbent with optimal performance characteristics significantly out-performed the commercial materials.

In the last years, our research group has also prepared hypercrosslinked polymers functionalized with ionic moieties to provide mixed-mode ion-exchange sorbents with outstanding retention capabilities thanks to the hypercrosslinked network. A revision of them is described in section 1.2 of this Thesis.

Carbon-based sorbents have gained the attention of researchers as alternative sorbents owing to their improved water dispersibility, versatile surface modifications, high mechanical strength, and high surface area. Carbon-based sorbents can also be considered as graphite sheets containing a broad range of functional groups, such as ketone and carbonyl groups. Carbon-based sorbents include graphene and their derivatives, carbon nanomaterials and metallic nanoparticles [47,48].

The first approaches of carbon-based sorbents used many years ago as SPE materials are the porous graphitic carbons (PGCs) and graphitized carbon blacks (GCBs), employed due to its exceptional properties including thermal conductivity, fracture strength and high surface area. PGCs in which a porous graphitic substrate is immobilized on silica have higher stability than GCBs, and greater extraction efficiency than C_{18} sorbents because of the hydrophobic and electrostatic interactions. Examples of GCBs include Envi-carb, applied to extract phenolic compounds, pesticides and their degradation products [49].

Graphene is another important material that has drawn the attention of analytical chemistry in the field of sample treatment. Nowadays the novel emerging forms of graphene such as carbon nanomaterials, metallic nanoparticles and metal organic frameworks (MOFs) take over PGCs and GCBs [25,50–54]. Graphene represents excellent support to anchor chemical functionalities or nanomaterials to prepare graphene-based nanocomposites and in combination with its superior properties, and the synthesis and evaluation of novel graphene-derived materials has attracted the interest of many researchers. Among the graphene-based nanomaterials, graphite oxide (GO) is definitely one of the most popular. GO is an oxidized form of graphene that is rich in oxygen functional groups (i.e., epoxy, carbonyl, carboxyl, and hydroxyl groups) arranged in the carbon lattice. GO can be produced from graphite by chemical oxidation, followed by exfoliation by ultrasonication [47,55–60]. Graphene and its derivatives are good candidates for the extraction of benzenoid form compounds through strong π-stacking interactions due to their large delocalized π-electron system. In order to enhance the applicability of those materials, functionalization with different groups can be employed, aiming to prevent the aggregation of the carbon sheets to extend their potential application [58].

Although all these carbon-based materials are characterized by their thermal and mechanical resistance, they present significant disadvantages such as low specific surface area (100 m^2/g) and high, or even irreversible, adsorption capacity for some compounds, so eluting them may be difficult [22,24,28,61,62].

Metal organic frameworks (MOFs) are novel materials with structures based on classical coordination bonds between metal cations and electron donor organic linkers. These materials have received great attention due to their unusual properties including high surface area, good thermal and mechanical stability, uniform structure, and nanoscale cavities. MOFs were introduced in analytical chemistry as stationary phases for GC and LC applications, and gradually break into sample treatment as extraction sorbents for organic compounds and metal ions. Currently there are some synthetic methods to prepare MOFs, including the solvothermal, the hydrothermal, the electrochemical, and the mechanochemical approach. It is essential to select MOFs that are stable under adsorption and desorption conditions since they have the limitation of poor stability in aquatic environments. However, in some cases functionalization of MOFs can be employed to increase their stability and to enhance their selectivity towards the target analytes. For example, a MOF was employed as SPE sorbent material for the quantification of thymol and carvacrol by GC with flame ionization detection in Lamiaceae plant extracts and a honey sample. The MIL-101(Cr) (MIL for Matérials of Institut Larvoisier), a three-dimensional porous coordination network, was hydrothermally synthesized, intercalated with polyaniline and doped with silica nanoparticles to obtain the polyaniline composite, MIL-101(Cr)/PANI/SiO_2 [63]. Another example of the use of MOF in SPE is Zhang's study [64], where a graphene aerogel supported MOF was prepared as adsorbent for the SPE followed by LC-MS of non-steroidal anti-inflammatory drugs and the selective enrichment of proteins. In both studies MIL-101 was selected as a model MOF because of its excellent solvent stability, abundant mesoporous cages and plentiful coordinatively unsaturated metal sites.

Highly selective sorbents, such as immunosorbents or molecular imprinted polymers (MIPs), were introduced to enhance selectivity in SPE when extracting complex matrices [4,21,36,50,65,66]. Over two decades ago, the improvements of SPE to achieve a better selectivity involved the application of conventional immunosorbents. However, they are used less widely for many different compounds because a selective antibody must be developed for each analyte [65]. Afterwards, MIPs appeared to solve the main disadvantages of immunosorbents. MIPs are highly stable polymers that possess recognition sites within the polymer matrix that are adapted to the three-dimensional shape and functionalities of an analyte of interest. These MIPs can be produced in a covalent or a non-covalent method. In covalent imprinting, the print molecule, called template, is chemically coupled with one of the building blocks of the polymer, and after the polymerization, the resulting bond must be cleaved to obtain free selective binding sites. The most common approach is non-covalent imprinting, which relies upon self-assembly between the template and carefully selected functional monomers. After polymerization in the presence of a crosslinking monomer, the resulting highly reticulated polymer will contain a binding site equipped with functional groups in a defined three-dimensional arrangement.

MIPs are used in many applications to determine compounds in all kind of samples, such as environmental samples or food samples [67]. For instance, MIPs employed as SPE materials were applied to determine amphetamine-type drugs in urine [68] and wastewater and sulphonamides in animal-origin foods [69].

In recent years, researchers have been developing novel sorbents that are suitable for selective SPE, focusing on mixed-mode ion-exchange sorbents, described in more detail in section 1.2, and new materials including nanomaterials and MIP composite materials that are capable of extracting selectively various organic compounds from complex samples [67,70]. These nanostructured materials, include electrospun nanofibers, dendrimes and magnetic nanoparticles [48,71,72].

1.1.1.2. Formats

Nowadays, various SPE formats combining different sorptive materials are commercially available, such as cartridges, disks (47 mm diameter or the standard filtration size), multi-well SPE plates, pipette tips, in-syringe SPE and dispersive SPE (dSPE) [18,19,50]. Cartridges are by far the most frequently chosen format by analytical chemists. The SPE cartridges are small polypropylene or glass open-ended syringe barrels filled with a layer of sorption bed, located between two polyethylene or stainless frits. The obtention of the highest extraction efficiency depends mainly on the selection of a suitable sorbent. Depending on the type and volume of the sample to be percolated, cartridges are commercially available on the market with different types, sizes and sorbents. Generally, commercially available cartridges are employed, ranging from 3 mg to 500 mg of sorbent amount. Moreover, the combination of different cartridges with the same or different types of sorbents increases the extraction efficiency and the recovery of analytes. A highly selective tool can be developed depending on the sorbents chosen for the isolation and enrichment of analytes.

The extraction disks, also called membranes, are an alternative SPE format which has been used for many years among analytical chemists. They were available for the first time in 1989 by 3M in an attempt to solve the limitations that present SPE cartridges related to isolation and enrichment of analytes from environmental samples of high volume. In this format, the particles of sorbent are embedded in the membrane. The protocol and sorption materials used in disks are the same than in cartridges. The sorbent particles of the disk adsorb the analytes present in the sample and then the target analytes are rinsed using a small amount of solvent. As in the case of SPE cartridges, the size of the disk used for the isolation of analytes is determined by the volume of the sample used. Thus, disks with small diameter are frequently used in clinical, forensic and pharmaceutical analysis, and the larger ones in environmental analysis. Disks between 4 mm and 96 mm of diameter are used in all kind of analyses. The most frequently used disk size is 47 mm, suitable for standard methods used for the analysis of water samples with volumes from 0.5 to 1 L, which is more amenable for environmental analysis. The differences between cartridges and disks are mainly due to a different packing of the bed and the structure of the particles. The sorbent particles embedded in the disks are much smaller than those used for filling the cartridges. When using small diameter sorbent particles, the extraction efficiency is increased [18].

The SPE disks have many fields of application in various analytical areas of interest due to the diversity of sorbent materials available achieving a selective extraction of interested substances. They can also be used for special applications, such as passive sampling or storage of analytes [73,74]. For example, graphitised carbon SPE sorbent Envi-Carb [73] and C_{18} Empore disk [74] were used as passive samplers for acidic herbicides in river water and triazines in water samples, respectively.

The undoubted progress made in the understanding of adsorption mechanisms resulted in a variety of novel sorptive materials, with different particle sizes and disk diameters able to retain different types of analytes. Nowadays the use of extraction disks is reduced due to the different alternative SPE formats [18].

As an attempt to miniaturize and make the SPE a more environmentally friendly technique, SPE pipette tips (SPE-PTs) were developed. In this miniaturized version of SPE, the sorbent material is packed inside plastic micropipette tips. Analytes are extracted by repeated aspiration and desorption of the sample solution using single channel and multichannel pipettors. Thanks to no centrifugation or solvent evaporation needed in SPE pipette tips, they can be readily automated and the resultant eluents directly injected into a chromatography system to be analysed. The principal advantages of SPE pipette tips respect to conventional SPE cartridges are the reduction in the amount of sorbent, consequently reducing the amount of organic solvent and the sample volume used, which lowered the costs, while the possibility to integrate extraction and purification in the same step shortened the time needed to perform the entire analysis. Due to a minimal sample volume used in SPE-PTs, a high concentration factor is not achieved. Because of these advantages, SPE pipette tips immediately gained recognition among analytical chemists and are nowadays one of the most promising methods in some application fields [75,76].

Currently there is a wide range of commercially available tips classified by volume of tips (from 1 to 200 µL) and sorbent volume. The same materials available in SPE cartridges are also available for SPE-PTs. Since the SPE-PT is designed for micro-scale extraction and concentration, they became widely used primarily in genomic, proteomic and metabolomic studies for purification and concentration of proteins and peptides. However, it has recently been increasingly used in environmental analysis, for the isolation of drugs from food samples and biological fluids and for the determination of fungicides from tap water and grape juice [77–80]. For instance, a C_{18} SPE pipette tip was used to determine propofol in human plasma by LC-APCI-MS/MS [80]. In another study, Tamandani et al. [78] determined profenofos in seawater and foodstuff samples using a MIP-based SPE-PT.

Some of the main trends in new extraction techniques involve miniaturization, automation, on-line coupling with analytical instruments and low-cost operation with an extremely low level of solvent consumption [4,81]. Miniaturized techniques are specially growing very fast these recent years due to their simplicity, effectiveness, low cost, minimal solvent use and excellent abilities to clean up samples. Microextraction techniques are generally defined as non-exhaustive sample preparation methods that use a very small volume of the extracting phase (in the range of µL) relative to the sample volume. Examples of microextraction sorptive-based techniques are solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) [12,18,81], or in a less extent fabric phase extraction (FPSE) or stir cake sorptive extraction (SCSE), all of which are covered in next sections.

1.1.2. Solid-phase microextraction

Solid phase microextraction (SPME) was developed in the 1990s by Prof. Janusz Pawliszyn and co-workers. This technique is based on the distribution of the analytes between the sample matrix and the sorbent phase coated on a support. When using SPME, the analytes are isolated based on the equilibrium between the sample matrix and the fiber coating after selecting an appropriate extractive phase [12,25]. The SPME technique has several advantages over conventional extraction methods. SPME is a simple non-exhaustive extraction technique, fast, reproducible and sensitive. Thermal desorption (TD) is one of the main advantages in the coupling between SPME and GC, which is one of the main reasons for the broad applicability of this coupling (SPME-GC). However, liquid desorption (LD) can be also employed in SPME when directly coupling to liquid chromatography. SPME can present some disadvantages, such as fiber breakage, bleeding from conventional coated fibers, sample carry-over problems and pH instability [18].

SPME has gained importance in analytical and environmental analysis, since it provides an efficient and green sample preparation technique and, when coupling in TD, without the need for an organic solvent during extraction. Moreover, in SPME the isolation and preconcentration of the analytes occurs in a single step, providing a simple sampling methodology.

SPME configurations can be classified into static and dynamic techniques. Static procedures are typically carried out in stirred samples, including fibre SPME, thin-film microextraction (TFME) and dispersive SPME. Fibre SPME, which is the most common format for this technique, uses a sorbent coating on the outer surface of a fused silica fibre to extract the analytes from the sample matrix for a predetermined amount of time. This process can be performed using three basic modes of operation, including direct immersion (DI-SPME), headspace in a closed container (HS-SPME) or membrane-protected (MP-SPME) [82]. The dynamic techniques include capillary microextraction techniques (CME), such as in-tube SPME (IT-SPME), in-needle and in-tip microextraction configurations.

There are various commercially available coated SPME fibres, including pure polymers such as polydimethylsiloxane (PDMS), polyacrylate (PA) and polyethylene glycol (PEG); and mixed-coatings, in which porous materials are embedded in the polymers (divinylbenzene (DVB), PDMS/DVB, carboxen (CAR), carbowax (CWX), and CAR in PDMS) [83]. For instance, a HS-SPME combined with GC-MS method was developed for the extraction and quantification of 4-nonylphenol in different water sources in Mexico, such as recreational water, wastewater and drinking water. Three types of SPME fibres were evaluated for nonpolar, semipolar and polar compounds (PDMS, DVB/CAR/PDMS, PA). Different parameters including type of fibre, extraction time, temperature and ionic strength were evaluated to optimize the analytical method. The DVB/CAR/PDMS fibre offered the best performance to extract the 4-nonylphenol [84]. Apart from commercially available SPME coatings, nowadays, there are numerous coating materials developed in-house, such as ionic liquids and MIPs, so as to improve capacity against the target analytes and reach high selectivity for certain groups of compounds [85–87].

Sol-gel technology has become one of the most popular approaches to prepare novel in-house coated SPME fibres. This technology has helped to synthesise many novel coatings for SPME with large surface areas, exceptional selectivity and high thermal and solvent stabilities, propitiating high sample preconcentration factors. The versatility of these materials enables the creation of surface-bonded sorbent coatings on unbreakable fibre materials and substrates. For instance, a sol-gel strategy was used to prepare a poly(ethyleneglycol) (PEG) grafted onto multi-walled carbon nanotubes (PEG-g-MWCNTs) SPME fiber to determine ibuprofen, naproxen and diclofenac in urine samples, by hollow-fibre liquid membrane-protected solid-phase microextraction (HFLM-SPME) followed by gas chromatography flame ionization detector (GC-FID). The SPME fiber exhibited porous surface structure, good precision and accuracy, high sensitivity and high thermal stability. Important parameters influencing the extraction efficiency such as desorption temperature and time, organic solvent, extraction temperature and time, pH, stirring speed and salt effect were evaluated [88].

Monolith materials are also used as strategies to prepare SPME fibers. Monoliths are homogeneous, highly porous materials with an internal assemble containing macropores and mesopores. This double-pore structure results in a sorbent with high porosity, low backpressure, and fast kinetics for mass transfer. Polymer monoliths enable a high permeability to flow for the development of analytical separations due to their structure based on networks of interconnected pores. Monolithic materials are typically synthesized in the inner structure of capillaries by in-situ polymerization, comprising monomer, crosslinker, a binary solvent mixture (porogens) and a polymerization initiator [89,90]. According to their chemical composition, monoliths are classified as organic polymers, inorganic polymers, generally based on silica, and hybrid organic-inorganic materials, which combine features from the first two groups.

Nevertheless, SPME coatings present some limitations when extracting polar organic analytes, but this may be solved with a derivatization step. Derivatization can increase the volatility and reduce the polarity of the polar organic analytes, and therefore their selectivity, detection and extraction recovery is improved. There are three different derivatization procedures: direct derivatization, derivatization on the SPME fibre (the preferred method in SPME) and derivatization in the GC injection port [91,92].

Another way of improving the retention of polar compounds is using in-house prepared sorbents, in particular in-house mixed-mode ion-exchange sorbents, which are afterwards detailed in section 1.2.

1.1.3. Stir bar sorptive extraction

SBSE emerged in the late 1990s' by Pat Sandra. Similar to SPME, stir bar sorptive extraction (SBSE) is also an equilibrium-based non-exhaustive sample preparation technique. However, the major difference between SPME and SBSE is the high sorbent loading on the stir bars, which imparts increased sample pre-concentration capabilities. During SBSE, a magnetic stir bar coated with the sorptive material is spun into an aqueous sample for a selected long extraction time. Once the extraction step is completed, the stir bar is removed, a step that is usually performed manually, and the bar is dipped into the elution solvent for some time. Then, a fraction of the concentrated extract is transferred to a chromatographic system [93–96]. The feasibility of SBSE for pre-concentrating analytes with medium to low polarity and divergent volatility from essentially aqueous samples has been demonstrated. Several advantages of SBSE compared to SPME have been described. However, this technique has not been as widely accepted as SPME due to the limited number of commercially available coatings and the difficulty of achieving full automation [93].

Like SPME, in SBSE either thermal or liquid desorption (LD) could be performed. Thermal desorption (TD) allows the quantitative transfer of all extracted analytes into the column and presents the highest possible sensitivity for trace analysis in GC. SBSE combined with TD is totally solvent-free and volatile solutes are not masked by the solvent peak in GC analysis. It also takes advantage of the excellent thermal stability of polydimethylsiloxane (PDMS) but limits the use of some coating materials [97]. Alternatively, using LD is preferred in combination with LC. LD is every time more applied since the novel coating materials are thermolabile and might be degraded during TD [93,98,99].

The SBSE coating is the key factor when extracting target analytes, which determines their extraction performance, selectivity and dynamics. Nowadays, polydimethyl-siloxane (PDMS) and polyethylene glycol (PEG)-modified silicone (EG Silicone) are the only commercially-available SBSE coatings, commercialized under the name Twister by Gerstel (GmbH & Co., Germany). Since PDMS is a non-polar coating, its retention for polar analytes is practically null and that has become the major disadvantage of SBSE coatings [100]. In that case, commercially available coatings with polar character, such as EG Silicone Twister coatings, are normally used when extracting polar analytes since hydroxyl groups from EG structures contribute to enhance their polarity [99]. However, this SBSE lacks of thermal stability and TD is an issue. This scenario opens up to the new trend in analytical chemistry of developing a variety of novel in-house SBSE coatings with polar behaviour to improve the extraction performance. Currently, efforts in this field are focused on the development of dual phase/hybrid coatings, where the conventional PDMS phase is combined with another material to increase the selectivity and/or efficiency of the extraction process, or alternative new coating materials with improved analytical features, promoting the retention of polar compounds from complex matrices [24].

In Yang's study, [101] an in-house ethylene glycol/polydimethylsiloxane (EG/PDMS)-stir bar was employed to quantify the concentrations of some smoke-related volatile phenols in wines by GC-MS. It was found that the smoke-tainted wine contained higher concentrations of phenol, o-cresol and guaiacol than the control wine. However, all the smoke-associated compounds determined were below the sensory detection thresholds. In another study [102], the effectiveness of two stir bars (PDMS and EG Silicone) was compared to extract volatile and semivolatile bacterial metabolites determined by GC-MS. The experiments demonstrated that simultaneous extraction with EG twister in headspace and PDMS in liquid phase, combined with simultaneous desorption, was the most suitable conditions for the extraction and identification of the bacterial compounds: acetamide, acetic acid, methyl aetate and benzaldehyde, among others.

Preparation of in-house SBSE coatings can be achieved via two approaches: using sol-gel technology or using adhesion techniques [93,96]. Many SBSE coatings have been prepared using sol-gel technology, which involves the transformation of a liquid colloidal solution (sol) into a solid matrix (gel), as discussed in section 1.1.2. Various successive steps take place in order to prepare a typical sol-gel based stir bar, such as hydrolysis of the chemically active precursors, polycondensation of the hydrolysed species to form a growing inorganic network, incorporation of the active organic ligands (e.g. hydroxy-terminated PDMS) into the growing inorganic network, and chemical bonding of the growing hybrid polymer network with the SBSE substrate. The SBSE substrate is typically pretreated with HCl/NaOH solutions to create superficial silanol groups for more efficient binding of the sorbent. The material obtained by sol-gel technology has high thermal and mechanical stability and a long life-time owing to the strong chemical bonding between the coating and the surface of the glass rod. Sol-gel approach is specially suitable for the preparation of thick films. For instance, an aromatic framework (PAF-47) anchored to a PDMS stir bar was prepared by sol-gel approach to determine target polychlorinated biphenyls (PCBs) in environmental water by LC-DAD [103].

On the other hand, adhesion techniques involve the immobilisation of materials on the SBSE substrates. The immobilisation can be attained by two methods: using physical adhesion techniques and chemical adhesion techniques [104].

Physical adhesion techniques involve basically two steps: the formation of a preliminary adhesive film on the SBSE substrate, typically using a PDMS as liquid colloidal solution or other polymer/epoxy glue; and the adhesion of particles by rolling the coated stir bar back and forth in the particles followed by incubation and post-incubation treatments. Examples of physical adhesion techniques are electrodeposition methods, magnetic association, flame deposition and by using suitable glue-like substances.

As for chemical adhesion techniques, a chemical modification of the substrate is achieved by covalent immobilisation of the sorbent phase [96]. For instance, in Wang's study [105], a covalent immobilisation approach was employed to anchor metal organic frameworks onto chemical resistant poly(ether ether ketone) (PEEK) surface to analyse three parabens including methyl paraben, ethyl paraben and propyl paraben in in cosmetics. Through reduction and substitution reaction, benzoic acid group was bonded onto the surface, which can be the anchor for in situ covalent immobilization of metal organic frameworks.

Another synthetic way is the use of a monolithic polymeric material for SBSE, like in SPE and SPME. The polymer monolith is prepared through polymerisation of functional monomer and cross-linker, in the presence of porogens and initiators. To manufacture a polymer monolith, first the glass is modificated by silylation of the stir bar surface, followed by the immersion of the stir bar in an ultrasonicated mixture of monomers, cross-linkers, solvents and initiators, and finally a polymerisation reaction either through thermal initiation, from 60 to 80^{o}C for 12 to 24 h, or photo initiation initiation using UV light for 2 h. Several precursor monomers can be used to prepare the monolithic coating. Stir bars acquired using a monolithic polymeric coating commonly offers various advantages, such as large pore structure, good permeability, favourable mass transfer characteristics and low cost [98,99,106–108]. For instance, a poly(1-vinylimidazole-ethyleneglycoldimethacrylate) (poly(VI-EDMA) monolith coated stir bar was synthesised by one-pot polymerization by Yao et al. [109] for the extraction of perfluoroalkyl acids from environmental water samples by LC-MS/MS. The stir bar exhibited higher extraction efficiency and faster extraction dynamics for the selected analytes than commercial EG Silicone Twister.

Recent studies have incorporated carbon-based materials, such as fullerene, graphene, graphite oxide (GO), carbon nanotubes (CNTs), carbon nanohorns, and their functionalized materials in the manufacture of SBSE coatings due to the good thermal stability, strong hydrophobicity and high π-electron possession of fullerene C_{60} [95]. Graphene is one of the most broadly used carbon-based materials, where the graphene is incorporated into the stir bar employing various approaches, such as sol-gel technology, in-situ growth, electrochemical polymerization and physically magnetic adsorption. The obtained stir bars acquire stable mechanical properties which can withstand stirring and ultrasound processes. Graphene oxide is also employed as SBSE coating. It is introduced into the stir bar by sol-gel approach, in-situ growth and solvent exchange method. CNTs are used as SBSE coating material thanks to its π-π interaction, Van der Waals forces and hydrophobic interaction that can stablish with the target analytes from different matrices [110]. CNTs coatings can be prepared by solvent conversion method, physical adhesion method and sol-gel method. However, the use of CNTs in SBSE coatings is limited, mainly because CNTs are insoluble in aqueous solutions and organic solvents. Currently, analytical scientists have attained the purpose of adsorbing different target analytes by modifying the surface of CNTs with different covalently or non-covalently functionalized groups [111–113].

Carbon functionalized materials, such as metal organic frameworks (MOFs) and porous organic polymers (POFs), are the most recent SBSE coatings based on carbon structures [72,95,105,114]. They are generally prepared by in-situ synthesis, chemical bonding and sol-gel technology. Since 2013, the use of MOFs as coatings has increased promptly, although some of them are sensitive to moisture and their structure is damaged due to the incorporation of water molecules inside, limiting their applications. To solve this limitation and improve the stability and dispersion of MOFs in aqueous solution, postsynthetic modification and combination with other materials are two valid methods [115–117]. Liu et al. [111] designed a covalent organic framework onto a polypropylene hollow fiber by polydopamine modification method as stir bar for the extraction of four benzophenones in soil and sunscreen samples. The main advantage of this method is to avoid using high concentrated sulfuric acid during the pretreatment process of the supporting material. Moreover, the immobilization of the covalent organic framework on the polypropylene fiber significantly provides it with multiple interaction abilities including hydrophobic interaction and π-π interaction.

In Wang's study [112], a SBSE/LC-UV method was applied for the determination of carbamate pesticides in corn and potato samples. As SBSE coating, three different porous organic frameworks were used, syhntesised by catalyst-free solvothermal method with different three aldehyde monomers containing different conjugated structures (1,4-phthalaldehyde, 4,4′-biphenyldicarbox-aldehyde and tris (4-formylphenyl) amine) and phloroglucinol as monomers. All of the porous organic frameworks were attached on glass stir bars by adhesion method. In another study [113], a sulfonic acid-functionalized covalent organic frameworks (COF-SO_3) was developed as SBSE coating to determine by LC-UV three fluoroquinolones from milk.

In view of this, exploring novel coatings for SBSE and its evolution is still an active filed of research. Like in SPE and SPME, mixed-mode ion-exchange materials have aroused attention as SBSE coatings and the different in-house materials are explained in detail in section 1.2.

1.1.4. Other techniques

Various techniques and miniaturized approaches, such as fabric phase sorptive extraction (FPSE) [118–120], stir cake sorptive extraction (SCSE) [121], stir bar sorptive-dispersive microextraction (SBSDME) [122], or the more recent stir extraction format capsule phase microextraction (CPME) [123], appeared from modifications of the previously described microextraction techniques mainly to solve some of their limitations and incorporate improvements.

FPSE, developed by Kabir and Furton, combines the features of SPE and SPME. FPSE utilizes sol-gel coating technology to immobilize a sorbent in a piece of fabric material, resulting in a microextraction device with high volume of porous sorbent chemically bonded to the substrate. When sol-gel sorbents are coated on fiberglass, they offer high thermal stability that allows thermal desorption of the extracted analytes. To extract the target analytes, FPSE membranes can be inserted directly into the sample matrix, such as whole blood, urine, whole milk, environmental water without any sample pretreatment [124–128].

SCSE combines the extractive capability of porous monolith with the advantages of stirring. SCSE utilizes a very simple preparation method. The monolithic cake is synthesized by in situ polymerization and then, the cake is inserted in an original unit (holder), which is constructed from a syringe cartridge and allows the magnetic stirring of the cake during the extraction process. Apart from is simplicity, the novel approach presents other benefits such as simple operation, high adsorbent capacity, cost-effective and environmentally friendly. Huang et al. [121] prepared a monolythic stir cake based on poly(vinylimidazole-divinylbenzene) to determine steroid hormones in milk samples by SCSE followed by LC-DAD.

SBSDME combines the principles of SBSE and dSPE, consisting in the use of a neodymium stir bar coated with a magnetic material as extraction device. The magnetic material of the promising hybrid solid phase-based microextraction approach acts as SBSE at low stirring rate, while at higher rate the magnetic nanoparticles are dispersed into sample solution, returning to the magnetic bar when the stirring is stopped. The magnetic material containing the target analytes is retrieved by the stir bar without requiring any additional magnetic field. Later, the analytes can be either desorbed in an appropriate solvent for further injection into LC or directly thermally desorbed into a GC [122]. SBSDME presents some advantages over SBSE and dSPE, such as reduced extraction time and more versatile sorbents by using different coated MNPs, easier extraction, easier post-extraction treatment and minimum manual intervention. For instance, SBSDME was applied for trace determination of triphenyl and diphenyl phosphate in urine of nail polish users [129] and pesticides from water samples [130].

Recently, Kabir and Furton introduced a novel of SBSE approach called CPME [123]. CPME is based on the principle of equilibrium extraction and uses a microextraction capsule as the extraction medium. The capsule encompasses two fused porous tubular polypropylene membranes, one accommodates the sorbent through sol-gel technology and the other encapsulates the magnetic metal rod. This microextraction format and their in-house prepared mixed-mode ion-exchange materials are explained in more detail in section 1.2 and section 3.2.2.

Sample preparation is in continuous growth and constantly novel trends of sample preparation are appearing. Therefore, the present scenario points out the rising of innovative approaches to carry out sample preparation procedures and new methodologies for sample pretreatment have been introduced [22,24,131]

1.2. Mixed-mode ion-exchange materials

As explained in the previous sections, many sorbents combined with the different extraction and microextraction techniques have been described. The research to attain more selective materials able to retain specific analytes is the up to date goal, being ionisable compounds a challenging task. In this way, the interest of novel materials such as mixed-mode ion-exchange sorbents appeared, to combine an improvement of capacity and selectivity towards ionizable compounds. They are developed by the incorporation of functional groups to a reverse-phase resin. They can be silica-based or polymer-based depending on the characteristics of the resin. The ionisable analytes can establish either ionic interactions with the ionic groups of the sorbent and reverse-phase interactions via the hydrophobicity of the resin, or polar interactions by Van der Waals forces, depending on the resin [21,36,132–134].

Different types of mixed-mode ion-exchange exist and feature different properties depending on the functional groups attached to the resin: weak and strong character positively and negatively charged (cationic or anionic exchanger). Weak anionic exchangers (WAX) comprise the functional groups formed by primary, secondary and tertiary amines, while strong anionic exchangers (SAX) are the ones where a quaternary amine is incorporated as the functional ionic group. Weak cationic exchangers (WCX) incorporate carboxylic acids and strong cationic (SCX) sulfonic acids. Weak ion-exchangers are charged on a specific pH range, having a pK_a value associated, and can tune their integration with ionic analytes by modifying the pH. Once either the functional group of the sorbent or the analyte is in neutral form the interaction is disrupted. On the other hand, strong ion exchangers are permanently charged regardless of the pH and solvent conditions used. Consequently, to disrupt sorbent-analyte interaction the analyte should be turned into a neutral form by controlling the pH, taking care about the pK_a values of the analytes.

Mixed-mode ion-exchange sorbents can be either universal or very selective depending on whether or not a washing step is used. They can be universal because the list of compounds that can be extracted from the samples is larger compared to other sorbents, thanks to the enhanced retention toward ionic compounds given by the presence of ionic functionalities. However, their selectivity can be increased towards ionic analytes if the compounds retained through interactions different from ion-exchange (for instance, reversed-phase interactions) are rinsed from the sorbents, using washing steps with organic solvents to disrupt these interactions. Thus, ionic compounds are eluted in the elution fraction separately from neutral ones.

Retention by ion-exchange interaction is influenced by the pH and ionic strength of the sample and elution solvent. The pH is crucial as the interaction that provides retention occurs between oppositely charged species. If the material presents a weak ion-exchange, we can change the ionization of the analyte or the sorbent by adjusting the eluent pH. Similarly to the effect of pH, we can weaken the electrostatic interaction between analyte and sorbent using an aqueous solution having appropriate ionic strength. Specific counter-ions can compete with the analyte for the binding sites on the sorbent, thus causing the analyte elution. The ionic strength of the eluting solvent and the sample are kept as low as possible to prevent competition for the charged sites on the sorbent [135].

1.2.1. Commercially available mixed-mode ion-exchange materials

There are several commercially available mixed-mode ion-exchange sorbents, and most of them have macroporous networks. To the best of our knowledge, all of them are based on previously commercialized polymeric sorbents modified with an ion-exchange group. The first commercially available mixed-mode polymeric sorbents were pioneered by Waters Corporation (Milford, MA,USA), which developed four novel sorbents for SPE: Oasis MCX (SCX), Oasis WCX (WCX), Oasis MAX (SAX) and Oasis WAX (WAX). All of these mixed-mode sorbents were based on the Oasis HLB skeleton and further chemically modified. For instance, Oasis MCX is prepared by sulfonation of the aromatic rings of Oasis HLB, whereas Oasis WCX is obtained from an intermediate chlorinated resin subsequently oxidized to carboxylic groups. Oasis MAX and Oasis WAX were also obtained from the chlorinated intermediate reacted with dimethylbutylamine (DMBA) and piperazine, respectively. Similarly, other mixed-mode polymeric sorbents have been commercialised, such as the Bond Elut Plexa family sorbents by Agilent Technologies (Santa Clara, CA, USA) the Strata-X family sorbents by Phenomenex (Torrence, CA, USA) the Evolute family sorbents by Biotage (Uppsala, Sweden), the AttractSPE (Polyintell) and the Chromabond HR (Macherey-Naagel). Commercialised mixed-mode polymeric sorbents present a macroporous structure with a specific surface area over 800 m^2/g. There are numerous examples in which these commercial mixed-mode sorbents have been used to exploit the selectively toward ionizable compounds and to minimize interferences from complex matrices such as biological fluid, foodstuff, or sewage water [133]. Table 2 details the commercially available mixed-mode ion-exchange sorbents and some of their applications in environmental, biological and food analysis. Some of them, from the studies reported in literature, have not been applied either to environmental samples or biological or food analysis.

In the next section a review devoted to the in-house mixed-mode ion-exchange materials prepared for SPE, SPME, SBSE and other related techniques is enclosed in paper format since it was already published in the first volume of the new journal Advances in Sample Preparation 1 (2022) 100008.

Table 2. Commercially available mixed-mode ion-exchange sorbents and their applications.

Sorbent	Supplier	Group	Application
Isolute Hax*	Biotage	C_8-SAX	Urine and plasma
Bond Elut Certify II*	Agilent Technologies	C_8-SAX	Blood
Oasis MAX	Waters	SAX	Aerosol water samples, harbour water, stormwater, hair
Strata X-A	Phenomenex	SAX	Harbour water
Bond Elute Plexa PAX	Agilent Technologies	SAX	Stormwater, urine
Cleanert PAX	Bonna-Agela Technologies	SAX	Sesame oil
Evolute AX	Biotage	SAX	
AttractSPE SAX	Affinisep	SAX	
Chromabond HR-XA	Macherey-Nagel	SAX	
Extrabond EAX	Scharlau	SAX	
HyperSep SAX	Thermo Scientific	SAX	
Oasis MCX	Waters	SCX	Tap and river water, effluent and influent wastewater, urine, chicken tissue, eggs
Strata X-C	Phenomenex	SCX	Tap and harbour water, effluent and influent wastewater, saliva, human serum, pharmaceuticals

Table 2. (Continued).

Bond Elute Plexa PCX	Agilent Technologies	SCX	Urine
Cleanert PCX	Bonna-Agela Technologies	SCX	Sesame oil
Evolute CX	Biotage	SCX	Surface and effluent wastewater
AttractSPE SCX	Affinisep	SCX	
Chromabond HR-XC	Macherey-Nagel	SCX	Surface and ground water, influent wastewater
Extrabond ECX	Scharlau	SCX	
HyperSep SCX	Thermo Scientific	SCX	
Oasis WCX	Waters	WCX	River water, effluent and influent wastewater, milk
Strata X-WC	Phenomenex	WCX	Tap and surface water, effluent wastewater
Abselut NEXUS WCX	Agilent Technologies	WCX	
Cleanert PWCX	Bonna-Agela Technologies	WCX	
Evolute WCX	Biotage	WCX	
AttractSPE WCX	Affinisep	WCX	
Chromabond HR-XCW	Macherey-Nagel	WCX	
Oasis WAX	Waters	WAX	Tap, surface, ground and river water, effluent and influent wastewater, jelly and gummy candy, fish, vegetables, amended soil
Strata X-WA	Phenomenex	WAX	Tap, surface and ground water, effluent and influent wastewater
Cleanert PWAX	Bonna-Agela Technologies	WAX	
Evolute WAX	Biotage	WAX	
AttractSPE WAX	Affinisep	WAX	
Chromabond HR-XAW	Macherey-Nagel	WAX	

[*] Silica-based

1.2.2. Novel in-house mixed-mode ion-exchange materials for sorptive phase extraction techniques

Novel in-house mixed-mode ion-exchange materials for sorptive phase extraction techniques

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Abstract

Mixed-mode ion-exchange materials have been implanted in different sorptive extraction techniques as they combine capacity and selectivity in a single material. They are a good option for concentrating ionic (or ionisable) compounds from complex samples using an appropriate washing step that simplifies the sample matrix. Research into the development of in-house mixed-mode ion-exchange materials is therefore a field of scientific interest. These materials are developed with the aim to improve the features of both the core and the ionic moieties attached. The study of the optimal extraction protocol to exploit the ionic interactions between the material and the target compounds is a key point in method development.

This review is an overview of the preparation, characterisation, evaluation and application of in-house mixed-mode ion-exchange materials. It mainly covers the technique of solid-phase extraction; however, the latest advances in solid-phase microextraction, stir bar sorptive extraction and other emerging microextraction techniques are also presented. Examples of different applications that cover the extraction of different compounds in several types of samples are also illustrated.

Keywords: *Mixed-mode ion-exchange; complex samples; solid-phase extraction; solid-phase microextraction; stir bar sorptive extraction; ionisable compounds*

1. Introduction

Trace analysis of organic contaminants is always challenging due to the complexity and diversity of the sample matrices. In spite of the recent advances in analytical instrumentation, especially in chromatography and mass spectrometry (MS), analysing complex samples requires sample treatment before the chromatographic analysis. By including a sample treatment, the complexity of the extract is simplified to make it easier to separate the target analytes from other components [1]. Several treatment techniques have been used in the past decades to extract organic compounds from different types of samples. For liquid samples, although solvent extraction techniques have been miniaturised to achieve high enrichment factors, minimise solvent consumption and reduce waste, sorptive extraction techniques are the preferred extraction techniques. The most commonly used sorptive extraction techniques are solid phase extraction (SPE) followed by solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE), but other techniques such as fabric phase soptive extraction (FPSE), stir bar sorptive-dispersive microextraction (SBSDME), rotating disk sorptive extraction (RDSE),disposable pippette extraction (DPX), capsule phase microextraction (CPME) and stir-cake sorptive extraction (SCSE), among others, have recently been developed [2–8].

The selectivity and capacity of the sorptive extraction techniques are mainly determined by the material, and a wide variety of them have been exploited. In SPE, the first materials were silica-based, which are usually modified with C_{18} to be used to extract apolar compounds. Nevertheless, they show limited recovery for polar and ionisable compounds [9]. Later on, carbon-based sorbents were introduced, although their high retention hinders the elution of analytes. Polymer-based sorbents were developed to improve the properties of the previous materials. The first one was based on polystyrene-divinylbenzene; however, its hydrophobic character is a limitation to trapping the most polar compounds [10]. New generations of polymeric materials, namely hydrophilic polymer sorbent and hypercrosslinked polymers, emerged to enhance capacity [11]. To promote selectivity, molecularly imprinted polymers were synthesised to extract selectively target analytes [12]. In addition, other emerging materials, such as metal-organic frameworks, magnetic materials, nanomaterials, etc., have been developed [13,14].

For other microextraction techniques, materials based on polydimethylsiloxane (PDMS) were the first to be introduced in both SPME and SBSE; however, they lack retention for the most polar compounds. Other materials have gradually appeared to solve this drawback, such as polyacrylate (PA), carbowax (CW) and carboxen (CAR) for SPME; and ethyleneglycol-Silicone and PA for SBSE [15].

The need to combine in a single material an improvement of capacity and selectivity towards ionisable compounds evidences the interest in developing novel sorbents, such as mixed-mode ion-exchange sorbents. These are developed by incorporating ionic functional groups in a hydrophobic and/or hydrophilic base. Depending on the properties of the material, the ionisable analytes can establish either ionic interactions with the ionic groups of the material and hydrogen bonding, dipole-dipole or Van der Waals forces with the skeleton [16,17]. The materials to prepare the mixed-mode ion-exchange sorbent are silica- and polymer-based, and most commonly are the polymeric ones [18,19]. Moreover, there are different types of mixed-mode ion-exchange materials that have different properties depending on the functional groups attached to the resin: strong anion-exchange (SAX), strong cation-exchange (SCX), weak anion-exchange (WAX) and weak cation-exchange (WCX). On one hand, strong ion-exchangers are charged throughout the pH range and comprise sulfonic acids or quaternary amines, and are cationic or anionic exchangers, respectively. On the other hand, weak exchangers tune their ionisation state in accordance with the pH, since they are functionalised with carboxylic acids or tertiary, secondary or primary amines, and are cationic or anionic exchangers, respectively.

It is crucial to select the appropriate SPE protocol to achieve good results when using mixed-mode ion-exchange sorbents. For instance, the common SPE protocol for SCX sorbents uses an acidic (pH 2-3) sample so that the basic analytes are in their protonated form, and uses a basic elution medium (normally 3-5% NH_4OH in MeOH) to break any ionic interactions between the analytes and the sorbent, neutralising the basic analytes. Conversely, in the general SPE protocol for SAX sorbents, the sample is always loaded at pH 7-9 to deprotonate the acidic compounds and establish ionic interaction with the quaternary amine groups of the sorbent. The elution of the acidic compounds is performed at acidic pH to protonate the compounds and disrupt the ionic interaction. In the case of WAX or WCX, the recommended protocol uses a neutral pH to load the sample so that both the functional groups of the sorbent and the analytes are in ionic form. As an elution solution, normally an acidified organic solution is employed for WCX sorbents to neutralise the carboxylic acid groups of the sorbent; and a basic elution solution in organic solvent is used for WAX sorbents to neutralise the amine groups of the sorbent. These are general protocols, but they should be optimised depending on the pK_a of the analytes and the sorbents.

Mixed-mode ion-exchange sorbents can be either universal or very selective. They can be universal because numerous compounds can be extracted from samples due to the enhanced retention towards ionic compounds given by the presence of ionic functionalities. In addition, their selectivity can be increased towards ionic analytes if the compounds retained through other type of interactions are rinsed from the sorbents using washing steps with organic solvents to disrupt these interactions [16,17,20].

Although several commercial mixed-mode ion-exchange sorbents have been developed, in recent years different in-house sorbents have been prepared. This review will focus on the synthesis, characterisation and application of in-house mixed-mode ion-exchange materials to different sorptive techniques, being the SPE the most used, in different types of samples.

2. Solid-phase extraction

There is an increasing amount of mixed-mode ion-exchange materials due to the challenging need to improve the SPE sorbents. Although commercial mixed-mode ion-exchange sorbents, based on Strata-X, Oasis, Discovery and the Bond Elut series, among others, are available and have been studied by the analytical chemistry community [21–25], the necessity to enhance their capacity and selectivity properties has led to the development of in-house mixed-mode ion-exchange sorbents. Various materials are applied as in-house sorbents for SPE, most commonly silica and polymer based materials. This section is divided into mixed-mode cation-exchange, mixed-mode anion-exchange material and zwitterionic sorbent subsections. Each subsection covers the preparation, characterisation, evaluation and application of each type of material. Table 1 details the different in-house mixed-mode ion-exchange materials used in SPE, their optimal conditions and their applications.

2.1. Mixed-mode cation-exchange sorbents

Mixed-mode cation-exchange sorbents contain sulfonic acid or carboxylic acid moieties as functional groups anchored to the backbone, and have a SCX or WCX character, respectively. There are diverse synthetic methods for obtaining in-house mixed-mode cation-exchange. Regarding the polymeric backbone, traditional polymerisation methods, such as precipitation polymerisation (PP) [26–28], bulk polymerisation [27], dispersion polymerisation [28] and non-aqueous dispersion polymerisation [28] are mainly used to prepare polymer-based mixed-mode materials. These can be prepared by copolymerisation followed by a second step of functionalisation to incorporate the functional group, or by copolymerisation with a monomer that already contains the functional group. Studies that apply these in-house materials as SPE sorbents for the extraction of analytes in complex matrices are presented in Table 1. For instance, Gilart et al. [29] prepared two different cation-exchange polymeric materials employing different polymerisation approaches. One was prepared by copolymerisation of the two monomers, 2-hydroxyethyl methacrylate (HEMA) and divinylbenzene (DVB), followed by a post-sulfonation with H_2SO_4 to obtain a SCX polymer. The monomers gave the polymer hydrophilicity due to the ester and hydroxyl groups in the HEMA and also allowed π-π interactions due to the aromatic rings in the DVB. The other material was prepared by the copolymerisation of three monomers: 2-acrylamido-2-methylpropane sulphonic acid (AMPSA), HEMA (functional monomers) and pentaerythritol triacrylate (PETRA) (cross-linker). HEMA and PETRA provided hydrophilicity to the material, and AMPSA provided an ionic character since it contains a secondary amide group and a sulphonic acid in its structure. The materials had different ion-exchange capacities, 0.84 mmol g^{-1} for the

Table 1. Examples of application of SPE in-house mixed-mode ion-exchange materials.

Type	Coating	Analytes	Sample	Conditions	Washing	% Recovery	Determination technique	Ref.
SCX	HXLPP-SCX sulphonated with H_2SO_4	Pivotal purine metabolites	Human serum	L: 1 mL with HAc (0.13%) E: 3 mL of 5% NH_4OH in MeOH	3 mL of H_2O + 3 ml of MeOH	91-103	LC-UV	[27]
SCX	HXLPP-SCX and HXLNAD-SCX functionalized with sulphonic groups	Drugs	EWW and IWW	L: 250 mL at pH 3 E: 5 mL of 5% NH_4OH in MeOH	5 mL of MeOH	75-100	LC–MS/MS	[28]
SCX	Sulphonated HEMA/DVB AMPSA/HEMA/PETRA	Drugs	EWW and IWW	L: 50 mL (EWW) and 25 mL (IWW) pH 3 E: 3 mL of 5% NH_4OH in MeOH	5 mL of MeOH	39-98	LC-MS/MS	[29]
SCX	GMA-EGDMA-SO_3^-	Alkylated purine adducts	Human urine	L: 2 mL pH acid E: 4 mL of 5% NH_4OH in MeOH	3 mL 2% HCOOH in H_2O+3 mL MeOH/H_2O (50/50, v/v)	90-105	LC-MS/MS	[30]
SCX	Si–C_8–SO_3^-	Sulphonamides	Milk	L: 1mL pH 2.5 (5mM phosphate buffer) E: 0.15 mL of mobile phase (ACN/20mM NH_4Ac, 24/76, v/v)	0.08 mL of0.2% HCOOH in H_2O	40-92	LC-UV	[32]
SCX	Sulphonic acid-functionalized covalent organic frameworks anchoring Ni particles	Benzimidazoles	Vegetables, fruits and juices	L: 10 mL acidic aqueous sonicated for 2 min E: 3 mL ACN/NH_4OH (95:5, v/v) for 2 min	2 mL of H_2O + 2 mL of MeOH	80-115	LC-UV	[31]
SCX	SBA-15-C_{18}-SO_3^-, SBA-15-C_8-SO_3^-	Veterinary drugs	Meat	L: 2g→10mL ACN/5% TCA in H_2O (50/50, v/v) E: 2 mL ACN + 2 mL MeOH + 2x2 mL 3% NH_4OH in MeOH	5 ml of acetate buffer	70-100	LC-MS/MS	[33]
WCX	HXLPP-WCX (MAA)	Basic pharmaceuticals	River water	L: 1000 mL at pH 7 E: 5mL of 2% TFA in MeOH	2 mL of 5% NH_4OH in MeOH	54-92	LC-UV	[26]

Table 1. (Continued).

WCX	Maleic acid-DVB	Basic pharmaceuticals	River, EWW and IWW	L: 250 mL (river) or 100 mL (EWW) or 50 mL (IWW) at pH 7 E: 5 mL of 15% HCOOH in MeOH	5 mL of MeOH	57-89	LC-MS	[34]
SAX	Mesoporous silica SBA-15 functionalised with 3-[2-(2- aminoethylamino)ethylamino]propyl-trimethoxysilane and phenyl glycidyl ether	NSAIDs	Tap and river and WW	L: 500 mL at pH 7.2~7.8 E: 3 mL 1% HAc in hexane/EtOAc (3:1)	4 mL of MeOH/25 mM pH 7 phosphate buffer (1:1, v/v)	81-111	LC-UV	[9]
SAX	SiO_2@DEAEMA-DVB func TEA-(DEAEMA)	Drugs	EWW	L: 500 mL pH 7 E: 9 mL MeOH + 3 mL 1% HCOOH in MeOH	3 mL 10% HAc H_2O + 9 mL MeOH	82-101	LC-UV	[20]
SAX	HXLPP-SAXa and HXLPP-SAXb (TEA)	Acidic pharmaceuticals	River and EWW	L: 100 mL at pH 7 E: 10 mL of 10% HCOOH in MeOH	10 mL of MeOH	60-90	LC-UV	[35]
SAX	HXLPP-SAX (TEA)	Fluoroquinolones	Milk	L:1 g→0.2 mL H_2O E: 3mL of 10% HAc in MeOH	3 mL of ACN +3 mL of MeOH/H_2O (75/25, v/v)	86-118	LC-UV	[36]
SAX	VBC-EGDMA func TEA, Imidazole, Piperidine, Pyrrolidone	Estrogens and NSAIDs	Tap and river	L: 200 mL at pH 7 E: 4.5 mL of MeOH + 4.5mL 2% TFA in MeOH	1mL of H_2O+15 min drying	53-94	LC-UV	[37]
SAX	SiO_2@DEAEMA-DVB func DMEA	NSAIDs	Urine	L: 3 mL E: 4 mL of 1% HCOOH in MeOH	5 mL of MeOH/50 mM NaAc aq (5/95, v/v) +5 mL of MeOH	85-104	LC-UV	[38]
SAX	Bi-functionalized hexagonal mesostructured silicas with C_8 and NR_4^+ groups	Polyphenols	Fruit and vegetables based HPP juice	L: 5 mL at pH 9 stirred for 20 min at 300 rpm E: 6 mL of MeOH/water (95/5,v/v) at pH 1		70-101	UHPLC-MS/MS	[69]
SAX	Periodic mesoporous organosilica with (styrylmethyl)bis(triethoxysilylpropyl)ammonium chloride (PMO-STPA) and with bis(3-triethoxysilylpropyl)amine (PMO-TEPA)	Phenoxy acid herbicides	River and EWW	L: 750 mL E: 2 x 4 mL of MeOH at pH 5.6 (PMO-STPA) or 1.8 (PMO-TEPA)		78-108	CE-DAD	[70]

Table 1. (Continued).

SAX	Ionic liquid crosslinked polymer-supported imidazolium trifluoroacetate salt (IL-CF_3COO^-) and VBC-DVB-IL (methylimidazolium)	Pharmaceuticals	Tap, river and EWW	L: 1000 mL (tap and river) or 250 mL (EWW) pH 7 E: 10 mL of 5% HCOOH in MeOH	20 mL of MeOH	55-101 and 63-100	LC-UV	[71]
SAX	Si–C_{18}/-SAX	Aristolochic acids	Herbs	L: 100 g herbs → 2L of 5 mM K_2HPO_4/MeOH (50/50, v/v) E: 7mL 5% HCOOH in MeOH	5 mL of H_2O + 5 mL of MeOH	67-105	LC-UV	[72]
SAX	Si-IL (1-Alkyl-3-(propyl-3-sulphonate) imidazolium)	Organic acids, amines and aldehydes	Atmospheric aerosol water	L: 0.5 mL E: 0.5 mL of 10% HAc in H_2O (acids) 0.5mL of 10% HAc in MeOH (amines)	1mL of H_2O	87-110	LC-MS GC-MS	[73]
SAX, SCX	Methacrylate based resin functionalized with sulphoethylbetaine	Ionic polar solutes (nucleobases, acids and zwitterions)	Standard solutions	L: 10 mL E:10 mL of H_2O/ 0.2% TFA/ 20 mM NH_4Ac	2 mL of ACN	4-105	LC-UV	[74]
SAX, SCX	1-Alkyl-3-(propyl-3-sulphonate) imidazolium-functionalised silica	Aromatic amines	EWW, IWW and soil (mineral and humus) samples	L: 500 µL, 6 mL or 10 ml E: 500 µL of H_2O	1 mL of hexane	9-97	LC-UV	[75]
WAX	HXLPP-WAX functionalized with 1,2-ethylenediamine and piperazine.	NSAIDs	River and EWW	L: 1000 mL (river) or 250 mL (EWW) at pH 7 E: 2 ml of 2% NH_4OH in MeOH:ACN (1:4, v/v)	4 ml of MeOH	77-101	LC-UV	[19]
WAX	Dendrimer aminopropyl functionalized mesoporous silica KIT-6	Acidic drugs	Human urine	L: 3 mL at pH 7 E: 3 mL of 1% HAc hexane/EtOAc (3:1, v/v)	5 mL 25 mM phosphate (pH 7.0) in 5% MeOH + 1 mL EtOAc	86-114	LC-DAD	[39]
WAX	Silica gel covered with polyaniline	Alkaloids	Roots and shoots of Chelidonium majus	L:1 mL E: 5 mL of 0.1 M NH_3 in MeOH	2 mL of H_2O/MeOH (1/1, v/v)	97-99	LC-DAD	[40]
WAX	Si-tris(2-aminoethyl) Amine-3-phenoxybenzaldehyde)	Chlorophenols	Tap and surface water	L: 20 mL at pH 8 E: 0.5 mL of 1% HAc in MeOH		89-110	LC-UV	[41]

Table 1. (Continued).

WAX	Si-tris(2-aminoethyl) Amine-3-phenoxybenzaldehyde)	Chlorophenols	Tap and surface water	L: 20 mL at pH 8 E: 0.5 mL of 1% HAc in MeOH		89-110	LC-UV	[41]
WAX	CB [6]-NH$_2$@SiO$_2$@Fe$_3$O$_4$ (Curcubit [6] uril)	Salvianolic acids	Natural plant water	L: 1 mL at pH 5.5 E: 1 mL 1% HAc in MeOH/H$_2$O (60/40, v/v)		95-106	LC-UV	[42]
WAX	APTES@SiO$_2$@Fe$_3$O$_4$ (3-aminopropyltriethoxysilane)	Chlorophenoxyacetic	Well and surface water	L: 25 mL at pH 5 E: 3x2 mL 1% NH$_4$OH in MeOH	2 mL of H$_2$O	80-100	LC-UV	[43]
WAX	Fe$_3$O$_4$@nSiO$_2$@C$_{18}$@NH$_2$	Lipophilic marine biotoxins	Shellfish	L: 4 mL at pH 7.0, vortexed for 30 s and shaken for 5.0 min E: 2.0 mL HCOOH/MeOH (5/95, v/v) for 1 min	2 mL H$_2$O + 2 mL MeOH	83-119	UHPLC-MS/MS	[76]
WAX	Nanocomposite of silica-polydiphenylamine doped with silver nanoparticles	Pesticides	Well water and tap water	L:50 mL at pH 7 at 4 mL/min E: 500 µL of MeOH at 0.1 mL/min	1 mL of H$_2$O	86-103	GC-MS	[77]
WAX/WCX	Si-ampholine	Acidic and basic compounds	Beverage samples	L: 10 mL pH 4–6 (acids), pH 6 (basics) E: 1 mL H$_2$O/MeOH (50/ 50, v/v) pH 11 (acids), 1 mL H$_2$O/MeOH (50/ 50, v/v) pH 1 (bases)	1mL of H$_2$O/MeOH (50/50, v/v)	84-110	LC-UV	[44]
WAX/WCX	HXLPP-WAX/WCX	Acidic and basic compounds	River and EWW	L: 100 mL at pH 6 E: 5 mL of 5% NH$_4$OH in MeOH	1 mL of MeOH	32-88	LC-UV	[45]
WAX/WCX	Ternary mixed-mode Si–COOH/-NH$_2$	Drugs	Human serum	L: 2 mL at pH 6 E:2 mL of MeOH followed by 2 mL of 5% HCOOH in MeOH	3 mL of 5% MeOH in 20 mM phosphate buffer (pH 6).	92-117	LC-UV	[46]
SAX/WCX and WAX/SCX	HXLPP-SAX/WCX and HXLPP-WAX/SCX	Acidic and basic compounds	River and EWW	L: 500 mL at pH 6 E: 5 mL of 5% NH$_4$OH in MeOH	1 mL of MeOH	18-105	LC-UV	[47]

AMPSA/HEMA/PETRA sorbent and 2.05 mmol g^{-1} for the HEMA/DVB sorbent. The last of these is the material with the most ion-exchange sites and surface area to keep the analytes strongly retained. This was confirmed by the high recoveries of the analytes when wastewater samples were extracted.

The sulfonation process to develop a SCX material was carried out beforehand mainly using alkyl sulfates, such as acetyl sulfate and lauroyl sulfate [28,31], but these methods have many drawbacks. Acetyl sulfate has a low cation-exchange capability, implying a limited number of functional groups that can be introduced through the sulfonation reaction. Although lauroyl sulfate has shown to be a better sulfonating reagent, it needs to be freshly prepared before the sulfonation process by using chlorosulfonic acid, a very toxic chemical, and there are some issues for obtaining and working with it. Another sulfonation method is to use concentrated H_2SO_4, which is much simpler than using alkyl sulfate reagents. Moreover, using H_2SO_4 does not involve sulphonating or highly toxic reagents. H_2SO_4 has been successfully applied for the sulphonation of conventional methods, such as PP [27,28,30].

Silica-based mixed mode ion-exchange materials are generally prepared in a single step by means of adding the reagent with an ionic moiety into the solution mixture. The procedure involves the previous activation of the silica with NaOH and/or HCl, followed by a polycondensation of the monomers via sol-gel technology. The sol-gel process offers high permeability, high mechanical strength, and good organic solvent tolerance. For instance, Zheng et al. [32] developed a hybrid silica monolith functionalised with octyl and thiol moieties in a single step with the sol-gel approach. After oxidisation using H_2O_2 to yield sulfonic acid groups, the mixed-mode ion-exchange silica monolith provided a hydrophobic and SCX character and was evaluated by µ-SPE to extract sulphonamides (amphoteric compounds) from milk samples. The sample matrix was adjusted to pH 2.5 since sulphonamides exist in protonated forms at acidic pH and they could interact with the sulfonic acid groups of the silica monolith via cation-exchange interactions. This material exhibited a good extraction capacity (recoveries ranging from 40 to 92 %) and simplified the milk matrix of protein and fat, thus improving sulphonamide determination.

Other networks for developing mixed-mode ion-exchange materials for SPE sorbents, like covalent triazine-based organic frameworks (CTFs), have recently appeared. CTFs are of increasing interest as separation media for chromatography and sample pretreatment due to their uniform and adjustable pore size, effective post-synthetic functionalisation and their stable triazine-benzene structure that allows the materials to endure acid or basic conditions, which is not the case for other porous materials. The CTFs are generally prepared via cyclotrimerisation of 1,4-dicyanobenzene in molten $ZnCl_2$. In one study [31], the authors generated a SCX sorbent using CTFs. Once the CTFs were synthesised, they were functionalised with chlorosulfonic acid to incorporate the sulfonic acid into the CTF matrix, obtaining CTF-SO_3H. Afterwards, Ni particles were anchored on the CTF support through a magnetising process followed by an in situ reduction. This generated a robust magnetic hybrid SPE sorbent (Ni/CTF-SO_3H) with high density and easily accessible ion-exchange sites in a three-dimensional nanospace. The Ni/CTF-SO_3H material showed very good results (recoveries of 80-115%) for the extraction of alkaline benzimidazoles in vegetables, fruits and juices, promoting efficient SCX and reversed-phase interactions, as benzimidazoles contain hydrophobic aromatic rings and protonated nitrogen atoms that interact through both ways with the sorbent.

Regarding the extraction protocol, as mentioned above, the loading conditions for SCX materials involve the sample being adjusted at acidic pH, while the elution conditions use basic additives. Moreover, the washing step is an important process of the SPE to keep in mind. Table 1 details the washing conditions used in the suggested examples. As it can be seen for mixed-mode SCX sorbents, organic solvent, such as MeOH [28,29] and acidified aqueous solution (acidified with HCOOH or HCl) followed by organic solvent [27,30,31] are generally used as washing solutions to remove the compounds retained in the sorbent by reverse-phase interactions (i.e. acidic and neutral compounds), keeping the basic compounds still bound to the sorbent by cation-exchange interactions. In a previous study by our research group [28], the HXLNAD-SCX that was applied to extract a group of basic drugs from effluent and influent wastewater, a washing step with 5 mL MeOH was employed to break the reversed-phase interactions and consequently remove the acidic drugs in the washing fraction. Negligible matrix effects (ME) (below 20%), were obtained when 50 mL and 25 mL of effluent and influent sewage water samples were analysed respectively. This is related to the high selectivity achieved towards the selected analytes. In another example, Hu et al. [30] used 3 mL of 2% HCOOH aqueous solution (pH 2.0) followed by 3 mL of 50% aqueous MeOH solution as washing solutions when extracting by SPE alkylated purine adducts in 2 mL of urine sample using a SCX sorbent (sulphonate functionalised poly(glycidyl methacrylate-divinylbenzene). The acidic aqueous washing solution removed the protein and salt from the urine sample and the organic washing solution was applied to wash off the weakly polar compounds and the undissociated acidic compounds [30]. Some studies only used aqueous-based washing solutions to remove interferents [32,33], and since these washing solutions are weaker than organic-based ones, less interferents are removed from the matrix and, thus, the selectivity is affected.

The synthetic methods to prepare WCX materials are similar to those used for SCX materials. There are fewer studies that prepare and apply WCX materials compared to SCX materials; they are shown in Table 1. In the SPE procedure of the WCX sorbents an acidic pH is not suitable for retaining the basic analytes because the carboxylic acid groups of the sorbent are primarily in their non-ionised form and no ionic interactions are established under this pH. Therefore, sample loading should be at neutral pH so that the carboxylic acid would be in the ionic form. Bratkowska et al. [26] prepared hypercrosslinked polymeric WCX resin by PP with the monomers vinylbenzylchloride (VBC), DVB and methacrylic acid (MAA), acquiring the cation-exchange character by means of the MAA residues. pH 7 was the optimal conditions to load the samples when a polymeric WCX sorbent was used to extract, in this case, basic pharmaceuticals in 500 mL of river water and 250 mL of effluent wastewater. To elute the basic analytes, an acidic solution, such as 5 mL of 2% trifluoroacetic acid (TFA) in MeOH was found to be the best eluent since acidification protonates the carboxylic acid residues in the sorbent, breaking the cation-exchange interactions. Basified MeOH was used as the washing solution to maintain the desired ionisation state of the analytes and the sorbent [26]. In another study [34], a WCX material was developed by functionalising a mesoporous DVB resin with maleic acid for extracting a group of basic drugs in environmental samples. The functionalisation was carried out through a Diels-Alder reaction with maleic anhydride, and posterior basic hydrolysis generated the carboxyl groups on the polymer surface. A neutral pH was used to load the sample, pure MeOH in the washing step, thus reducing the %ME to values between -30% and 7%, and eluting the analytes with 5 mL of 15% HCOOH in MeOH. The apparent recoveries in 250 mL of river water, 100 mL of effluent wastewater and 50 mL of influent wastewater ranged from 63-89%, 62-80% to 57-74%, respectively.

To the best of our knowledge, no in-house mixed-mode WCX using silica-based materials has been developed so far.

2.2. Mixed-mode anion-exchange sorbents

To prepare mixed-mode anion-exchange sorbents for SPE, similar synthetic procedures to the cation-exchange procedures for functionalising silica and polymeric supports are employed but obviously using different reagents/monomers to obtain the materials with an anion-exchange character. Table 1 shows studies that prepare and evaluate anion-exchange materials applied as SPE sorbents, as well as their SPE protocol conditions. For polymer-based anion-exchange materials, most of the authors chose PP using DVB and VBC as monomers [35,36]. However, Meischl et al. [37] employed PP using VBC and ethylene glycol dimethacrylate (EGDMA) as monomer and crosslinker, respectively, since the incorporation of EGDMA is a suitable tool for interacting with more polar compounds, obtaining high recoveries and enrichment of non-steroidal anti-inflammatory drugs (NSAIDs). The use of VBC made it possible to further functionalise the material through nucleophilic substitution reactions. In this case, the functionalisation was carried out with imidazole, but other authors used dimethylbutylamine (DMBA) [35] or trimethylamine (TMA) [36]. Liang et al. [36] achieved the quaternisation and hypercrosslinking reactions in one single step, reducing the reaction time and simplifying the procedure. In most cases these reactions need two or more steps. Other authors [38] obtained SAX materials by copolymerisation of 2-(diethylamino)ethyl methacrylate (DEAEMA) and DVB generating aminated polyDVB microparticles, which were then functionalised with hyperbranched macromolecules and successive reactions to produce multiple cationic quaternary amines. In this way, a highly branched three-dimensional framework with large mesopores, well-defined particle sizes and a large number of functional groups was developed. The Pickering emulsion polymerisation, used by Huang [20], is another approach for preparing a spherical and porous SAX sorbent. Pickering emulsion is the process in which solid particles are employed to stabilise the emulsion. It requires the self-assembly of the particles at the interface of two immiscible liquids to reduce the interfacial energy of the system. Compared to conventional surfactants, Pickering emulsifier possesses a number of advantages, including the reduced foaming problem and lower toxicity. The reagents 1,4-butanediol diglycidyl ether (BDDE) followed by triethylamine (TEA) were used to functionalise the material.

The recommended SPE protocol for SAX resins suggests loading sample at neutral pH so that the acidic analytes (in their ionic form at this pH) can cationically interact with the quaternary amine of the SAX sorbent. Li et al. [9] applied this protocol and used pH 7.2-7.8 to deprotonate the acidic analytes (a group of NSAIDs) to be retained in the SAX. The eluent, 1% HAc in hexane/EtOAc (3/1, v/v), was used to protonate the acidic analytes and disrupt the ionic interaction with the resin. All the SPE conditions are presented in Table 1.

As discussed previously for the cation-exchange materials, the washing step, normally using organic solvents, should be included to promote selectivity. In one study [38], described in Table 1, two different washing solutions were used when acidic NSAIDs were extracted in 5 mL of urine sample using a SAX cartridge. Since the interferences were retained either by ionic or reversed-phased interactions, they could be selectively washed off. Therefore, two separate washing solutions were employed: 3 mL of 15% acetic acid in water to remove polar interferences, followed by 5 mL of 5% MeOH in 50 mM NaAc (pH 7.0) to further deprotonate the acidic analytes to enhance interactions with the sorbent; and a second washing with 5 mL of MeOH to remove basic and neutral interferences retained via hydrophobic interaction. The results obtained (%R ranged from 85% to 104%) indicated the excellent ability of the sorbent to selectively extract NSAIDs from complex samples. In another study [20], where a SAX resin was used to extract a group of neutral, acidic and basic compounds from complex environmental water, similar washing solutions (aqueous with acidic additive plus a methanolic one) were also applied; however, the second one was used to elute the neutral and basic compounds and not to remove them.

Metal organic frameworks (MOFs) have recently increased in interest for the analytical community due to their mesmerising structures and unique properties, including high surface areas, good chemical resistance, favourable thermal stability and well-defined pore structures. A study by Li et al. [39] prepared a SAX dendrimer-functionalized mesoporous silica material from a MOF material (KIT-6). The SAX material was evaluated by SPE to extract acid drugs (ketoprofen, naproxen and ibuprofen) from 3 mL of urine sample. Two washing solutions, 5 mL of 25 mM phosphate (pH 7.0) in 5% MeOH and 1 mL of ethyl acetate (EtOAc), were applied. In the loading and the first washing step, high molecular weight matrix components, such as proteins, were size-excluded thanks to the small pore diameter (7.9 nm) of the sorbent. In the second washing step, the neutral matrix interferences were washed off. At the end, most matrix interferences were removed and a very clean extract was obtained. Recoveries were in the range of 86 to 114% for the selected acidic compounds.

Similar methodologies used for SAX sorbents were used for WAX sorbents. Table 1 lists some examples of WAX sorbents and their applications. For instance, Wójciak-Kosior [40] prepared a silica WAX sorbent based on polyaniline by in situ polymerisation of aniline on the silica gel resin. The sorbent was applied for the SPE of leaf and root extracts from Chelidonium majus to determine alkaline plant metabolites such as benzophenanthridine, protoberberine and protopine alkaloids. The SPE protocol used 2 mL of H_2O/MeOH (1/1, v/v) as a washing solution and 5 mL of 0.1M of NH_4OH in MeOH as elution solvent. Using optimised conditions, the selected alkaloids attained recoveries over 96%. In another study, Gao et al. [41] developed a silica-based material with WAX properties functionalised with tris(2-aminoethyl)amine and 3-phenoxybenzaldehyde, introducing amino and phenyl groups attached to the surface of silica. The material was functionalised in two sequential steps by adding the functional molecules to the SiO_2-Cl resin to obtain the mixed-mode ion-exchange material. The retention properties of the material were evaluated with chlorophenols, some of them with an acidic character, in 20 mL of tap, river, wetland and lake water. This demonstrated that the established protocol was applicable to water sample analysis (%R between 89% and 110%).

Our research group [19] prepared two different WAX polymer-based materials based on hypercrosslinked polymer resins modified chemically with 1,2-ethylenediamine and piperazine moieties by PP using VBC and DVB as monomers. Under optimal conditions (see Table 1), the 1,2-ethylenediamine (EDA) functionalised material attained better recoveries (67%-109%) than the piperazine material for determining the selected acidic model compounds from river water and effluent wastewater samples. The basic elution solution (2 mL of 2% NH_4OH in 1:4 MeOH:ACN) ensured the deprotonation of the functional moieties of the sorbent and consequently the elution of the acidic compounds.

WAX materials using magnetic nanoparticles were also prepared and evaluated. For instance, Zhang et al. [42] developed a novel amino-terminated WAX material based on magnetic nanoparticles through cucurbit [6] uril promoted azide-alkyne cycloaddition using 2-azidoethylamine as the functional monomer. The material was synthesised following different steps: preparation of Fe_3O_4 magnetic nanoparticles, incorporation of SiO_2 and modification with NH2, functionalisation with alkynyl and incorporation of cucurbit [6] uril and 2-azidoethylamine in the alkyne-modified Fe_3O_4 particles. The proposed material was employed as dispersive SPE (dSPE) sorbent to extract salvianolic acids in 1 mL of Danshen water extract samples. The recoveries were between 95% and 107% for most compounds. 1 mL of sample solution at pH 5.5 was loaded for 20 min and then the analytes were eluted using 1 mL of 1% HAC in MeOH/H_2O (60/40, v/v) solution at 30 °C for another 20 min. The same synthetic method was used by Ghambarian et al. [43] to prepare Fe_3O_4 silica coated nanoparticles modified with amino groups for the extraction of two chlorophenoxyacetic acids (2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid) by dSPE from 25 mL of well and surface water samples with good recoveries.

2.3. Zwitterionic sorbents

Zwitterionic materials were developed with the aim of retaining both acidic and basic analytes simultaneously, thus improving the properties of the mixed-mode ion-exchange materials. Some studies have prepared and evaluated zwitterionic materials used as sorbents for SPE [44–47].

Some authors have prepared mixed-mode resins that combine WCX and WAX features. For instance, Jin et al. [46] prepared a ternary mixed-mode silica sorbent with carboxyl and amino groups via a Cu(I)-catalysed azide-alkyne cycloaddition (CuAAC) click reaction and a subsequent reduction of the remaining azide to primary amine (WAX character). The incorporation of 10-undecynoic acid conferred a WCX character to the silica network. In this case the sample was loaded at pH 6. In the first elution step, 2 mL of MeOH was chosen to elute the acidic and neutral analytes, retained by reversed-phase interactions. In a second elution, a solution of 2 mL of 5% HCOOH in MeOH was selected to elute the basic analytes because formic acid is able to make the carboxyl groups on the sorbent protonated and breaks the cation-exchange interactions between the analytes and the sorbent. Very good recoveries (all around 100%) were obtained in 2 mL of human serum for all the analytes at three different concentration levels. Neverthless, in spite of developing a zwitterionic sorbent, only the WCX interactions were exploited.

In other examples, different combinations of hypercrosslinked zwitterionic polymeric materials were synthesised and evaluated by SPE to retain ionisable compounds with acidic and basic properties from river and effluent wastewater samples [45,47]. The zwitterionic materials were synthesised via a three-step procedure: PP of DVB and VBC to give poly(DVB-co-VBC) microspheres; hypercrosslinking of poly(DVB- co -VBC) microspheres to give hypercrosslinked poly(DVB-co-VBC) microspheres (HXLPP); functionalisation of HXLPP with either sarcosine further hydrolysed (HXLPP-WAX/WCX) [45], or quaternised sarcosine groups (HXLPP-SAX/WCX) or taurine moieties (HXLPP-WAX/SCX) [47]. Figure 1 shows the three different structures of the zwitterionic materials prepared in-house and evaluated, the HXLPP-WAX/WCX, HXLPP-SAX/WCX and HXLPP-WAX/SCX. In all materials, pH 6 was selected as the optimal pH to load the sample so that all the acidic and basic analytes and the different functional groups of the sorbent would be in ionic form. Excellent recoveries were attained in all sorbents evaluated for all the compounds (from 80% to 108% when 100 mL of ultrapure water was loaded), except for the strongly acidic analytes (acesulfame and saccharin), which were only retained in the HXLPP-SAX/WCX material, with recoveries of 106% and 108%, respectively. This ability of the HXLPP-SAX/WCX material to retain the strongly acidic compounds could be attributed to the SAX character of the sorbent, as the other two have a WAX character.

Likewise, Wang et al. [44] evaluated a zwitterionic material that includes both primary and secondary amines, and carboxylic acid groups into a silica resin, to retain acidic and basic compounds. The material was synthesised by chemical immobilisation of ampholine on the surface of the hybrid organic-inorganic silica network. A glutaraldehyde solution in phosphate buffer was added to the silica resin and subsequently, ampholine containing $NaCNBH_3$ was added on the activated support to generate the carboxylic acid groups. Like other WCX studies, 10 mL of sample was adjusted at pH 6 and different elution conditions were adopted depending on the type of compounds to be eluted. A basic aqueous solution at pH 11 and acidic aqueous solution at pH 1, both in MeOH 50/50 (v/v), were used to elute the acidic and basic compounds, respectively. The average recoveries of the ten analytes obtained when a beverage sample was extracted ranged from 82% to 98%.

3. Solid-phase microextraction

Although there are several commercially available SPME fibres, none of them contain a mixed-mode ion-exchange coating. However, the need to extract ionisable analytes has led to mixed-mode ion-exchange SPME coatings being developed. As can be seen in Table 2, different in-house mixed-mode ion-exchange SPME materials have been developed and evaluated to determine analytes from environmental and biological samples. In this section, we have not included the subsections about anionic and cationic materials since less studies have been published compared to SPE in-house mixed-mode ion-exchange materials.

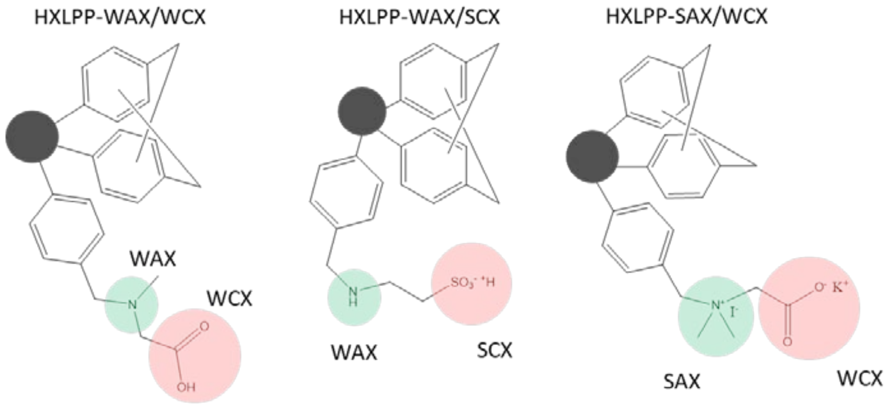

Figure 1. Structures of 3 different zwitterionic polymeric materials: HXLPP-WAX/WCX, HXLPP-SAX/WCX and HXLPP-WAX/SCX.

In general, in-house mixed-mode ion-exchange sorbents for SPME can be developed using various methodologies, such as in situ immobilisation [48–53] or sol-gel deposition [54] and they can be polymer- or silica-based.

In-house sorbents on stainless steel fibres can be immobilised in situ using adhesives [48,51] via sonification [50,52] by photopolymerisation [49] or even by electro-co-deposition using potentiostatic polymerisation [53]. Adhesives such as Loctite 349 impruv and Kasil 1 were used to immobilise a thin film of extraction phase on a flat stainless steel blade. The metal blades were dipped into the adhesives under a UV lamp while rotating to ensure all sides of the coatings were exposed to the UV lamp [51]. In other cases, polydopamine was used as an adhesive for further incorporation of the functional groups [48]. Sometimes an etching pretreatment with sulphuric acid [48] or concentrated nitric acid [51] or both [52] is necessary before the functionalisation to improve adhesion and porosity of the stainless steel wire. In situ photopolymerisation needs a UV crosslinker for the covalent bonding of the monomer on the stainless steel wire. For instance, Cudjoe et al. [51] compared different in-house SPME fibres by applying two different adhesives (Loctite 349 impruv and Kasil 1) to commercially available SPE particles: polymeric SCX, SAX, WCX and WAX; silica Discovery DPA-6S; C_{18} + B (C_{18} with benzenesulphonic); C_8 + B (C8 with benzenesulphonic); C_{18}; silica Clean Screen DAU and GHB and silica Chromabond

Table 2. Examples of application of SPME mixed-mode ion-exchange materials.

Type	Coating	Analytes	Sample	Conditions	% Recovery	Determination technique	Ref.
SCX	C_{18}/SCX (benzenosulphonic acid)	Pharmaceutical and illicit drugs	Blood	E: 1200 µL 90 min W: H_2O, 3 steps (5s each) D: MeOH/ ACN (4/1) 20 min	80-150	LC-MS/MS	[55]
SCX	Several sorbents C_{18} and C_8 with benzenosulphonic acid	36 polar metabolites	Human plasma	E: 300 µL 5min D: 250 µL of ACN/ H_2O (1/1)	0-45	LC-MS HILIC-MS	[78]
WCX	Graphene oxide and polydopamine modified etched PEEK tube	Quaternary alkaloids	Herb and plasma	E: 5 mL at pH 6 at the rate of 40 mL h^{-1} D: ACN/20 mM NH_4Ac at pH = 3 (20/40, v/v)	95-103	LC-MS/MS	[48]
WAX	Hollow fibre membrane-coated polymeric ionic liquid functionalized with 1-(3- aminopropyl)-3-(4-vinylbenzyl)imidazolium 4-styrenesulphonate and 1,6-di(3-vinylimidazolium) hexane as IL-crosslinking agent.	Estrogens	Milk	E. 30 mL at 800 rpm at 70 ºC for 40 min D: 500 µL of ACN shaking for 4 min.	86-112	LC–UV	[50]
WAX	Graphitic carbon nitrides modified hollow fibre (g-CNs-HF)	Uric acid	Urine and serum	E: 1 mL in ultrasonic bath at 20 °C for 30 min D: 100 µL of BSTFA at 80 °C for 45 min	81-122	GC-MS	[52]
SCX and SAX	C_{18} benzenesulphonic acid (B), C_8–B, MAX, MCX, WCX, WAX	Neurotransmiters	Artificial cerebrospinal fluid, rat brain tissue	E: 50 ng/mL, 1h D: 180 µL of H_2O/ ACN (3/2) with 0.1% HCOOH 1h rotated at 800rpm	20-85	LC-MS	[51]
WAX and SAX	Ti-APTES and C_{18}-TMOS	PFASs	River water	E: 20 mL at pH 2 for 60 min D: 100 µL of MeOH for 15 min	91-102	LC-MS	[54]

for the quantitative LC-MS/MS analysis of four polar neurotransmitters in two biological matrices, cerebrospinal fluid and rat brain tissue, shown in Table 2. For the synthesis of the fibres, the metal blade was dipped into the adhesive, and subsequently rotated in the pile of particles. The mixed-mode sorbents (C_{18} + B; C_8 + B; SCX); SAX); WCX; WAX) were able to extract all four neurotransmitters in quantitative amounts, showing multiple interaction modes, whereas the rest of the sorbents did not extract all target compounds because they did not interact with analytes through ionic interactions. Comparing the mixed-mode coatings, those with stronger ion-exchange properties performed better in the extraction of the polar neurotransmitters (see Figure 2). There were no considerable differences between the silica and polymer-based supports, meaning that the extraction efficiency of the sorbent was not dependent on the type of base support.

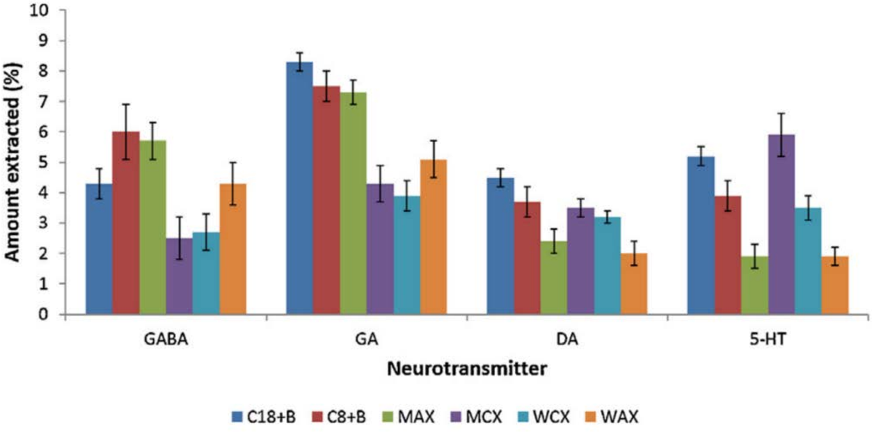

Figure 2. Comparison of the extraction efficiency of the proposed SPME coatings (polymer-based MAX, MCX, WCX and WAX; silica-based C18+B and C8+B) for the extraction of neurotransmitters in cerebrospinal fluid. Reprinted from [51] with permission from Elsevier.

Zhang et al [49] used the vinyl polyhedral oligomeric silsesquioxane (POSS) as a crosslinker for bonding the guanidyl-functionalised L-cysteine (G-Cys) monomer on the thiolated stainless steel fibre. The thiol functionalised stainless steel fibre was dipped into the polymerisation solution and pulled out quickly so that a thin layer of hybrid coating was formed. Then it was placed into the UV crosslinker for in situ photopolymerisation to complete the chemical bonding between thiol and vinyl. Electrochemical techniques normally need the presence of an electrolyte for the electropolymerisation of the polymers. However, in the study by Behzadi et al. [53] the potentiostatic polymerisation of poly(o-anisidine) and graphene oxide nanosheets (GONSs) was carried out without any supporting electrolyte. The carboxylic acid and hydroxyl groups in GONSs can act as the necessary electrolyte. In other cases, graphene oxide nanosheets have been demonstrated to be suitable material for incorporating the functional groups into the stainless steel fibres [48,53].

As previously mentioned, sol-gel technology is a well-known method for preparing organic-inorganic hybrid materials, synthesising polymer or composite materials under extraordinarily mild conditions. The sol-gel process makes it possible to incorporate additives, such as nanoparticles, into the first stage of the sol preparation without inhibiting the formation of the silica network. Silica has been reported to be a suitable supporting matrix for immobilising noble metal nanoparticles and avoiding their aggregation. For instance, Chen et al. [54] prepared a mixed-mode SPME fibre with SAX character by sol-gel deposition of a mixed-mode coating to an anodised Ti wire support for the extraction of perfluorooctane sulphonate and perfluorooctanoic acid from river water. The mixed-mode coating was composed of 3-(trimethoxysilyl)-1-propanamine and dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride. The SPME fibre was prepared by covalently bonding the designed mixed-mode coating to a Ti wire, with TiO_2 nanotube arrays on the surface, through the sol-gel process. The target analytes were extracted at pH 2, as both analytes are mainly present as anions at this pH and the coating is more positively charged in a more acidic solution. Therefore, a higher extraction efficiency is achieved in a more acidic solution. The authors compared the extraction efficiency of the selected anion-exchange material to commercially available coatings, such as PDMS, PA, and C_{18}/NH_2-functionalised fibres (see Figure 3). They found that when the in-house anion-exchange coating was used, the extraction efficiency was 4 and 55 times higher than the commercial PDMS and PA, respectively. This significant improvement can be attributed to the cooperative effects of hydrophobic and ionic interaction originated from the functional groups of the mixed-mode coating, which leads to strong adsorptions toward the acidic analytes.

As seen in the SPE section, pH has a crucial influence on achieving a good ionic interaction between the analytes and the coating. Moreover, the ionic state of the analytes and the charge of the fibre coating are pH-dependent. As pointed out in the introduction, different sample and elution pH conditions have been reported depending on the functional group incorporated into the mixed-mode ion-exchange material, as seen in Table 2. Desorption can be achieved by liquid desorption (LD) [50,54] or thermal desorption (TD) [49,52], and the last one is limited to the further GC analysis.

For LD, the influence of pH is shown in the study by Wang et al. [48], who developed an mixed-mode WCX coating to extract quaternary alkaloids in 5 mL of herb and plasma samples by SPME. They selected pH 6 as the optimal pH to load the sample and pH 3 as the optimal pH value to desorb the analytes. The authors observed that the extraction efficiency of the WCX coating decreased as the elution pH increased.

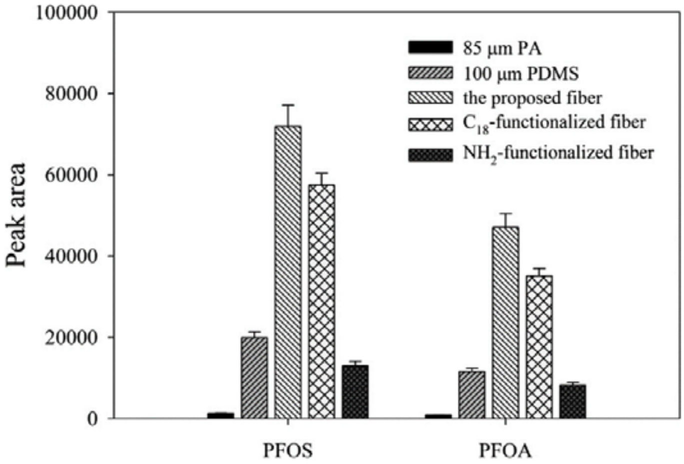

Figure 3. Comparison of the extraction efficiencies of the selected SPME fibre to commercially available PDMS, PA, and C18/NH2-functionalised fibres for the extraction of perfluorooctane sulfonate and perfluorooctanoic acid. Reprinted from [54] with permission from Elsevier.

For those with TD, increasing the desorption temperature led to a decrease in the partition coefficient of the targets between the coating and the gaseous phase, minimising the desorption time and carry-over. However, excessive temperature can damage the coating and result in a shortened lifetime. For instance, some authors chose 280 °C as the optimal temperature to desorb acidic compounds when an anion-exchange mixed-mode sorbent was used [49], although lower temperatures (80 °C) have also been used [52]. The temperature also obviously depends on the stability of the fibre.

SPME is an equilibrium-based extraction procedure and therefore a time-dependent process. As can be seen in Table 2, in most cases the equilibrium time was reached at 30-40 min, and then the peak areas remained steady with a longer extraction time [49,50,52]. In other cases, the equilibrium time was longer, 60 min [54].

Another important extraction parameter is the addition of salt. For ionic compounds, increasing the ionic strength of the sample solution can usually improve its activation state in water, and thus increase the solubility in water. For positively charged coatings, such as the SAX mixed-mode coating mentioned previously [54], increasing the ionic strength tends to screen the electrostatic attraction between the negatively charged analytes and the surface due to the electrical double layer compression, and consequently no salt is added to the sample.

When mixed-mode ion-exchange SPME coatings are used, few methods include a washing step. Reyes-Garcés et al. [55] introduced a washing step consisting of three consecutive washes of 1500 µL of nanopure water for 5 s each for determining pharmaceuticals and illicit drugs in blood using a SCX sorbent. In this way, the presence of proteins, salts, and other interferences that could affect the instrumental analysis are easily avoided. The rest of the extraction conditions are presented in Table 2.

Important parameters are the stability and robustness of the materials, evaluated by performing several extractions or testing the materials in different extraction solvents or temperatures. For instance, Feng et al. [50] reported a decrease in peak areas of 22-27% in the 50th–70th extractions compared to the first 20 extractions when the SPME fibre was applied. Chen et al. [54] tested the robustness of the fibre by dipping it into organic solvents, such as MeOH, ACN, acetone and n-hexane. The authors observed no losses in the extraction efficiency after exposing it to these organic solvents overnight. The SPME fibre's great robustness towards organic solvents can be attributed to the chemical bonding between the mixed-mode coating and the anodised Ti wire. At very high temperatures the fibres can be unstable. For instance, a weight loss of about 20 wt% was observed at 380°C in the study by Zhang [49], implying the decomposition of the monomer covalently bonded on the surface of the fibre. However, despite the high stability of the fibres, for metabolomic applications the authors suggest using the biocompatible SPME fibres only once to avoid carry over due to the traces of metabolites from previous extractions remaining in the coating.

4. Stir bar sorptive extraction

Although commercially available SBSE materials, such as PDMS for the extraction of non-polar compounds and EG-silicone for polar compounds, have been used for SBSE applications, new in-house mixed-mode ion-exchange materials have been developed to improve the retention of ionic or ionisable compounds. In-house mixed-mode materials are mainly developed using two different techniques: sol-gel technology [56] or one pot polymerisation [56–59]. Examples of these materials applied in SBSE are presented in Table 3.

Sol-gel is employed to prepare SBSE surface coating materials taking advantage of their properties. Using this synthetic method, OH-PDMS is added to the sol-gel mixture due to its ability to lengthen the silica network (increasing the surface area of the coating material), distribute the stationary phase uniformly, and reduce the coating layer fragility, preventing the cracking of the coating layer [56]. Hu et al. [56] developed a WAX coating with amino ionic functional groups for SBSE based on multi-walled carbon nanotubes-4,4'-diaminodiphenylmethane/polydimethylsiloxane for the extraction of seven phenols in lake water, which were then determined using HPLC-UV. The mixed-mode ion-exchange material was synthesised by sol-gel technology. The pretreated bars were immersed vertically into the sol solution containing all the reagents. However, since the sample was loaded at pH 4, no ionic interactions were stablished but hydrophobic, π-π and intermolecular hydrogen bonds between 4,4'-diaminodiphenylmethane and phenols. The LD solvent, MeOH/1mM NaOH (8/2), was selected to neutralise the amino moieties of the coating, disrupting the interactions between the phenols and the coating (%R of 79–123% in the water sample and 71–119% in the soil sample).

In one pot polymerisation, the reaction is carried out successively in only one reactor, where all the reactants are included. It is implemented due to its simplicity, simple workup and short reaction time. For instance, in the study by Huang [59] a SBSE with SAX monolithic coating was prepared by in situ copolymerisation of DVB and 2-(methacryloyloxy)ethyltrimethylammonium chloride, whose quaternary amines provide the ionic character to the material. The developed material displays SAX interactions with the selected analytes, inorganic anions such as Br^-, NO^{3-}, PO_4^{3-} and SO_4^{2-}, since it contains quaternary ammonium groups in the monolith structure. Hence, 50 mL of commercial purified water samples was loaded and NaOH aqueous solvent was the suitable desorption solvent, reaching recoveries of 70-93% for all the anions in all water samples analysed. In this study the SAX monolithic coating was not exploited as mixed-mode material since only ionic interactions are present between the analytes and the coating. Another example was recently presented by our research group [60], in which a polytetrafluoroethylene (PTFE)-based magnet was coated with a weak anion exchanger monolith (glycidyl methacrylate-EGDMA polymer further modified with EDA). Later, this SBSE-EDA was successfully applied under optimal conditions (10 mL of ultrapure water adjusted to pH 6 stirred at 600 rpm for 180 min; elution with 3 mL ACN containing 5% NH4OH in the ultrasonic bath for 20 min) to extract a group of acidic compounds with recoveries ranging from 37% to 74%.

Recently, magnetic materials, mentioned previously, such as functionalised nanoparticles, or composite materials made of nanoparticles embedded in polymeric sorbents, have gained popularity in SBSE as they can be recovered by applying a magnetic field. In addition, stir bars made with magnetic nanoparticles have lower extraction times and acquire more versatility than the commercial stir bars. Some examples of in-house mixed-mode ion-exchange stir bar devices based on magnetic materials are shown in Table 3. Wang et al. [61] employed a novel covalent modification method for the in situ

Table 3. Examples of application of SBSE mixed-mode ion-exchange materials.

Type	Coating	Analytes	Sample	Conditions	% Recovery	Determination technique	Ref.
SCX	Poly (MAA-3-sulphopropyl ester potassium salt-DVB)	Quinolones	WW	E: 100 mL at pH 5, stirred at 400 rpm at r.t. for 60 min D: 3 mL of MeOH/water at pH 1.3 (80/20, v/v) for 40 min at 400 rpm	64-73	LC/DAD	[58]
SCX	Poly(MAA-3-sulphopropyl ester potassium salt-co-DVB)	5-nitroimidazole-based drugs	Honey	E: 100 mL at pH 5, stirred at 300 rpm for 3 h at r.t. D: 3 mL of MeOH/water at pH 2 (90/10, v/v) stirred for 1 h	71-114	LC–DAD	[63]
SAX	Poly(2-(methacryloyloxy) ethyltrimethylammonium chloride-co-DVB)	Inorganic anions	Commercial purified water	E: 50 mL at pH 8 stirred at 400rpm for 2.5 h at r.t. D: 3 mL of 10mM NaOH at 400rpm at r.t. for 3 h	70-90	LC-IC/CD	[59]
WAX	Poly(1-vinylimidazole-ethyleneglycol dimethacrylate)	Perfluoroalkyl acids	Surface water samples	E: 10 mL at pH 3, stirred at 800 rpm for 60 min D: 300 µL of MeOH containing 0.4% NH_4OH (v/v) under sonication for 10 min	80-122	LC-MS/MS	[57]
WAX	MOFs of aluminium-based functionalised with 4-aminobenzoic acid immobilised onto PEEK tube	Parabens	Cosmetics and rabbit plasma	E: 20 mL at pH 6 stirring at 300 rpm for 2 h D: 250 µL of MeOH by vortex for 10 min.	75-102	LC-MS/MS	[61]
AX	Zn-Al layered double hydroxide	Organic acidic compounds	Grape juice and non-alcoholic beer	E: 10 mL at pH 7, stirred at 200 rpm for 20 min (25 °C) D: 50 µL of 10 mM NaOH MeOH sonicated for 2 min.	90-95	LC-DAD	[62]

immobilisation of aluminium-based MOF (MIL-68) onto a poly (ether ether ketone) (PEEK) tube by bonding with benzoic acid groups. They applied this to extract three parabens at pH 6, including methyl paraben, ethyl paraben and propyl paraben from cosmetics, sunscreen and day cream. However, once again the MIL-68@PEEK material is not used as ion-exchange mixed-mode coating, since at pH 6 the selected parabens are protonated and could not establish ionic interactions with the coating.

Ghani et al. [62] prepared in situ Zn-Al layered double hydroxide (LDH) on the surface of a nanoporous anodised aluminium bar to be applied in the SBSE of three organic acidic compounds, including p-hydroxybenzoic acid, 3,4-dihydroxybenzoic gallic acid and quercetin from 10 mL of commercial and natural white and red grape juice, cranberry juice and non-alcoholic beer samples. A porous anodised aluminium bar was electrochemically prepared and used as nanoporous substrate for in-situ growth of LDH. The incorporation of LDH gave the stir bar attractive properties such as high anion exchange capacity, compositional flexibility and biocompatibility. The target analytes were desorbed by LD employing 50 µL of MeOH containing 10 mM NaOH, and good recoveries were obtained.

The SBSE conditions are shown in Table 3. It can be seen that low agitation speeds of 200-400 rpm were preferred for most of the studies [58,59,61–63], considering that a higher speed would cause abrasion loss of the coating. The studies by Hu et al. [56] and Yao et al. [57] are exceptions since the robustness of the stir bars made it possible to submit them to 700-800 rpm. Most of the authors reported an extraction time between 1 h and 2.5 h necessary for the stir bars to reach equilibrium, except Ghani et al. [62] whose stir bars were based on Zn-Al LDH and required only 20 min.

The desorption step is generally carried out by LD or TD. As in SPME, the desorption of thermal-sensitive and polar compounds from the coating is usually performed by LD. The desorption solution is chosen to obtain an effective and strong elution of the compounds, using the minimal volume for entirely immersing the stir bar in the desorption tube. Another reason to select the suitable desorption solvent concerns the chromatographic behaviour. The solvent should be compatible with the mobile phase and column used for the analysis. If not, an evaporation to dryness followed by a reconstitution with a more compatible solvent is necessary [57,63]. Acetone, MeOH and/or ACN in acidic or basic medium are the preferred desorption solvents used in an LD [56–58,61–63]. For instance, when WAX material is used as coating, normally the desorption solvent is basified with NaOH or ammonia to neutralise the functional groups of the coating (amine groups) and therefore disrupt the ionic interactions between the acidic compounds and the sorbent [56,57,59,62].

An acidic medium was introduced to the desorption organic solvent for strong cation-exchangers [58,63]. For instance, Huang et al. [63] performed an SBSE to determine nitroimidazoles in 100 mL of honey employing a SCX stir bar based on poly(methacrylic acid-3-sulphopropyl ester potassium salt-co-divinylbenzene) monolithic material with sulphonic groups (see Table 3). When the optimal desorption solvent, MeOH:H_2O (pH 2) (90:10), is used, recoveries from 71% to 114% were attained for the selected analytes in real honey samples. Tests with 100% MeOH as desorption solvent showed that MeOH disrupts the hydrophobic interactions between coating and analytes; however, if there is no acidified water in the desorption solvent, the extraction efficiencies decreased, as the ionic interactions were not disrupted and the nitroimidazoles were not completely eluted.

Many types and sizes of in-house stir bars are obtained depending on the synthetic method. Rods of around 0.1-0.5 µm of thickness are obtained when sol-gel technology is used, while thicker rods (1 mm) are obtained when in situ polymerisation methods are used. They can be reused several times (more than 25-50 extractions) without losing their efficiency, which supports their good stability and proper mechanical fulfilment to prevent their degradation and facilitate their bonding to the glass layer that covers the magnetic rod.

In terms of extraction conditions, most authors reported sample loading pH values between 6 and 8 [59,61,62] for mixed-mode anion-exchange materials. However, Yao et al. [57] chose pH 3 to extract perfluoroalkyl acids from river and lake water samples using a WAX stir bar based on poly(1-vinylimidazole-ethyleneglycol dimethacrylate) monolith (poly(VI-EGDMA)), because at weak acidic pH the imidazole groups of the coating are in an ionic state, as well as the perfluoroalkyl acids. The SBSE conditions are described in Table 3. Regarding strong cation-exchangers, as mentioned in the introduction section, the loading solution is normally acidified to pH 2-3 to protonate the basic analytes, although a value of 5 has also been used depending on the analytes [58,63].

As can be seen in some of the examples shown, successful mixed-mode ion-exchange stir bars have been developed. However, in some applications, the extraction and desorption conditions are not suitable for exploiting the mixed-mode ion-exchange interactions and a washing step was not included in any of the examples, which also limits the selectivity of the method. Therefore, a lot of research can be conducted to improve the development and application of mixed-mode ion-exchange SBSE.

5. Other techniques

Mixed-mode ion-exchange materials have been used in other microextraction techniques, mainly based on modifications of the previously described microextraction techniques, such as stir bar sorptive-dispersive microextraction (SBSDME) [64] or the more recent stir extraction formats, such as capsule phase microextraction (CPME) [65] and stir-cake sorptive extraction (SCSE) [66], or fabric phase sorptive extraction (FPSE). Figure 4 shows the schematic configuration of these emerging techniques. For instance, SBSDME unifies the principles of SBSE and dispersive solid-phase extraction (dSPE) using a neodymium stir bar coated with a magnetic material as the extraction device. As magnetic materials can be recovered by applying a magnetic field, composite materials made of nanoparticles embedded in polymeric sorbents have been widely applied in microextraction techniques. In the study by Grau et al. [64], a magnetic composite made of $CoFe_2O_4$ magnetic nanoparticles embedded in a mixed-mode WAX sorbent (Strata-X-AW) was used as coating to extract triphenyl phosphate (TPP) and diphenyl phosphate (DPP) in urine samples. A wet chemical co-precipitation method was used to prepare the magnetic nanoparticles, which were then inserted into the polymeric resin. $CoFe_2O_4$ magnetic nanoparticles were preferred to the classical Fe_3O_4 due to their higher chemical stability. As expected, DPP (pK_a=1.12) was the only analyte to establish WAX interactions with the sorbent, as TPP is not ionisable. Similar results were obtained when loading at a pH below 9, as the amines of the sorbent and DPP are both in ionic state.

Huang et al. [66] prepared a new monolithic sorbent for SCSE from poly(allyl thiourea-co-divinylbenzene) (ATDB) by in-situ polymerisation to provide the ATDB-SCSE device. The novel material, which presents primary and secondary amines as functional groups, was used to extract phenols in different types of water, such as tap, lake and reservoir water. Similar to the study by Grau et al. [64], the extraction of phenols improved when the pH was increased from 3 to 8, and then decreased when the pH was increased further. This is due to the competition between the hydroxyl groups in the sample and phenol groups of the analytes to interact with the activated amino groups of the coating, weakening the anion-exchange interactions. This demonstrates that the amino groups of the sorbent conferred a WAX character to the ATDB-SCSE device.

Another new microextraction technique is the CPME, which consists of two fused porous polypropylene membranes, one accommodates the sorbent and the other encapsulates a magnetic metal rod. In one CPME study [65], the authors prepared two mixed-mode ion-exchange materials combining C_{18} with sulphonic moieties (SCX) and C_{18} with quaternary amines (SAX) using sol-gel technology to retain selectively basic and acidic analytes, respectively. First the tubular membranes were prepared, then the monolithic sorbent was developed in situ inside the membrane using the sol-solution. During extraction, for the material with an SCX character, pH 5 was selected to load the sample, while for the SAX, it was pH 7. Regarding the desorption conditions, MeOH containing 5% HCOOH was selected for the material with a C_{18} and SCX character, and

MeOH containing 5% NH_4OH for the other material with a C_{18} and SAX character. Under these conditions, each material provided suitable retention and selectivity.

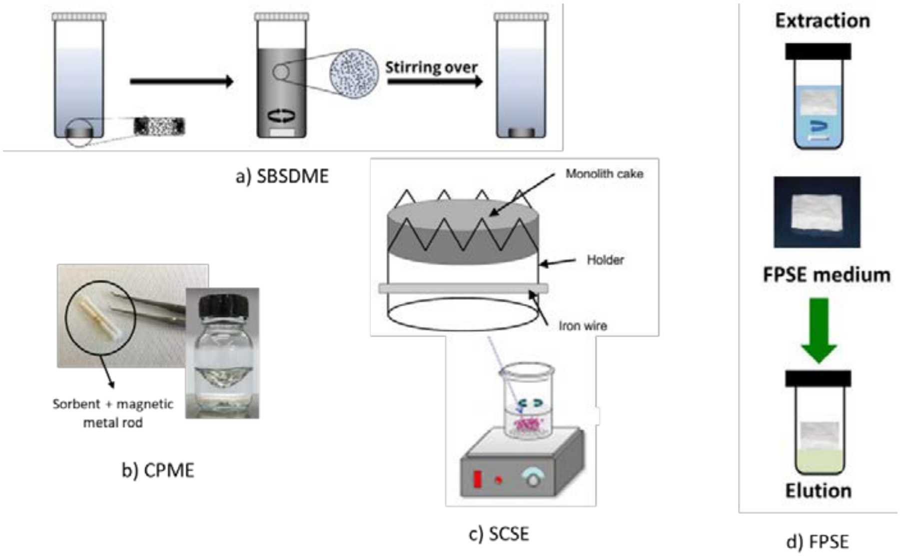

Figure 4. Schematic configuration of some emerging techniques: a) SBSDME [79]; b) CPME; c) SCSE; d) FPSE [80].

Mixed-mode ion-exchange materials have also been used in FPSE [67,68]. Agadellis et al. [67] prepared a mixed-mode strong cation-exchange membrane to determine four tetracycline antibiotic residues (oxytetracycline, tetracycline, chlortetracycline, doxycycline) in milk, after protein precipitation, by HPLC-UV. The membrane, coated on 100% cellulose cotton, contained propyl sulfonic acid, (strong cation exchanger) and C18. The material was synthesised using the sol-gel technique. The use of a mixed-mode material was essential for this study since the structure of tetracyclines present both positive and negative charge centres at different solution pH values, and for this reason the determination of tetracyclines makes it a major challenge. At lower pH values tetracyclines exist mainly as cation, and at higher solution pH values they exist mainly as anionic species. At the natural pH of milk (6.7–6.9), tetracyclines mostly exist as zwitterions due to the simultaneous ionization of both cationic and anionic functional groups. Zwitterionic FPSE materials were also prepared and evaluated [68] for the extraction of five triazine herbicides (simazine, atrazine, prometryn, terbuthylazine and propazine) from fruit juice samples to be analysed by HPLC-DAD. The novel zwitterionic membrane was prepared by combining C_{18}, propyl sulfonic acid (strong cation-exchanger), and propyl-N,N,N-trimethylammonium chloride (strong anion-exchanger) in a sol-gel siloxane network, chemically coated on 100% cotton cellulose fabric substrate. Triazines exist in neutral state in juice samples, while a large number of ionic species, mainly organic acids and bases, are present in ionic state as interferents of the matrix. The zwitterionic material could efficiently help cleaning all the acidic interferences from fruit juices thanks to their ability to retain the ionic species present in the fruit juice sample trough the ionic functional groups attached to the material. By this way, the concentration of ionic species of the matrix is reduced, enabling the retention of neutral triazines by the C_{18} groups of the material.

Thus, these examples evidence that mixed-mode ion-exchange is spreading through a broad range of sorptive extraction techniques.

Conclusions

New in-house mixed-mode ion-exchange materials have been developed mainly for SPE but they are also gaining importance for other sorptive extraction techniques. Precipitation polymerisation, sol-gel technology and in situ immobilisation are the main synthetic approaches for creating the basis that is further functionalised with ionic moieties.

Zwitterionic materials recently appeared with the purpose of extracting acidic and basic compounds simultaneously, and are mainly applied in SPE.

These in-house mixed-mode ion-exchange materials were successfully applied to selectively extract acidic and basic analytes from complex samples when optimal extraction protocols that take into account the pHs in the different steps are applied. However, it should to be mentioned that promising mixed-mode ion-exchange materials have been developed but in some cases not used at the optimum conditions to exploit the potential of these materials.

One of the future trends in in-house mixed-mode technology is to apply it to emerging extraction techniques, so that the mixed-mode ion-exchange materials extend the application field of the techniques. Furthermore, mixed-mode ion-exchange materials will be applied in the future to other extraction techniques and supports and will expand the application field to other kinds of samples and analytes.

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CHAPTER 2. OBJECTIVES

This Doctoral Thesis aims the development, evaluation and application of different in-house mixed-mode ion-exchange materials for extracting contaminants using different sorptive extraction techniques, such as solid-phase extraction, stir bar sorptive extraction and capsule phase microextraction. A group of compounds from three different families (pharmaceuticals, sweeteners and illicit drugs) were selected as model compounds to evaluate the sorptive materials, since these compounds present a wide range of physicochemical and acidic and basic properties. Since these compounds are widely used and present in the environment, the methods based on the different extraction techniques followed by liquid chromatography with mass spectrometry in tandem were validated and applied in environmental waters, such as river water, effluent and influent wastewater.

CHAPTER 3. EXPERIMENTAL PART, RESULTS AND DISCUSSION

This chapter includes the experimental part, results and discussion of the studies developed in this Doctoral Thesis. The results have already been published in international scientific journals. The Doctoral Thesis has been developed in the research group of Chromatography and Environmental Applications at Universitat Rovira i Virgili in Tarragona, which for years has focused its research on the development and evaluation of novel in-house sorbents.

The following sections are organized according to the different type of sorptive extraction technique used. In each section, a brief introduction is included to establish the context of the research followed by the results presented in paper format. In addition, the most important results are also discussed after the papers. Section 3.1 describes the use of the solid-phase extraction technique to evaluate two in-house hypercrosslinked mixed-mode ion-exchange zwitterionic materials, structured in two studies. These studies were performed in collaboration with Prof. P.A.G. Cormack, in the Department of Pure and Applied Chemistry, at University of Strathclyde (Glasgow, Scotland), where the materials were synthesized during a stay as a part of my PhD. Contaminants from three different groups of families, pharmaceuticals, illicit drugs and sweeteners, were selected for the evaluation of the new materials. In the second study some analytes were added and others were removed from those evaluated in the previous study, depending on the previous results obtained. Since the target contaminants are found at low concentration levels in analyzed samples, the detection and quantification was performed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Complex samples, particularly water samples such as river water and effluent wastewater were analyzed.

In section 3.1.1 an in-house synthesized hypercrosslinked weak cation-exchange and weak anion-exchange zwitterionic sorbent (HXLPP-WCX/WAX) was evaluated by SPE for the retention of 11 model compounds with acidic, basic and amphoteric properties. The HXLPP-WCX/WAX polymer was functionalized with sarcosine ethyl ester hydrochloride, to include the anion-exchange properties to the particles, followed by the hydrolysis of the ester group to allow the polymer get the cation-exchange properties. All sorbents in section 3.1 were synthesized using poly(divinylbenzene-co-vinylbenzylchloride) (poly(DVB-co-VBC)) as the precursor, which was prepared by precipitation polymerization. Before the functionalization, a hypercrosslinking reaction was used in the polymers to increase the specific surface area and so the capacity of the sorbent. All the different SPE parameters were optimized and thoroughly discussed so as to get the optimum extraction conditions. Special attention was paid to the pk$_a$ values of the analytes and the functional groups of the sorbents, as well as the pH conditions, in order to promote effective interactions between them. The method was applied and validated in river water and wastewater samples to demonstrate that it is capable for the extraction of compounds with different proprieties from complex samples.

In section 3.1.2 two hypercrosslinked zwitterionic polymers were synthesized and evaluated by SPE for the retention of acidic, basic and amphoteric analytes simultaneously. The hypercrosslinked strong anion- and weak cation-exchange polymer (HXLPP-SAX/WCX) has a quaternary amine and a carboxylic acid as the anionic- and cationic-exchangers, respectively. A secondary amine and a sulfonic acid are the weak anion- and strong cation-exchangers incorporated in the hypercrosslinked weak anion- and strong cation-exchange polymer (HXLPP-WAX/SCX).

In section 3.2, divided in two different studies, different in-house mixed-mode ion-exchange materials were also evaluated for stir bar sorptive extraction (SBSE) and capsule phase microextraction (CPME) techniques. The first study was performed in collaboration with Dr. Mónica Catalá-Icardo in the Instituto de Investigación para la Gestión Integrada de Zonas Costeras from Universitat Politècnica de València and Prof. José Manuel Herrero-Martínez, in the Department of Analytical Chemistry from Universitat of Valencia, who prepared the stir bars. The second study in this section was performed in collaboration with Dr. K. G. Furton and Dr. A. Kabir from Florida International University in Miami (USA), where the mixed-mode ion-exchange coatings were synthesized. Liquid chromatography followed by tandem mass spectrometry was employed to determine the target compounds selected. In both studies, the optimized methods followed by LC-MS/MS were used to determine the ionizable compounds in environmental water samples, including river water and effluent wastewater.

Section 3.2.1 presents the first example of a polytetrafluoroethylene (PTFE)-based magnet coated with weak anion-exchanger monolith as novel support for

SBSE. 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 (or in-situ polymerized) and modified (or reacted) 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 as target compounds. Firstly, the SBSE conditions were optimized to promote the weak anion-exchange interactions with the target compounds and enable the selective extraction of these compounds.

In the second section, section 3.2.2, CPME was introduced as novel sportive extraction technique. CPME is performed by means of a microextraction capsule (MEC). Two different MECs (MEC-C_{18}/SAX and MEC-C_{18}/SCX) based on mixed-mode ion-exchange technology were prepared and evaluated for the selective extraction of a group of ionizable compounds, including acidic and basic analytes. Sulfonic acid moieties were used as the cation-exchange group in MEC-C_{18}/SCX, and a quaternary amine as the anion-exchange group in MEC-C_{18}/SAX. The extraction parameters optimized were sample pH, elution solvent, sample/elution volume and extraction/elution time

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3.1. Synthesis and evaluation of mixed-mode ion-exchange amphoteric materials for the SPE of ionizable compounds in environmental waters

As detailed in the introduction of the Thesis, solid-phase extraction (SPE) is the most widely used extraction technique for several types of analytes and matrices in liquid samples due to its enrichment of analytes and high capability of elimination of matrix interferences. Different materials with a broad range of properties are available to be used as SPE sorbents [1]. Among them, mixed-mode ion-exchange sorbents combine capacity and selectivity in a single material. They are based on a polymeric or silica phase that contains ionizable functional groups specifically designed to interact with ionic species by combining effective reversed-phase chemistry with ion-exchange groups. There are four types of mixed-mode sorbents depending on the functional groups attached to the polymeric or silica support classified as cationic or anionic exchangers with strong or weak properties. The strong ion-exchangers include functional groups that are charged in the entire pH range, while those with weak properties have groups with a more reduced working pH range depending on their pK_a values. Strong cation-exchangers (SCX) usually have sulfonic acid groups, whereas weak cation-exchangers (WCX) have carboxylic acid groups. Otherwise, sorbents with strong anion-exchange (SAX) properties present quaternary amine groups and the ones with weak anion-exchange (WAX) properties include tertiary, secondary or primary amines. Mixed-mode ion-exchange sorbents have been applied in environmental samples, like river or wastewater samples, to successfully extract ionic or ionizable analytes like pharmaceuticals and drugs of abuse [2,3].

Nevertheless, the necessity of extracting various types of analytes with acidic and basic properties led to use new ion-exchange sorbents. Research to demonstrate the application of various mixed-mode ion-exchange SPE cartridges in tandem to simultaneously extract analytes with different properties was ongoing [4]. However, the simultaneous extraction of both acidic and basic compounds within the same cartridge is the main issue of mixed-mode sorbents. To deal with this topic, combination of well-known SPE materials in one cartridge (multi-layer solid-phase extraction (mlSPE)) were tested for the determination of polar organic chemicals in three different aqueous matrices [5]. Another example employing the same strategy achieved successful results when mixing together and packing four commercial mixed-mode ion-exchange sorbents (Oasis MCX, Oasis WCX, Oasis MAX and Oasis WAX) into 100 mg cartridges to give four different complementary combinations (SCX/SAX, SAX/WCX, SCX/WAX and

WCX/WAX) with similar ion-exchange capacities to determine acidic and basic compounds [6].

To enhance the properties of the sorbents, the incorporation of cation and anion exchange moieties in the same functional group attached to the polymeric network also came out, called zwitterionic polymers. However, only few authors have developed and evaluated in-house mixed-mode ion-exchange materials used as SPE sorbents with both the cation- and anion-exchangers incorporated in the same material, but they did not get the aim of retaining all acidic and basic analytes simultaneously by ionic interactions. Jin et al [7] developed a ternary mixed-mode silica-based SPE sorbent bearing C_8 groups, a primary amine and a carboxylic acid to retain acidic, neutral and basic compounds. In this case, only the basic compounds were retained by ion-exchange mechanisms, whereas the acidic and neutral compounds were retained primarily by hydrophobic interactions.

In sections 3.1.1 and 3.1.2 the two developed studies focusing on development of mixed-mode ion-exchange materials that contain both anionic and cationic moieties and their evaluation by SPE are presented. The results of the studies have been published in the Journal of Chromatography A 1626 (2020) 461348, and Journal of Chromatography A 1661 (2022) 462715.

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3.1.1. Microporous polymer microspheres with amphoteric character for the solid-phase extraction of acidic and basic analytes

Microporous polymer microspheres with amphoteric character for the solid-phase extraction of acidic and basic analytes

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Abstract

Solid-phase extraction (SPE) is a widely-used and very well-established sample preparation technique for liquid samples. An area of on-going focus for innovation in this field concerns the development of new and improved SPE sorbents that can enhance the sensitivity and/or the selectivity of SPE processes. In this context, mixed-mode ion-exchange sorbents have been developed and commercialised, thereby allowing enhanced capacity and selectivity to be offered by one single material. The ion-selectivity of these materials is such that either anion-exchange or cation-exchange is possible, however one limitation to their use is that more than one sorbent type is required to capture both anions and cations. In this paper, we disclose the design, synthesis and exploitation of a novel SPE sorbent based on microporous polymer microspheres with amphoteric character. We show that it is possible to switch the ion-exchange retention mechanism of the sorbent simply by changing the pH of the loading solution; anion-exchange dominates at low pH, cation-exchange dominates at high pH, and both mechanisms can contribute to retention when the polymer-bound amphoteric species, which are based on the α–amino acid sarcosine (N-methylglycine), are in a zwitterionic state. This is an interesting and useful feature, since it allows distinctly different groups of analytes (acids and bases) to be fractionated using one single amphoteric sorbent with dual-functionality. The sarcosine-based sorbent was applied to the SPE of acidic, basic and amphoteric analytes from ultrapure water, river water and effluent wastewater samples. Under optimised conditions (loading 100 mL of sample at pH 6, washing with 1 mL of MeOH and eluting with an acidic or basic additive in MeOH) the recoveries for most of the compounds were from 57% to 87% for river water and from 61% to 88% for effluent wastewater. We anticipate that these results will lay the basis for the development of a new family of multifunctional sorbents, where two or more separation mechanisms can be embedded within one single, bespoke material optimised for application to challenging chemical separations to give significant selectivity advantages over essentially all other state-of-the-art SPE sorbents.

Keywords: *Amphoteric sorbent; mixed-mode ion-exchange; solid-phase extraction; acidic analytes; basic analytes; environmental water samples*

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1. Introduction

Solid-phase extraction (SPE) is a widely used sample preparation technique for liquid samples by virtue of its ability to enrich analytes and eliminate matrix interferences, with the popularity of this technique being influenced positively by the range of SPE sorbents available among other advantages [1,2]. Although a number of sorbents have been available commercially for several years, a recent trend in the field concerns the development of new and improved sorbents [1–4]. These sorbents aim to enhance the sensitivity and/or the selectivity of SPE based methods, thereby enabling increasingly complex matrices to be handled. Polymer-based sorbents having high-specific surface areas and/or hydrophilic character, such as hypercrosslinked polymers and hydrophilic macroreticular polymers, respectively, address the demand for improved sensitivity [5–7], whilst immunosorbents [8,9] and molecularly imprinted polymers were developed to improve selectivity [2,10,11].

Sorbent technology has evolved to a point in time where enhanced capacity and selectivity can be offered by a single material - mixed-mode ion-exchange sorbents. These sorbents are normally either silica-based or organic polymer-based, with the ion-selectivity arising from the presence of ionisable functional groups designed to interact selectively with ionic species present in samples [3,4,12–14].

Mixed-mode ion-exchange sorbents are classified into four distinct groups depending upon whether they are cation- or anion-exchangers and whether they offer strong or weak exchange properties. Strong ion-exchangers bear functional groups that are charged across the entire pH range of operation, whereas weak ion-exchangers bear functional groups that are ionised over a narrower pH window depending on their pK_a. Strong cation-exchangers (SCX) normally bear sulfonic acids (strong acids), whereas weak cation-exchangers (WCX) are decorated with carboxylic acids (weak acids). In contrast, sorbents with strong anion-exchange (SAX) character are normally based on immobilised quaternary ammonium species, whereas sorbents with weak anion-exchange (WAX) properties are functionalised with tertiary, secondary or primary amines [4,15]. Commercial offerings of mixed-mode ion-exchange sorbents and research works focused on the development of new materials have been reported [4,16]. Hypercrosslinked mixed-mode ion-exchange sorbents were developed and reported by our group [14,17–19]; these materials offer high capacity, which is ascribed to their high specific surface area (SSA) (typically >1000 m2 g^{-1}), in combination with tuneable ion-selectivity (which is governed by the chemical nature of the ion-exchange groups present). Published examples of hypercrosslinked mixed-mode ion-exchange sorbents include SAX materials decorated with quaternary ammonium species [19] and WCX materials which derive their selectivity from carboxylic acid groups installed during polymer synthesis involving a functional monomer [18].

Mixed-mode ion-exchange sorbents have been applied successfully within various analytical chemistry fields for the extraction of a variety of ionic analytes, including pharmaceuticals, drugs of abuse and compounds of biological interest, among others. They are well-accepted by the analytical chemistry community [4,12,14,16–24].

Given the chemical basis for the ion-selectivity offered by mixed-mode ion-exchange sorbents, one limitation to their use is that any given sorbent cannot be used to extract both acidic and basic compounds. One practical solution to this limitation is to use a pair of mixed-mode ion-exchange sorbents in combination, where one sorbent captures the cations and the second sorbent captures the anions. In one demonstration of this approach, Lavén et al. [25] used mixed-mode cation- and anion-exchange SPE sorbents (Oasis MCX and MAX) in tandem to separate basic, neutral and acidic pharmaceuticals from wastewater samples. An alternative solution is to use multi-layer SPE, where two (or more) chemically distinct sorbents are combined within a single SPE cartridge. In one example of multi-layer SPE, an SPE cartridge was filled, from bottom-to-top, with graphitised carbon black (GCB), a WCX sorbent, and a WAX sorbent, with polyethylene frits separating the layers, and the multi-layer SPE set-up then used for the determination of polar organic chemicals in aqueous matrices [26]. In a second example, mixed-mode ion-exchange sorbents with strong or weak cation- and anion-exchange properties were combined within single SPE cartridges, with a balance of positive and negative charges, for the capture of basic and acidic pharmaceuticals through ionic interactions [27].

Of course, there is no chemical reason for why cation-exchange and anion-exchange cannot be combined within a single sorbent material, however this is a relatively unexplored area to date. Thus far, this strategy has been adopted only for silica-based mixed-mode ion-exchange materials for use as either mixed-mode LC stationary phases [28,29] or as mixed-mode SPE sorbents [30]. In the study by Jin et al. [30], a ternary mixed-mode silica sorbent bearing C_8 groups, a primary amine and a carboxylic acid, was used to retain acidic, neutral and basic compounds. The results from the latter study showed that electrostatic repulsions and hydrophobic interactions between the sorbent and the analytes influenced the retention behaviour. To the best of our knowledge, organic polymers bearing amphoteric moieties, optimised for use as mixed-mode SPE sorbents, have not yet been described in the literature, although a methacrylate-based material bearing zwitterionic sulfoethylbetaine groups was used as an SPE sorbent to retain hydrophilic solutes by a mechanism which relies upon the water retention capacity of the sorbent surface [31] rather than ionic exchange interaction mechanisms.

In the present study, a mixed-mode polymeric sorbent decorated with amphoteric moieties was designed and synthesised, to explore the opportunities that may accrue from having cation- and anion-exchange groups present simultaneously within a single, bespoke SPE sorbent. To exemplify this strategy, hypercrosslinked polymer microspheres were synthesised and functionalised with sarcosine residues to yield a hybrid material that offers both WAX and WCX character. The new material (named HXLPP-WAX/WCX) was evaluated as an SPE sorbent for the extraction of acidic, basic and amphoteric analytes from aqueous media. Operational parameters relating to the SPE procedure were optimised, such as the pH of the loading sample and washing/elution solvents. The optimised SPE method was then applied to river water and effluent wastewater samples.

2. Experimental

2.1. Reagents and standards

The monomers used for the synthesis of the polymer microspheres were divinylbenzene (DVB) (80% technical grade) and 4-vinylbenzyl chloride (VBC) (90% technical grade), both supplied by Sigma Aldrich (St. Louis, MO, USA). Prior to use, they were passed through a short column of alumina (activated, neutral, also supplied by Sigma Aldrich) to remove inhibitors. 2,2-Azobisisobutyronitrile (AIBN) (97%), used as initiator, was purchased from BDH Lab Supplies (Poole, UK) and recrystallised from acetone at low temperature. For the hypercrosslinking reactions, iron (III) chloride (96% anhydrous) was used as received from BDH LabSupplies. Acetonitrile (ACN) (99.9% HPLC grade), methanol (MeOH) (≥ 99% analytical specification), toluene (99.3% LabReagent), acetone, 1,2-dichloroethane (DCE) (99.8% anhydrous), diethyl ether (99.8% ACS reagent) and ethanol (EtOH) (≥ 99.8%) were all supplied by Sigma-Aldrich. For the preparation of washing solutions, the reagents used were nitric acid (65%, provided by Sigma-Aldrich), potassium carbonate (K_2CO_3) and sodium hydrogen carbonate ($NaHCO_3$), supplied by VWR International (Leuven, Belgium). Sarcosine ethyl ester hydrochloride (≥ 99%), purchased from Sigma-Aldrich, and potassium hydroxide (KOH), obtained from VWR International, were used for the polymer-analogous reactions.

The group of 11 model compounds selected for the SPE evaluation included artificial sweeteners, illicit drugs, pharmaceuticals and metabolites. Potassium acesulfame (ACE), alitame (ALI), clofibric acid (CLO AC) (a metabolite of clofibrate), diclofenac (DICLO), fenoprofen (FEN), methadone (MET), neotame (NEO), propranolol (PROP), saccharin (SAC) and trimethoprim (TRI) were purchased as pure standards from Sigma-Aldrich. Mephedrone (MEP) was purchased from LGC Standards. All standards were of >96% purity.

Stock solutions of individual standards were prepared in MeOH at a concentration of 1000 mg L^{-1} and stored at −20 °C. Working solutions of a mixture of all compounds were prepared weekly in a mixture of ultrapure water and MeOH (50/50) and were stored at 4 oC in the dark. Ultrapure water was provided by a water purification system (Veolia, Sant Cugat del Vallès, Spain) and HPLC grade MeOH and ACN were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid (HCOOH) and ammonium hydroxide (NH4OH) from Sigma-Aldrich, and hydrochloric acid (HCl) from Scharlab (Barcelona, Spain), were used to prepare the mobile phase and the solutions for SPE.

2.2. Synthesis and characterisation of HXLPP-WAX/WCX sorbent

The HXLPP-WAX/WCX sorbent was synthesised via a three-step procedure: i) Precipitation polymerisation (PP) of DVB with VBC to give poly(DVB-co-VBC) microspheres; ii) Hypercrosslinking of poly(DVB-co-VBC) microspheres to give hypercrosslinked poly(DVB-co-VBC) microspheres, HXLPP; iii) Chemical treatment of HXLPP with sarcosine ethyl ester hydrochloride via a polymer-analogous reaction (to give HXLPP-WAX), followed by ester hydrolysis, to give HXLPP-WAX/WCX.

The C, H and N contents of the polymers were determined by elemental microanalysis using a Perkin Elmer 2400 Series II CHNS Analyser, whereas the chlorine contents were measured using standard titration methods. Fourier-Transform infrared (FT-IR) spectra were acquired using an Agilent Technologies 5500 Series Compact FT-IR instrument with a scanning range of 4,000-650 cm^{-1} in ATR mode. Polymer microspheres were imaged by scanning electron microscopy (SEM) using a Cambridge Instruments Stereoscan 90, with the microspheres being sputter-coated with gold using a Polaron SC500A sputter coater prior to imaging. Image analysis of the SEM micrographs using ImageJ software (1.52a version, Wayne Rasband, USA) enabled mean particle diameters to be determined (n = 100). Nitrogen sorption analysis was performed using a Micromeritics ASAP 2000 instrument.

A published synthesis protocol was followed for the preparation of the hypercrosslinked polymer microspheres [32]. For the synthesis of poly(DVB-co-VBC) microspheres by PP, DVB (5.033 g, 38.7 mmol) and VBC (15.021 g, 98.4 mmol) (25/75, w/w), were dissolved in ACN (500 mL) in a Nalgene bottle (1 L). The monomer solution was ultrasonicated at r.t. for 15 min. and then sparged with oxygen-free N_2 for a further 15 min. at ice-bath temperature. AIBN (0.560 g, 3.4 mmol, 2 mol% relative to the number of moles of polymerisable double bonds) was then added, the bottle sealed immediately under nitrogen and placed on a low-profile roller housed inside a temperature controlled incubator. The incubator temperature was ramped from ambient to 60 °C over a period of around 2 h and then held at 60 °C for 48 h, after which time a milky suspension of polymer particles had formed. The product was isolated by vacuum filtration on a 0.45 µm nylon membrane filter, washed with ACN and acetone, and dried overnight in vacuo (70 °C, 60 mbar). The product was isolated in the form of a free-flowing, white powder (3.268 g, 16 %). The characterisation data for the poly(DVB-co-VBC) microspheres can be found in the Supplementary Information.

For the hypercrosslinking of the poly(DVB-co-VBC) microspheres, poly(DVB-co-VBC) microspheres (3.210 g, 9.0 mmol of Cl) and DCE (50 mL) were placed into a three-necked, round-bottomed flask equipped with a reflux condenser and an overhead stirrer, and the flask immersed in an temperature-controllable oil bath. The suspension of microspheres was stirred gently at 100 rpm, for 1 h at room temperature and under N2. Iron (III) chloride (2.067 g, 12.7 mmol) and a second portion of DCE (40 mL) were added, the mixture heated to 80 °C and then left to react for a further 10 min. Thereafter, the flask contents (which were dark purple in colour) were cooled to r.t. and filtered by vacuum on a 0.2 µm nylon membrane filter. The product on the filter was washed sequentially with MeOH, 2 M aqueous HNO3, MeOH and acetone, and then washed with acetone via Soxhlet extraction for 24 h. The product was re-filtered by vacuum on a 0.45 µm nylon membrane filter and re-washed with MeOH and diethyl ether prior to drying overnight in vacuo (70 °C, 60 mbar) to give HXLPP as a brown-coloured, free-flowing powder (2.945 g, 92 %). See Supplementary Information for the characterisation data.

For the chemical functionalisation of HXLPP with sarcosine ethyl ester hydrochloride to give HXLPP-WAX, HXLPP (2.907 g, 3.2 mmol Cl g-1) and EtOH (70 mL) were introduced into a three-necked, round-bottomed flask equipped with a reflux condenser and an overhead stirrer, and the flask immersed in a temperature-controllable oil bath. The suspension of microspheres was stirred gently at 100 rpm for 1.5 h, then K_2CO_3 (3.883 g, 28.1 mmol) and sarcosine ethyl ester hydrochloride (4.388 g, 28.6 mmol) dissolved in H2O (70 mL) added. The flask contents were heated at 75 °C for 18 h. After cooling, the product was isolated by vacuum filtration on a 0.45 µm nylon membrane filter and washed sequentially with EtOH, MeOH, 1:1 MeOH:H2O, 0.01 M aqueous $NaHCO_3$, H2O and acetone, prior to drying for 24 h in vacuo (70 °C, 60 mbar). HXLPP-WAX was isolated as a free-flowing, orange-coloured powder (2.958 g). See Supplementary Information for the characterisation data.

The ester groups in HXLPP-WAX were hydrolysed under basic conditions to give HXLPP-WAX/WCX. For this, HXLPP-WAX (2.920 g, 2.3 mmol of sarcosine ethyl ester groups) was placed into a glass Duran bottle together with a solution of KOH (4.353 g, 77.6 mmol) in ethanol (100 mL). The tube was sealed and a low-profile roller used to mix the contents at r.t. for 24 h. The product was filtered by vacuum on a 0.45 µm nylon membrane filter, washed with large volumes of EtOH and then dried overnight in vacuo (70 °C, 60 mbar). HXLPP-WAX/WCX was isolated as its potassium carboxylate and in the form of a free-flowing, orange-coloured powder (3.274 g). FT-IR $\bar{v}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1587 (carboxylate $(CO_2)^-$ asymmetric stretch), 1400 (carboxylate $(CO_2)^-$ symmetric stretch), 1040 (amine C-N stretch), 827 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Found for HXLPP-WAX/WCX: C, 73.4 %; H, 8.5 %; N, 1.2 %; Cl, 1.9 %; Sarcosine loading level = 0.9 mmol g-1. SEM microscopy: mean particle diameter = 3.02 µm; Cv = 18.2 %. N2 sorption analysis: Langmuir SSA = 1,140 m2 g-1; specific pore volume = 0.45 cm3 g-1; mean pore width = 2.7 nm.

2.3. Solid-phase extraction procedure

An empty 6 mL SPE cartridge (Symta, Madrid, Spain) was fitted with a 10 µm polyethylene frit (Symta) followed by a 2 µm stainless steel frit (Sigma-Aldrich). The cartridge was then manually packed by weighing 200 mg of sorbent, and a second 10 µm polyethylene frit placed at the top of the sorbent bed. An SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump was used for all the subsequent SPE steps.

The SPE protocol started with the conditioning of the sorbents, and this involved passing 10 mL of MeOH through the cartridges followed by 10 mL of ultrapure water adjusted to the same pH as the sample. The volumes of river water samples and effluent wastewater samples used for the loading step was 100 mL, with all samples being adjusted to pH 6 with HCl. After loading of the samples onto the sorbent, the washing step involved washing with 1 mL of MeOH, and the elution step involved washing with 5 mL MeOH containing 5% NH_4OH. A miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) was used to evaporate the extracts to dryness prior to reconstitution with 1 mL of mobile phase (H_2O/ACN, 90/10, v/v). All reconstituted extracts were filtered using 0.45 µm PTFE syringe filters (Scharlab) before injection into the chromatographic system. The SPE cartridges were reused more than 20 times when analysing environmental waters.

Prior to SPE, the river and effluent wastewater samples were filtered through a 1.2 µm glass-fibre membrane filter and then through a 0.45 µm nylon membrane filter (Fisherbrand, Loughborough, UK).

2.4. Instrumentation and chromatographic conditions

The chromatographic system was an Agilent 1200 UHPLC equipped with a binary pump, an autosampler, an automatic injector and a DAD detector (Agilent, Waldbronn, Germany). The chromatographic column used was 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). The mobile phase was a mixture of ultrapure water (adjusted to pH 2.8 with HCl) (solvent A) and ACN (solvent B). 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 column was kept at 30 °C and the flow rate was 600 µL min^{-1}. The injection volume was 20 µL.

3. Results and discussion

3.1. Synthesis of the HXLPP-WAX/WCX sorbent

In order to prepare an amphoteric sorbent with good separation efficiency and capacity, the polymer synthesis approach taken was to use PP to deliver high quality polymer microspheres in the low micron size range, and then to subject the polymer microspheres to a hypercrosslinking process (Fig. 1). Hypercrosslinking normally leads to dramatic increases in the SSA of polymers, as has been reported in the literature for a number of mixed-mode ion-exchange polymers [5,14,17,18,33], and this results in an upturn in capacity when the polymers are used in SPE. The amphoteric moieties were installed via a polymer-analogous reaction, and were based on the α–amino acid sarcosine (N-methylglycine).

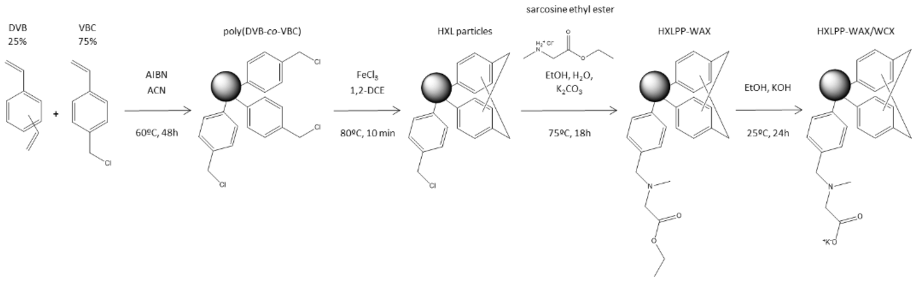

Figure 1. Synthetic procedure used to prepare the HXLPP-WAX/WCX sorbent.

For the PP, DVB was copolymerised with VBC, under textbook PP conditions, to give an acceptable yield of high quality poly(DVB-co-VBC) microspheres. The microspheres were gel-type, thus non-porous in the dry state but swellable in compatible solvents such as DCE. The polymer microspheres were hypercrosslinked in a DCE-swollen state, with the pendent chloromethyl groups providing the source of internal electrophiles for methylene bridge formation. As anticipated, the hypercrosslinking led to a dramatic increase in SSA and retention of the high quality of the microspheres.

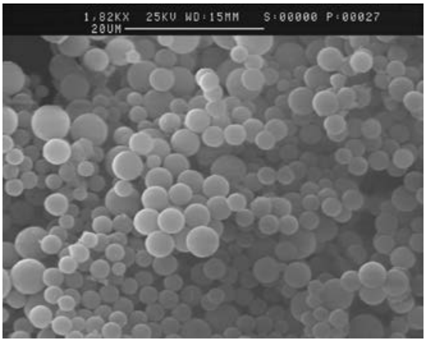

Figure 2. SEM micrograph of the HXLPP-WAX/WCX sorbent (the applied acceleration voltage of the incident electron beam was 20 kV).

The very fast hypercrosslinking reaction was not taken to full conversion of chloromethyl groups into methylene bridges – for this, the hypercrosslinking reaction time was restricted to 10 minutes – and this left a good number of pendent chloromethyl groups (1.1 mmol g^{-1}) unreacted and available for use as chemical handles in subsequent polymer-analogous reactions. The synthetic strategy taken to install amphoteric moieties into the polymers was to use a sarcosine derivative as a nucleophile to displace chloride in a nucleophilic aliphatic substitution reaction. This approach is very attractive with regards to the future development and elaboration of a family of amphoteric polymers, since many α–amino acids are available and the polymer-analogous reactions are efficient. Of course, the methodology is not restricted to α–amino acids, although having the amino groups in close proximity to the carboxylic acid groups, as is the case for α–amino acids, may allow for fine-tuning of sorbent selectivity. In the present case, sarcosine residues were installed into the hypercrosslinked microspheres by treatment of the microspheres with sarcosine ethyl ester hydrochloride under basic conditions (to give HXLPP-WAX, which is a new weak ion-exchanger in its own right), followed by ester hydrolysis to give the amphoteric polymer target, HXLPP-WAX/WCX. This high quality polymer was isolated in a good yield (close to full recovery of the microsphere product across the two polymer-analogous reactions) in a convenient beaded format (mean particle diameter = 3 µm; CV = 18%). Fig. 2 shows an SEM image of the HXLPP-WAX/WCX microspheres. HXLPP-WAX/WCX had a well-developed porous morphology (Langmuir SSA = 1,140 m^2 g^{-1}; mean pore diameter = 2.7 nm); the high SSA and low mean pore diameter arise from the hypercrosslinking process, and the values are consistent with a permanently porous polymeric material which contains a significant proportion of micropores. The loading level of sarcosine residues was calculated from the elemental microanalytical data to be 0.9 mmol g^{-1}.

3.2. Optimisation of the SPE protocol

The HXLPP-WAX/WCX polymer was evaluated as an SPE sorbent by using a complex aqueous mixture of three different groups of test compounds as a sample. The test compounds comprised artificial sweeteners, illicit drugs and pharmaceuticals, and included compounds with acidic, basic and amphoteric properties, chosen to allow the selectivity of the sorbent to be evaluated in detail. Care was taken to elucidate the retention mechanism of each analyte, taking into account the pK_a values of the analytes (listed in Table 1) and the ionisation state of the sorbent as a function of pH. Control of the pH in the loading and washing steps is crucial, since the analytes and the sarcosine residues on the sorbent must be in appropriate ionisation states for the analytes to be retained. The sorbent is expected to function as an anion-exchanger at low pH and as a cation-exchanger at high pH, however at intermediate pH values the sarcosine residues are expected to be in a zwitterionic form and this opens up the possibility of analyte retention by the sorbent via both ion-exchange mechanisms. Estimates of the pK_a values of polymer-bound Brønsted acids and bases can be made by consideration of small molecule analogues, however such estimates are complicated when the polymers are heterogeneous and micro-environmental effects are likely, as in the case for HXLPP-WAX/WCX.

The initial SPE conditions evaluated used 100 mg of sorbent and, for the loading step, 25 mL of a standard solution of the analytes at a range of different pH values. The washing step comprised 2 x 2 mL MeOH, and was included to remove non-selectively bound analytes, i.e., analytes bound by hydrophobic interactions rather than by ionic interactions. Based on results from previous studies [14,18], two different elution solvents were applied: firstly, an acidic elution solvent (5 mL MeOH containing 5% HCOOH) to disrupt cation-exchange interactions and, secondly, a basic elution solvent (5 mL MeOH containing 5% NH4OH) to disrupt anion-exchange interactions.

Table 1. %R obtained with HXLPP-WAX/WCX when using different volumes of ultrapure water at pH 6.

			pK_a	25 mL	100 mL	250 mL
Acidic	Strong	ACE	-0.3	11	3	3
		SAC	1.6	66	13	15
	Weak	CLO AC	3.4	93	91	94
		FEN	4.0	92	94	101
		DICLO	4.0	98	95	99
Amphoteric		ALI	3.4/8.2	12	10	10
		NEO	4.2/9.1	25	29	26
Basic		MEP	8.1	86	80	78
		MET	9.1	92	84	94
		PROP	9.7	95	84	94
		TRI	10.8	94	89	97

% RSD (n=3) <18%

During the evaluation, the washing and elution fractions were collected and diluted with ultrapure water (to 5 mL for the washing fraction, and to 10 mL for the elution fractions) and analysed in order to evaluate possible losses of analytes. The elution recoveries were taken as the sum of the recoveries of the acidic and basic elution steps, and are discussed in Section 3.2.4. The parameters optimised were: the mass of sorbent in the SPE cartridge, the sample pH, the washing volume, the elution conditions and the sample volume.

3.2.1. Sample pH

The first parameter evaluated was the pH of the loading solution. Initially, three distinct pH values (pH 3, 6 and 9) were investigated to provide insights into the properties and retention mechanisms of the amphoteric sorbent for the range of test analytes. With the exception of strongly acidic ACE, for which around 50% of analyte was lost when loading at pH 6 and pH 9, the retention of the analytes was very high and essentially quantitative.

Thereafter, the sorbent was washed with MeOH. Unsurprisingly, it was found that the retention (or loss) or analytes in the washing step depended upon the pH of the loading solution. For loading at pH 3, it was found that all of the analytes were lost in the washing step, except for the strongly acidic ACE (pK_a= − 0.3) and SAC (pK_a= − 1.6). For loading at pH 6, the basic compounds TRI, PROP and MET were retained successfully and eluted subsequently with recoveries ranging from 71% to 97%, although the recovery of MEP (marginally less basic) was lower at 40%, whereas the acidic and amphoteric compounds

(ACE, SAC, ALI, NEO, CLO AC, FEN and DICLO) were lost completely to the washing step. For loading at pH 9, only TRI, PROP, MET were recovered effectively, with recoveries ranging from 55 % to 88 %; the rest of the compounds were lost to the wash.

To provide more insight into the pH-dependent retention behaviour, samples were loaded at three more pH values (pH 2, 5 and 7) and the sorbents washed, as before, with MeOH. For loading at pH 2, it was observed, yet again, that only the most acidic analytes (ACE and SAC) were retained after washing with MeOH; this is because the sorbent displays WAX character but not WCX character at low pH, and ACE and SAC are the only two analytes in anionic form at this pH. The other analytes were not retained at pH 2, either because they were acidic analytes in a neutral state (CLO AC, FEN and DICLO) or else because they were amphoteric/basic analytes in a cationic state. ACE and SAC were lost during the loading/washing steps when the loading pH was pH 5 or above since the amine moieties in the sorbent are not in an ionic form.

In order to turn on the WCX character of the sorbent and capture analytes that are cationic, the pH of the loading solution must be higher than the pK_a of the polymer-bound carboxylic acid groups but not so high that the basic compounds are neutral. In this regard, it was found that the optimal conditions required to ensure good retention of the basic compounds were when the sample was loaded at pH 6 or pH 7.

The weakly acidic compounds (CLO AC, FEN and DICLO) were not retained under any of the pH conditions tested when the mass of sorbent used was 100 mg and the sample volume was 25 mL. For the retention of this group of analytes, a fundamental requirement for their capture via anion-exchange is that the sample be loaded at a pH which is above the pK_a of the analytes, but below the pK_a of the polymer-bound amine groups; the SPE results suggested that these conditions were not realised in practice, possibly because the polymer-bound amine groups are less basic than expected due to a negative inductive effect from the carboxylic acid groups in the sarcosine residues.

The amphoteric artificial sweeteners, ALI and NEO, showed poor retention under all the conditions tested; they were always lost during the washing step. This is unsurprising since ALI and NEO are dipeptides derived from α–amino acids and have ionisable groups that are chemically similar to the polymer-bound ionisable groups in sarcosine; there is therefore no strong driving force for retention under any of the conditions tested.

Upon gathering all of these results together, it is clear that strongly acidic analytes can be retained by loading samples at lower pH (2 or 3) whereas basic analytes can be retained by loading samples at a higher pH (9) or intermediate pH (6 or 7); the analytes remain bound during washing, and can be eluted with good recoveries. This ability to switch between ion-exchange retention mechanisms simply by changing the pH of the loading solution is interesting and useful, since it allows different groups of analytes to be fractionated using one single amphoteric sorbent with dual-functionality. WAX dominates at low pH, WCX dominates at high pH, and both mechanisms can contribute to retention when the polymer-bound amphoteric species are in a zwitterionic state.

For the further optimisation work, the pH of the sample was fixed at pH 6, thereby targeting acidic and basic analytes with the HXLPP-WAX/WCX sorbent.

In a study by Salas et al. [27], two commercial mixed-mode ion-exchange sorbents (Oasis WCX and Oasis WAX) were mixed together and packed into 100 mg cartridges to give a hybrid WCX/WAX sorbent bed with similar ion-exchange capacities; comparing the data generated at pH 5, similar behaviour was observed in the case of basic compounds. However, regarding the acidic analytes, the results achieved at pH 5 were distinct since in the Salas study [27] they were not lost in the washing step. A possible explanation for this finding is that the tertiary amine groups in the sorbent are influenced by the adjacent carboxylic acid groups such that they are not in their ionic form at pH 5.

In the study by Jin et al. [30] where a ternary mixed-mode silica SPE sorbent bearing C8 groups, a primary amine and a carboxylic acid was developed, at pH 6 only the basic compounds were retained by ion-exchange mechanisms, whereas the acidic and neutral compounds were retained primarily by hydrophobic interactions.

3.2.2. Volume of washing solvent

The next parameter optimised for the SPE protocol was the volume of MeOH used in the washing step. Since the weakly acidic compounds, as well as the amphoteric compounds (ALI, NEO), were lost during the washing step, the volume of MeOH was reduced from 2 x 2 mL to 1 mL. Simultaneously, the mass of sorbent per cartridge was increased from 100 mg to 200 mg (all the following experiments were performed using 200 mg sorbent). As can be seen in Fig. 3, the effect of these two changes to the SPE method was to reduce the loss of analytes in the wash, leading to better recoveries for all analytes. All of the basic compounds (TRI, MEP, PROP, and MET) were retained and eluted successfully, with %R between 93% and 111%. Interestingly, the recoveries of weakly acidic compounds improved considerably too (the recoveries of FEN and DICLO were 73% and 74%, respectively, whereas the recovery of CLO AC was 48%). Moving forward, the volume of washing solvent was fixed at 1 mL for all subsequent experiments.

3.2.3. Elution conditions

As was outlined earlier, two different elution solvents were applied in series: firstly, an acidic elution solvent (5 mL MeOH containing 5% HCOOH) to disrupt cation-exchange interactions and, secondly, a basic elution solvent (5 mL MeOH containing 5% NH_4OH) to disrupt anion-exchange interactions. Accordingly, the elution recoveries are reported as the recovery from the acidic elution followed by the elution from the basic elution, or vice versa, with the overall recovery value for any given analyte being the sum of both recovery values. The data is presented in Table S1.

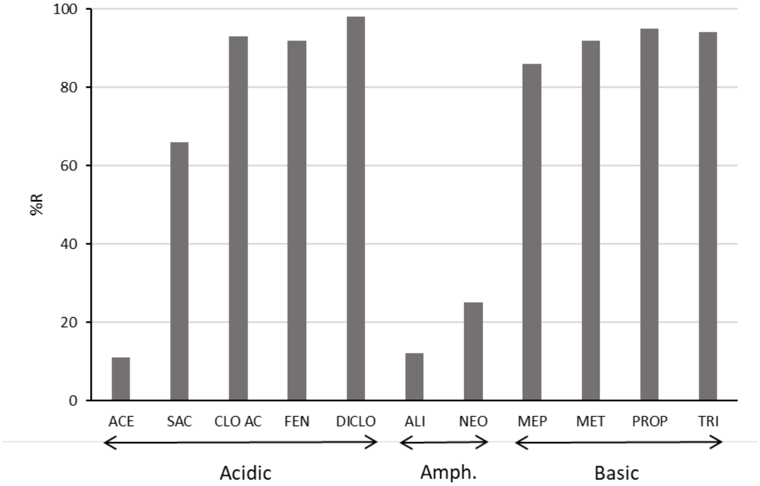

Figure 3. % R of each analyte when 25 mL of sample was loaded trough the HXLPP-WAX/WCX and 1 mL MeOH was applied as washing solution.

For acidic elution followed by basic elution, with the exception of ACE and SAC (which appeared in both elution fractions) the analytes were collected in the acidic fraction only. This is because formic acid switches off cation-exchange interactions between basic analytes and polymer-bound carboxylates by protonating the carboxylate residues and protonating any weakly acidic analytes bound through anion-exchange. For basic elution followed by acidic elution, all of the analytes were eluted quantitatively by the base. This is because ammonium hydroxide neutralises any polymer-bound amines, thereby turning off WAX behaviour, and deprotonates and neutralises any basic analytes, which means that they can no longer be retained on the sorbent by a WCX mechanism. Consequently, for the optimised SPE protocol an acidic elution step was not required; going forward, the optimised elution step used 5 mL MeOH containing 5% NH_4OH.

3.2.4. Sample volume

The effect of increasing the sample volume, from 25 mL to 100 mL and then to 250 mL, was investigated. The data is presented in Table 1. For a loading volume of 100 mL, compared to a loading volume of 25 mL there was little effect on the weakly bound acidic and amphoteric compounds; whilst the recoveries of the basic compounds decreased slightly, from 93-99% to 80-89%, the recoveries were still satisfactory. Moreover, the recoveries attained when the sample volume was 250 mL were very similar to those obtained with 100 mL of sample. Given these observations, yet higher sample volumes were not tested, and 250 mL was established as the preferred loading volume for the SPE protocol with ultrapure water sample. Nonetheless, we established 100 mL as the preferred volume for the percolation of more complex samples of environmental origin, such as river and wastewater samples.

Once all the parameters had been optimised, the optimal conditions for the SPE protocol were fixed as follows: 200 mg of sorbent; 100 mL of sample adjusted to pH 6; 1 mL MeOH as washing solution; elution with 5 mL MeOH containing 5% NH_4OH. Moreover, in order to increase the sensitivity of the method, the elution extract was evaporated to dryness and reconstituted with 1 mL of mobile phase. It should be noted that no losses of analytes were observed during the evaporation step.

3.3. Analysis of environmental samples

The optimised protocol was applied to the SPE of complex aqueous matrices, specifically river and effluent wastewater samples. In every case, any responses from analytes present in the blanks was subtracted from the responses obtained from the spiked samples.

Table 2 shows that the recoveries of all the compounds for the Ebre river water sample are slightly lower than the recoveries obtained with ultrapure water, with the exception of CLO AC and MET, where the recoveries decreased to 47% and 58%, respectively; this may be due to the presence of interferences that block the retention sites. For the effluent wastewater sample, the recoveries of the basic and weakly acidic analytes are good, especially given the complexity of such samples, although it should be borne in mind that the presence of ionic species in the samples that are not eliminated in the washing step may block the retention sites for the analytes. Fig. 4 shows the chromatograms of the SPE extracts obtained for two effluent wastewater samples; to illustrate the benefit of including a washing step in the SPE protocol, and to highlight the ion-exchange character of the amphoteric sorbent used for the extraction, one of the chromatograms is for the extract obtained when the washing step was omitted from the SPE protocol.

Table 2. %R obtained with HXLPP-WAX/WCX when using 100 mL of river water and effluent wastewater samples at pH 6 spiked at 20 µg L^{-1}.

			River	Effluent
Acidic	Strong	ACE	8	19
		SAC	32	68
	Weak	CLO AC	47	66
		FEN	73	73
		DICLO	78	83
Amphoteric		ALI	25	29
		NEO	38	39
Basic		MEP	87	88
		MET	58	61
		PROP	75	79
		TRI	85	86

% RSD (n=3) < 20%

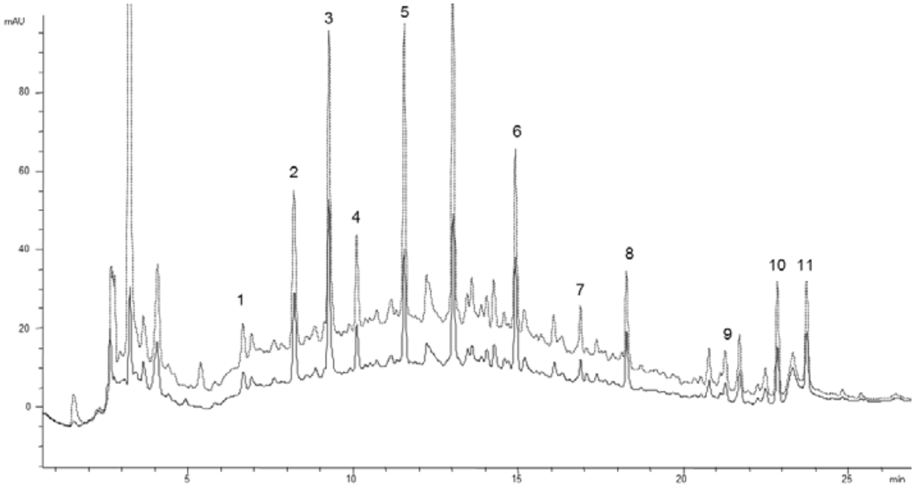

Figure 4. Overlapped chromatograms of an effluent wastewater sample spiked at 150 µg L-1 with a washing step of 1 mL of MeOH (solid line) and without washing step (dotted line). Peak identities: (1) acesulfame, (2) saccharin, (3) trimethoprim, (4) mephedrone, (5) alitame, (6) propanolol, (7) neotame, (8) methadone, (9) clofibric acid, (10) fenoprofen, (11) diclofenac.

Similar recoveries were obtained in other studies that use weak cation- or weak anion-exchange mixed-mode sorbents to determine these compounds in environmental waters [14,18]. In Fontanals' study [14], where an HXLPP-WAX polymer was used as SPE material, recoveries of 83% and 81% were reported for FEN and DICLO in river water, respectively, and 103% and 99% in effluent wastewater, respectively, similar to the present study where % recoveries of 73% were obtained in river water and effluent wastewater for FEN, and 78% and 83% were obtained in river water and effluent wastewater for DICLO, respectively. In another study [18], % recoveries of 90% and 92% were reported for PROP in river and effluent samples, respectively, when an HXLPP-WCX sorbent was used, while in the present study recoveries of 75% and 79% were obtained for PROP in river water and effluent wastewater. The repeatability of the method on the same day was also evaluated for both samples, and is expressed as the relative standard deviation (%RSD) of five replicates of river and effluent samples spiked at a concentration level of 20 µg L^{-1}. In river water the %RSDs were from 5% to 17% for the basic and amphoteric analytes, and from 10 to 18% for the acidic compounds when %R was larger than 8%. In effluent wastewater the %RSDs were from 8% to 19% for all the compounds when the %R was larger than 19%.

4. Conclusions

A distinctive approach to a multi-functional SPE sorbent (HXLPP-WAX/WCX) was devised, and this has led to the development of a microporous polymer in a convenient microsphere format where the innovative feature is the inclusion of amphoteric moieties. This was achieved through the immobilisation of sarcosine (N-methylglycine) residues within microporous polymer microspheres that are well-suited for high performance separation science work where both enhanced capacity and selectivity are demanded. The microspheres have a low mean particle diameter (3 µm), high SSA (1,140 m^2 g^{-1}) and a good functional group loading level (0.9 mmol g^{-1} sarcosine residues). Most interestingly of all, the retention mechanism of the HXLPP-WAX/WCX sorbent can be switched reversibly between anion-exchange and cation-exchange by control of pH, or placed into a zwitterionic state at intermediate pH values where both ion-exchange mechanisms can potentially contribute to the retention of analytes.

The HXLPP-WAX/WCX sorbent was applied to the SPE of acidic, basic and amphoteric analytes from ultrapure water, river water and effluent wastewater samples. When the samples were loaded at low pH an anion-exchange mechanism dominated the retention, such that strongly acidic analytes could be fractionated with excellent recovery. When the samples were loaded at high pH the basic analytes could be fractionated through cation-exchange. At intermediate pH values, it was found that acidic and basic analytes could be captured, enabling a range of analytes to be recovered very effectively, even for the most complex environmental water samples tested. However, the amphoteric analytes were not retained due to charge repulsion effects.

We believe that these results lay the ground for the development of a new family of multifunctional sorbents, where two or more ion-exchange mechanisms can be embedded within one single, bespoke mixed-mode material. The sorbents are promising candidates for challenging chemical separations and applications where high capacity and selectivity is essential.

Acknowledgments

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Supplementary data

Characterisation of the polymers

Poly(DVB-co-VBC)

FT-IR $\bar{v}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1266 (CH_2-Cl wag), 827 (1,3-disubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Expected C, 76.1 %; H, 6.5 %; N, 0.5 %; Cl, 17.0 %; Found for poly(DVB-co-VBC): C, 76.9 %; H, 6.3 %; N, 0.4 %; Cl, 10.0 %; Chlorine loading level = 2.8 mmol g^{-1}. SEM microscopy: mean particle diameter = 3.08 µm; C_v = 21.5%.

HXLPP

FT-IR $\bar{v}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1266 (CH2-Cl wag), 827 (1,3-disubstituted and 1,2,4 trisubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Found for HXLPP: C, 75.1 %; H, 6.2 %; N, 0.4 %; Cl, 3.9 %; Chlorine loading level = 1.1 mmol g^{-1}. SEM microscopy: mean particle diameter = 3.36 µm; C_v = 17.2 %.

HXLPP-WAX

FT-IR $\bar{v}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1708 (ester C=O stretch), 1182 (ester C-O stretch), 1040 (amine C-N stretch), 827 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Found for HXLPP-WAX: C, 82.4 %; H, 7.0 %; N, 1.1 %; Cl, 2.0 %; Sarcosine ethyl ester loading level = 0.8 mmol g^{-1}. SEM microscopy: mean particle diameter = 3.22 µm; C_v = 19.7 %.

Table S1. %R obtained for HXLPP-WAX/WCX when using an acidic elution followed by a basic elution, or else a basic elution step followed by an acidic elution step.

			Acidic	Basic	Basic	Acidic
Acidic	Strong	ACE	8	7	11	-
		SAC	12	53	66	-
	Weak	CLO AC	91	-	93	-
		FEN	97	-	92	-
		DICLO	95	-	98	-
Amphoteric		ALI	12	-	12	-
		NEO	22	-	25	-
Basic		MEP	103	-	86	-
		MET	101	-	92	-
		PROP	99	-	95	-
		TRI	107	-	94	-

% RSD (n=3) <16%.

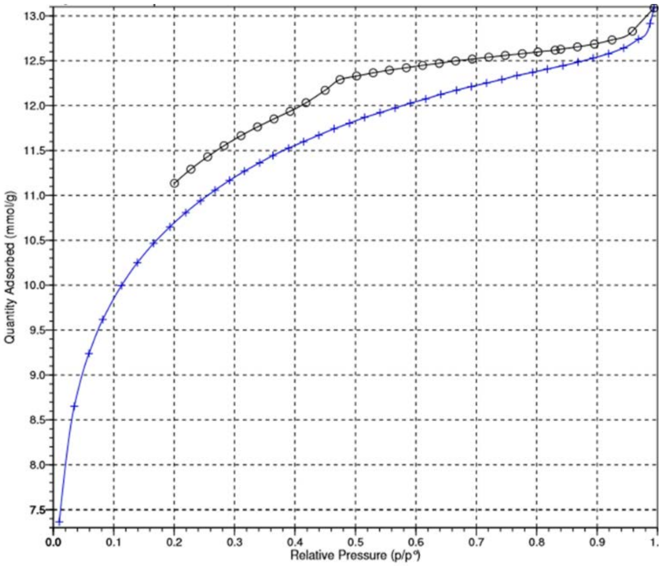

Figure S1. N_2 gas sorption isotherm of HXLPP-WAX/WCX displaying the adsorption (blue) and desorption (black) obtained at 77 K.

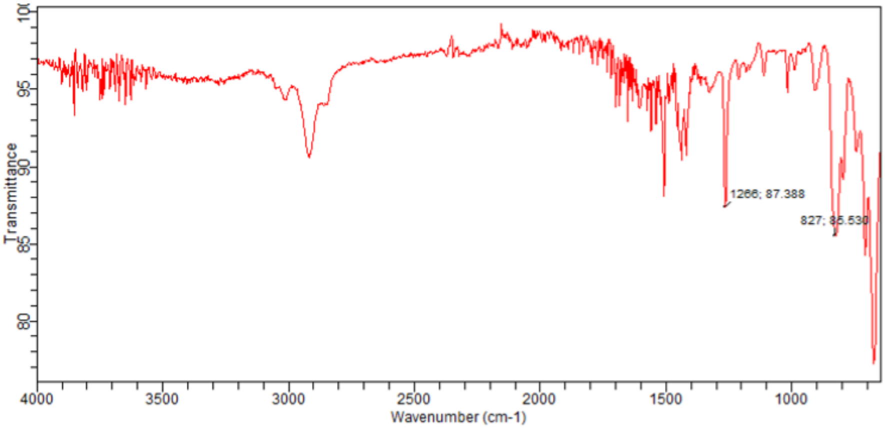

Figure S2. ATR FT-IR spectrum of poly(DVB-co-VBC).

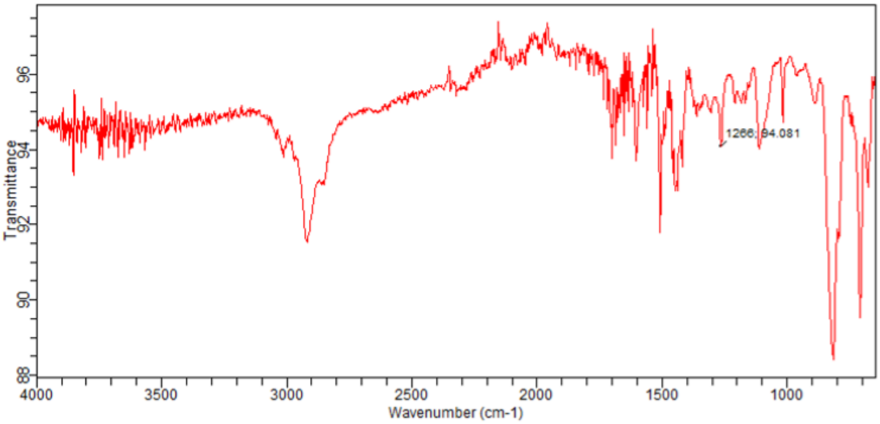

Figure S3. ATR FT-IR spectrum of HXLPP.

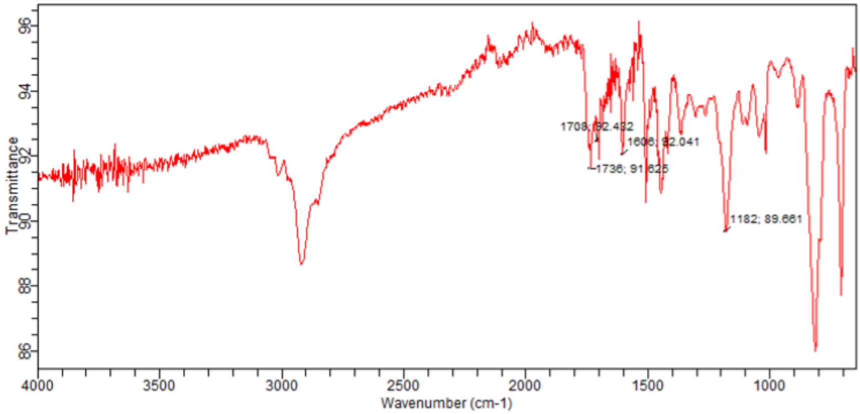

Figure S4. ATR FT-IR spectrum of HXLPP-WAX.

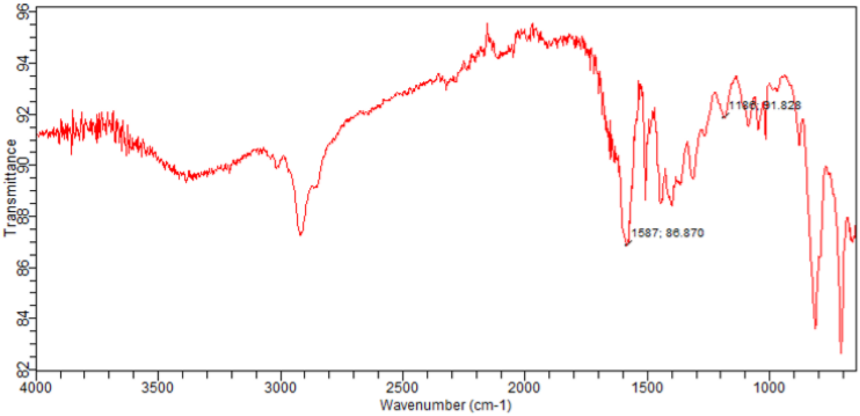

Figure S5. ATR FT-IR spectrum of HXLPP-WAX/WCX

3.1.2. Hypercrosslinked polymer microspheres decorated with anion- and cation-exchange groups for the simultaneous solid-phase extraction of acidic and basic analytes from environmental waters

Hypercrosslinked polymer microspheres decorated with anion- and cation-exchange groups for the simultaneous solid-phase extraction of acidic and basic analytes from environmental waters

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Abstract

Mixed-mode ion-exchange sorbents were introduced to improve the selectivity and retention of solid-phase extraction (SPE) sorbents. Mixed-mode ion-exchange sorbents integrate reversed-phase chemistry with ion-exchange groups to promote favourable interactions with ionic species. Nevertheless, a need to extract analytes with acidic and basic properties simultaneously within the same SPE cartridge led to the introduction of novel amphoteric/zwitterionic sorbents, which incorporate cation- and anion-exchange moieties within the same functional group attached to the polymeric network.

In the present study, the development, preparation and SPE evaluation of two novel hypercrosslinked zwitterionic polymeric sorbents, functionalised with either strong anion-exchange (SAX) and weak cation-exchange (WCX) or weak anion-exchange (WAX) and strong cation-exchange (SCX) groups (namely HXLPP-SAX/WCX and the HXLPP-WAX/SCX), is presented for the simultaneous retention of acidic and basic compounds. The sorbents were prepared by a precipitation polymerisation route which yielded poly(divinylbenzene-*co*-vinylbenzylchloride) as a precursor polymer; subsequently, the precursor polymer was hypercrosslinked, to increase the specific surface areas and capacities of the sorbents, and then functionalised to impart the zwitterionic character. The HXLPP-SAX/WCX sorbent was decorated with quaternised sarcosine groups and the HXLPP-WAX/SCX sorbent was decorated with taurine moieties. The SPE parameters were optimised to exploit the ionic interactions between compounds and the functional groups. The optimal conditions involve a washing step to remove the compounds retained by hydrophobic interactions, thus increasing the selectivity. The optimised SPE protocol used the quaternised sarcosine-based sorbent followed by liquid chromatography and tandem mass spectrometry, and was applied to determine compounds with acidic and basic properties from environmental samples, such as river water and effluent wastewater samples, with excellent selectivity and matrix effect values below -30% and apparent recovery results ranging from 52% to 105% for most of the compounds. The analytical method was validated for environmental water samples and used in the analysis of samples in which some of the target compounds were found at ng L^{-1} concentration levels.

Keywords: *Mixed-mode ion-exchange sorbents; hypercrosslinked polymers; microspheres; zwitterionic sorbents; solid-phase extraction; environmental water samples*

1. Introduction

Sample pre-treatment is an essential step in the determination of organic contaminants present at low concentrations in complex samples. This is because it is necessary to not only preconcentrate the contaminants prior to analysis, but also to remove the interferences that are present in complex samples which can lead to signal interference during analysis (e.g., ion-suppression in mass spectrometry (MS) analysis). Sorptive extraction techniques, such as solid-phase extraction (SPE), solid-phase microextraction and stir bar sorptive extraction, can achieve high degrees of preconcentration and selectivity (by decreasing matrix effects (ME%) and thereby improve sensitivity and reproducibility. SPE is the pre-eminent technique due to its simplicity, high preconcentration factors and the availability of a range of distinct sorbents [1–4].

Nowadays, the range of SPE sorbents available is very broad, ranging from sorbents that promote capacity enhancements, such as hypercrosslinked polymers, to sorbents that promote selectivity enhancements, such as molecularly imprinted polymers. However, capacity and selectivity enhancements can be offered in a single material by mixed-mode ion-exchange sorbents [5]. Mixed-mode ion-exchange sorbents can be silica-based or polymer-based, with immobilised functional groups conferring the ion-exchange properties onto the sorbents. The sorbents are classified in four main groups: strong anion-exchange (SAX), strong cation-exchange (SCX), weak anion-exchange (WAX) and weak cation-exchange (WCX). Strong ion-exchangers are charged throughout the entire pH range, and are normally based upon sulfonic acids or quaternary amines to give strong cationic and anionic exchangers, respectively. Conversely, the ionisation state of weak ion-exchangers vary depending upon the pH, and the sorbents are normally functionalised with carboxylic acids or tertiary, secondary or primary amines to give weak cation- and anion-exchangers, respectively [5]. Commercial polymeric mixed-mode sorbents, such as Strata-X-A or Oasis WCX, are available and have been used to extract ionisable analytes [6–8]. Alternatively, commercial silica sorbents have been developed, albeit to a lesser extent, including Bond Elut Certify II among others [9].

Non-commercial, silica-based mixed-mode sorbents have also been developed [10–13]. For instance, Wójciak-Kosior et al. [12] developed a polyaniline-based sorbent by the in situ polymerisation of aniline on silica; the sorbent was used to extract three alkaline plant metabolites (benzophenanthridine, protoberberine and protopine alkaloids) from Chelidonium majus extracts. The protonated polyaniline suggested that retention of the analytes was by π-π and ionic interactions. Excellent recoveries (> 96%) were reported for the compounds investigated.

Regarding non-commercial, polymer-based mixed-mode ion-exchange sorbents, most of them are based on hypercrosslinked polymer networks. Mixed-mode hypercrosslinked materials were developed and evaluated in our group as SPE sorbents for the extraction of ionisable compounds in aqueous samples [14–17]. The fact that they are hypercrosslinked means that they typically have high micropore contents and correspondingly high specific surface areas, which enhances the capacity and leads to an effective retention of analytes when loading samples by SPE [18]. For instance, Bratkowska et al. [14] prepared two different hypercrosslinked sorbents (HXLPP-SAX$_a$ and HXLPP-SAX$_b$) in a microsphere format and applied these materials as SAX sorbents in SPE for the extraction of acidic analytes from environmental samples.

All of the mixed-mode materials described thus far focus on the retention of one type of ionic species, either cationic or anionic. In order to retain both acidic and basic compounds simultaneously, different strategies have been devised, such as multi-layered and tandem approaches. Studies using various mixed-mode ion-exchange sorbents in tandem for sample clean-up have been published recently [19–22]. For example, the combination of Oasis MAX with Oasis MCX was reported to be the optimal SPE configuration, with excellent recoveries ranging from 91% to 99% [19]. Additionally, Salas et al. [22] combined pairs of commercially available mixed-mode sorbents (including SCX/SAX, SCX/WAX, WCX/SAX, and WCX/WAX) bearing opposite charges within one SPE cartridge to achieve simultaneous extractions of acidic and basic analytes with results comparable to other studies. A washing step consisting of 15 mL MeOH was used in the SPE protocol, which proved to be sufficient to remove the neutral interferences.

An alternative strategy towards the simultaneous extraction of acidic and basic analytes is to bring cation- and anion-exchange groups together in a single SPE sorbent, as was reported recently by our group [23]. In this study, a sorbent based on hypercrosslinked polymer microspheres bearing WAX and WCX moieties (HXLPP-WAX/WCX) was developed to extract acidic and basic analytes from environmental waters, where the sorbent displays amphoteric character by virtue of immobilised sarcosine (N-methyl glycine) residues. In a separate study, an amphoteric methacrylate-based material with sulfoethylbetaine groups was synthesised and applied as SPE sorbent to retain hydrophilic solutes by the water retention capacity of the sorbent surface [24].

The current paper presents the design, preparation and SPE evaluation of two novel hypercrosslinked polymeric sorbents functionalised with amphoteric groups. The immobilised groups are either quaternised sarcosine groups, in which case the sorbent offers SAX and WCX character and is referred to as HXLPP-SAX/WCX, or taurine residues in which case the sorbent offers WAX and SCX character and is referred to as HXLPP-WAX/SCX. An optimisation of the SPE parameters was conducted, including the elution conditions, washing step and sample volume. An SPE method using the best-performing sorbent was then validated and applied to environmental waters, with quantification by LC-MS/MS.

2. Experimental

2.1. Reagents and standards

The polymer microspheres were synthesised using the vinyl monomers 4-vinylbenzyl chloride (VBC) (90% technical grade) and divinylbenzene (DVB) (80% technical grade), both provided by Sigma Aldrich (St. Louis, MO, USA). The monomers were purified by passing them through a short column of neutral alumina (Sigma Aldrich). The solvents used were toluene (99.3% LabReagent), methanol (MeOH) (≥ 99% analytical specification), acetonitrile (ACN) (99.9% HPLC grade), acetone (≥ 99% analytical specification), dimethylsulfoxide (DMSO) (≥ 99% analytical specification), 1,2-dichloroethane (DCE) (99.8% anhydrous), ethanol (EtOH) (≥ 99.8%) and diethyl ether (99.8% ACS reagent), also purchased from Sigma-Aldrich. Iron (III) chloride (96% anhydrous), purchased from BDH LabSupplies, was employed for the hypercrosslinking reactions. 2,2-Azobisisobutyronitrile (AIBN) (97%), purchased from BDH Lab Supplies (Poole, UK), was recrystallized at low temperature from cold acetone and used as initiator. Sarcosine methyl ester hydrochloride and taurine, sourced from Sigma-Aldrich and of high purity (≥99%), tetra-n-butylammonium bromide (TBAB) (>98%) purchased from Alfa Aesar, iodomethane (MeI) (≥ 99%) from Sigma-Aldrich, THF (>99.7%) and potassium hydroxide (KOH), both supplied by VWR International, were employed as received from the suppliers. Tetrahydrofuran (THF) and nitric acid (65%), both supplied by Sigma-Aldrich, potassium carbonate (K_2CO_3) and sodium hydrogen carbonate ($NaHCO_3$), both provided by VWR International (Leuven, Belgium), were the reagents used to prepare the washing solutions.

The compounds selected to evaluate the retention properties were: saccharin (SAC), naproxen (NAP), ibuprofen (IBP), fenoprofen (FEN), diclofenac (DICLO), clofibric acid (CLO AC) (a metabolite of clofibrate) and potassium acesulfame (ACE) as acid compounds; and trimethoprim (TRI), ranitidine (RAN), propranolol (PROP), metoprolol tartrate salt (MTP), methadone (MET), mephedrone hydrochloride (MEP) and atenolol (ATE) as basic compounds. They were all purchased as pure standards with > 99% purity from Sigma-Aldrich, except MEP from LGC Standards. The compounds selected and their pK_a values are shown in Table 1.

The main stock solutions of the acidic and basic standards were prepared individually in methanol and stored at -20 °C in the dark. The stock concentration level was 1000 mg L^{-1}. Mixed working solutions including all the compounds were prepared weekly by mixing with proper amounts of each stock individual solution followed by dilution with ultrapure water (50/50, v/v), and kept at 4 °C in the dark.

The solvents used to prepare the mobile phase and the solutions for the SPE procedure were as follows: MeOH and ACN HPLC-grade, supplied by J. T. Baker (Deventer, The Netherlands), ammonium hydroxide (NH_4OH) and formic acid (HCOOH) purchased from Scharlab (Barcelona, Spain). Ultrapure water was provided by a Synergy UV water purification system (Merck Millipore, Burlington, USA).

Six water samples, three from Ebre river water and three from effluent wastewater from a treatment plant near Tarragona, were taken to be analysed. Prior to the SPE protocol, the river and effluent wastewater samples were filtered using a 1.2 µm glass-fibre membrane filter and then using a 0.45 µm nylon membrane filter (Fisherbrand, Loughborough, UK).

2.2. Synthesis and characterisation of the polymer microspheres

The hypercrosslinked polymer-based materials decorated with ion-exchange groups were prepared following a three-step procedure: firstly, the synthesis of poly(DVB-co-VBC) microspheres (PP) by the precipitation polymerisation of the monomers DVB and VBC; secondly, the hypercrosslinking of the poly(DVB-co-VBC) microspheres to give hypercrosslinked polymer microspheres (HXLPP); thirdly polymer-analogous reactions on HXLPP to yield hypercrosslinked polymer microspheres decorated with ion-exchange groups. The polymer-analogous reactions involved either the reaction of HXLPP with sarcosine methyl ester hydrochloride under basic conditions (to give an HXLPP-WAX material) followed by methylation of the amine and ester hydrolysis to yield the HXLPP-

Table 1. Analyte structure, pKa, MRM transitions and MS/MS parameters for the model compounds.

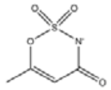
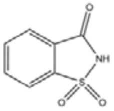
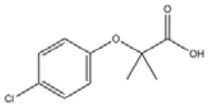
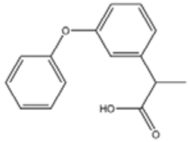
		Analyte	Formula	Structure	pK_a	Ion. mode	Precu. ion (m/z)	Frag. (V)	Prod. ion (m/z)	CE (eV)	Prod. ion (m/z)	CE (eV)
ACIDIC	STRONG	ACE	$C_4H_4NO_4S$		-0.3	NEG	162	75	82	11	78	15
		SAC	$C_7H_5NO_3S$		1.6	NEG	182	75	105	22	62	24
	WEAK	CLO AC	$C_{10}H_{11}ClO_3$		3.37	NEG	213	75	127	8	85	5
		FEN	$C_{15}H_{14}O_3$		3.96	NEG	241	75	197	2	93	5

Table 1. (Continued).

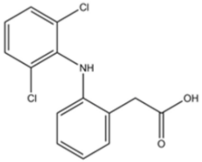
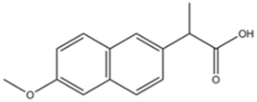
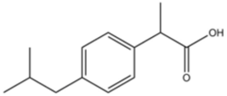
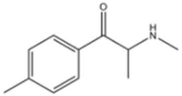
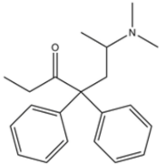
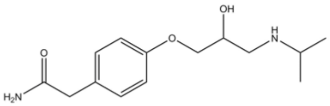
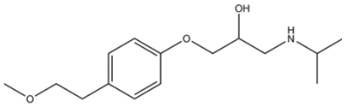
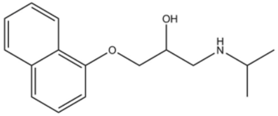
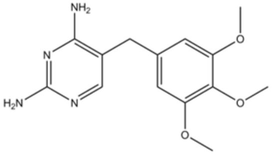
		DICLO	$C_{14}H_{11}Cl_2NO_2$		4.00	NEG	294	75	250	5	214	8
		NAP	$C_{14}H_{14}O_3$		4.19	NEG	229	75	185	2	169	4
		IBP	$C_{13}H_{18}O_2$		4.85	NEG	205.1	75	161	2		
BASIC		MEP	$C_{11}H_{15}NO$		8.0	POS	178.1	100	160	8	145	12
		MET	$C_{21}H_{27}NO$		9.1	POS	310.2	100	265	12	223	16

Table 1. (Continued).

	ATE	$C_{14}H_{22}N_2O_3$		9.67	POS	267.2	100	145	22	74.2	22
	MTP	$C_{15}H_{25}NO_3$		9.67	POS	268.2	100	116	22	74	22
	PROP	$C_{16}H_{21}NO_2$		9.7	POS	260.1	100	183	22	116	22
	TRI	$C_{14}H_{18}N_4O_3$		10.8	POS	291.1	100	261	22	230	22

SAX/WCX sorbent, or treatment of HXLPP with taurine to give the HXLPP-WAX/SCX sorbent.

2.2.1. Preparation of poly(DVB-co-VBC) microspheres

The synthesis of the hypercrosslinked polymer microspheres was based on a published synthetic method [25]. The monomers DVB (5.027 g, 38.4 mmol) and VBC (15.049 g, 98.4 mmol) (25/75, w/w) were used in a precipitation polymerization to give poly(DVB-co-VBC) microspheres, employing ACN (500 mL) as solvent and mixing all the reagents in a Nalgene bottle (1 L). The monomer mixture was introduced in an ultrasonic bath for 15 min. at room temperature (r.t.) and then introduced in an ice-bath and sparged with oxygen-free N_2 for 15 min. AIBN (0.542 g, 3.3 mmol, 2 mol% relative to the number of moles of polymerisable double bonds) was added then the Nalgene bottle sealed under nitrogen and placed on a low-profile roller housed inside a temperature-controlled incubator. The temperature of the incubator was raised from ambient to 60 °C for a period of 2 h and kept at 60 °C for 48 h, whereupon a milky suspension of polymer particles formed. The particles were filtered on a 0.45 µm nylon membrane filter by vacuum, isolated and washed with acetone and ACN and dried overnight in vacuo (70 °C, 60 mbar), yielding free-flowing, white poly(DVB-co-VBC) microspheres (5.217 g, 26%). A replicate synthesis yielded 6.108 g (30%) of product. See Supplementary Information (SI) for the polymer characterisation data on this intermediate product.

2.2.2. Preparation of hypercrosslinked poly(DVB-co-VBC) microspheres (HXLPP)

Poly(DVB-co-VBC) microspheres (4.605 g, 20.5 mmol of Cl) and DCE (90 mL) were introduced into a 250 mL round-bottomed, three-necked flask equipped with an overhead stirrer and reflux condenser, and immersed in a temperature-controllable oil bath. The mixture was stirred at 100 rpm for 1 h at r.t. and left to swell in the solvent under N2. The addition to the flask of a second portion of DCE (70 mL) and iron (III) chloride (3.057 g, 18.9 mmol) was conducted and the mixture was heated to 80 °C and left to react for a further 10 min. After that time, the dark purple mixture was cooled to r.t. and filtered by vacuum on a 0.45 µm nylon membrane filter. The filtered product was washed first with MeOH, 2 M aqueous HNO_3, MeOH and acetone, and secondly with acetone by Soxhlet extraction for 24 h. A 0.45 µm nylon membrane filter was used to filter the product by vacuum and washed again with MeOH and diethyl ether. Then, the particles were dried overnight in vacuo (70 °C, 60 mbar) to obtain brown-coloured, free-flowing HXLPP particles (4.325 g). A replicate synthesis used 4.577 g of the second batch of the poly(DVB-co-VBC) microspheres and yielded 4.242g of HXLPP product. See SI for the polymer characterisation data on this intermediate product.

2.2.3. Preparation of the HXLPP-SAX/WCX sorbent

The HXLPP particles (2.514 g, 6.064 mmol Cl) were subjected to chemical functionalisation with sarcosine methyl ester hydrochloride to yield an HXLPP-WAX material. Sarcosine methyl ester hydrochloride (3.317 g, 23.8 mmol), HXLPP (2.514 g, 6.064 mmol Cl), sodium hydrogen carbonate (3.980 g, 47.4 mmol) and tetra-n-butylammonium bromide (1.850 g, 5.740 mmol) were introduced into a round-bottomed, three-necked flask fitted with an overhead stirrer and a reflux condenser. A temperature-controllable oil bath was used to immerse the flask. Toluene (50 mL) was added into the flask and it was heated at 80 °C for 24 h. The product was cooled and isolated by vacuum filtration on a 0.45 µm nylon membrane filter. Soon after, the mixture was washed with water and acetone, before drying for 24 h in vacuo (70 °C, 60 mbar) to obtain free-flowing, orange-coloured HXLPP-WAX particles (2.586 g). See ESI for the polymer characterisation data on this intermediate product.

The tertiary amine groups in the polymer-bound sarcosine methyl ester residues were methylated in order to convert the WAX groups into SAX groups and deliver an HXLPP-SAX material. For this, HXLPP-WAX (2.224 g, 2.891 mmol of sarcosine methyl ester groups) and tetrahydrofuran (50 mL) were added to a round-bottomed, three-necked flask fitted with a reflux condenser and an overhead stirrer, and the flask immersed in a temperature-controlled oil bath. The mixture was stirred at 100 rpm for 30 min at r.t. prior to the addition of MeI (1.731 g, 12.195 mmol), after which time the flask contents were refluxed for 48 h. After allowing the contents to cool to room temperature, the product was filtered by vacuum on a 0.45 µm nylon membrane filter and washed with a 0.1 M aqueous NaOH solution, water, and acetone before drying for 24 h in vacuo (70 °C, 60 mbar). HXLPP-SAX was isolated as a free-flowing, orange-coloured powder (2.061 g). See SI for the polymer characterisation data on this intermediate product.

To obtain the HXLPP-SAX/WCX particles, the methyl ester groups of the HXLPP-SAX product were hydrolysed. For this, HXLPP-SAX (1.969 g, 1.2 mmol of quaternised sarcosine methyl ester groups) was placed into a round-bottomed, three-necked flask equipped with a reflux condenser and overhead stirrer, and immersed in a temperature-controllable oil bath. To this, a solution of KOH (4.276 g, 76.4 mmol) in MeOH (100 mL) was added and the suspension was left stirring at r.t. for 24 h at an agitation speed of 100 rpm. The resulting product was filtered using a 0.45 µm nylon membrane filter by vacuum and subsequently washed with large volumes of MeOH and dried overnight in vacuo (70 °C, 60 mbar), yielding orange-coloured HXLPP-SAX/WCX particles in their potassium carboxylate form (1.902 g). A reaction scheme outlining the synthetic procedure used to prepare the HXLPP-SAX/WCX sorbent is presented in Figure 1a.

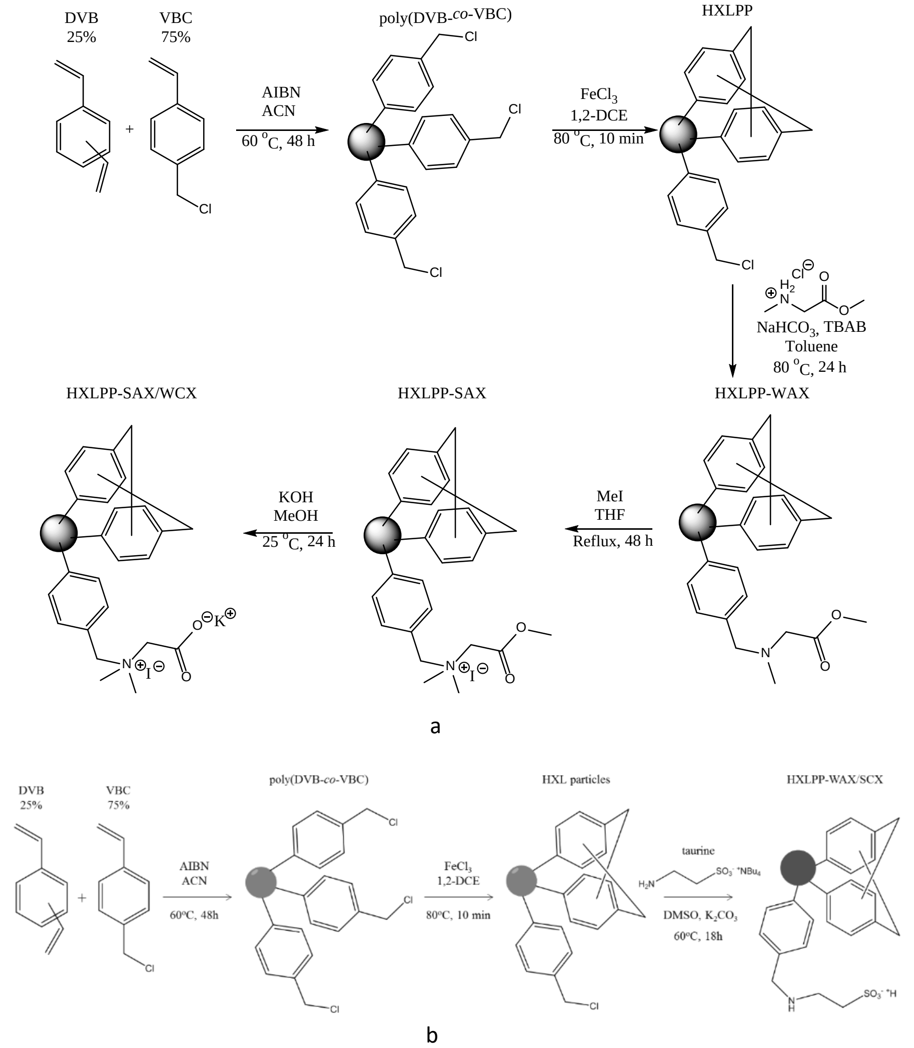


Figure 1. Reaction schemes showing the synthetic methods used to prepare the HXLPP-SAX/WCX sorbent (a) and the HXLPP-WAX/SCX sorbent (b).

Figure 1SA shows the FT-IR spectrum of HXLPP-SAX/WCX with the following bands (ν̄/cm-1): 3012 (aromatic C-H stretch), 2911 (aliphatic C-H stretch), 2850-2700 (broad, quaternary ammonium C-H stretch), 1750-1650 (broad, carboxylate C-O stretch), 819 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 709 (aromatic ring bend). Elemental microanalysis found for HXLPP-SAX/WCX: C, 84.6 %; H, 7.2 %; N, 0.6 %; Sarcosine loading level = 0.4 mmol/g. SEM microscopy: mean particle diameter = 4.31 µm; C_v = 18.79 %. N_2 sorption analysis: Langmuir specific surface area = 860 m^2/g; specific pore volume = 0.35 cm^3/g; mean pore width = 2.7 nm.

2.2.4. Preparation of the HXLPP-WAX/SCX sorbent

The second batch of poly(DVB-co-VBC) microspheres was used for the preparation of the HXLPP-WAX/SCX sorbent. For this, hypercrosslinked poly(DVB-co-VBC) microspheres (2.001 g, 2.201 mmol Cl) were introduced together with DMSO (60 mL) into a round-bottomed, three-necked flask fitted with a reflux condenser and overhead stirrer, and the mixture stirred under an N_2 atmosphere for 30 min. Taurine (0.603 g, 4.818 mmol) and K_2CO_3 (0.653 g,4.725 mmol) were dissolved in DMSO (40 mL), the solution added into the round-bottomed, three-necked flask and the mixture stirred and heated at 60 °C for 18 h. After this period, the particles were filtered on a 0.45 µm nylon membrane filter and then washed successively with ACN, MeOH, acetone, THF and acetone before being dried overnight in vacuo (70 °C, 60 mbar). HXLPP-WAX/SCX was isolated as a free-flowing, orange-coloured powder (2.304 g). A reaction scheme outlining the synthetic procedure used to prepare the HXLPP-WAX/SCX sorbent is presented in Figure 1b.

Figure 1SB shows the FT-IR spectrum of HXLPP-WAX/SCX with the following bands ($\bar{\nu}$/cm-1): 3350-3310 (amine N-H stretch), 3017 (aromatic C-H stretch), 2919 (aliphatic C-H stretch), 1600 (aromatic C=C stretch), 1364 (O=S=O asymmetric stretch), 1260 (CH_2-Cl wag), 1260 (amine C-N stretch), 1183 (O=S=O symmetric stretch), 822 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 707 (aromatic ring bend). Elemental microanalysis found for HXLPP-WAX/SCX: C, 82.4 %; H, 7.2 %; N, 0.7 % S, 0.3%; N loading level = 0.5 mmol/g; S loading level = 0.1 mmol/g. SEM microscopy: mean particle diameter = 6.1 µm; C_v = 5.9 %. N_2 sorption analysis: Langmuir specific surface area = 1310 m^2/g; specific pore volume = 0.51 cm^3/g; mean pore width = 2.8 nm.

2.3. Solid-phase extraction procedure

200 mg of sorbent (either HXLPP-SAX/WCX or HXLPP-WAX/SCX, as appropriate) was packed into a 6 mL SPE tube (Symta, Madrid, Spain) using an upper 10 µm polyethylene frit (Symta) and a lower 10 µm polyethylene frit followed by a 2 µm stainless steel frit (Sigma-Aldrich) to prevent sorbent loss. The cartridge was mounted on the vacuum manifold (Teknokroma, Barcelona, Spain) and conditioned with 10 mL of MeOH followed by 10 mL of ultrapure water adjusted to pH 6 for both sorbents. The samples were adjusted to pH 6 with HCl. A washing step, consisting of 1 mL of MeOH, was introduced to remove interferents from the matrix. The nature of the elution step depended upon which sorbent was in use. For the HXLPP-SAX/WCX sorbent, the elution step used 5 mL of 5% NH_4OH in MeOH. For the HXLPP-WAX/SCX sorbent, the elution step used 7 mL of 5%

NH_4OH in ACN. The HXLPP-SAX/WCX sorbent was evaluated for environmental water samples, and for this work 250 mL of river water and 100 mL of effluent wastewater were loaded thorough the cartridge.

The elution fractions were evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) prior to reconstitution with 1 mL of mobile phase (H2O/ACN, 90/10, v/v). and filtered through 0.45 µm PTFE syringe filters (Scharlab) for UHPLC analysis.

2.4. Instrumentation and chromatographic conditions

For the evaluation of both sorbents and optimisation of the SPE protocol, an Agilent 1200 UHPLC instrument equipped with autosampler, oven, binary pump, automatic injector and a DAD detector was used (Agilent Technologies, Waldbronn, Germany). Ultrapure water (adjusted to pH 2.8 with HCl) (solvent A) and ACN (solvent B) were used as the mobile phases. A Tracer Excel 120 C_8 (150 mm × 4.6 mm i.d., 5 µm particle size) provided by Teknokroma (Sant Cugat del Vallès, Spain), set at 30 °C, was used as the chromatographic column. The flow rate was 600 µL min^{-1} and the injection volume was 20 µL. The gradient starting conditions were ultrapure water (pH 2.8)/ ACN (90/10, v/v). Then, 10% ACN was ramped to 40% at 12 min and then to 100% ACN at 16 min, kept for 3 min and returned to the initial conditions in 3 min. The signal was measured at 210 nm for all the compounds.

For the method validation, which was performed with the HXLPP-SAX/WCX sorbent only, an Agilent 1200 series LC coupled with a 6460 QqQ mass spectrometer (MS/MS) detector with electrospray ionisation (ESI) interface was used (Agilent Technologies). The LC system was equipped with an autosampler, a degasser, an oven and a quaternary pump (Agilent Technologies, Waldbronn, Germany). The chromatographic conditions in LC-MS/MS were the same as used for LC-DAD.

Regarding the MS/MS detection, the basic compounds (ATE, TRI, MEP, PROP, MET, MTP) were analysed under ESI positive mode, whereas the acidic compounds (ACE, SAC, CLO AC, FEN, DICLO, IBP, NAP) were analysed under ESI negative mode, since better ionisation is achieved in each case. The optimum source conditions for basic compounds were: capillary voltage of 2500 V; nebuliser pressure of 60 psi; nitrogen flow rate of 13 L min^{-1}; source gas temperature of 350 ºC. For the acidic compounds they were: capillary voltage of 3000 V; nebulizer pressure of 25 psi; nitrogen flow rate of 12 L min^{-1}; source gas temperature of 350 ºC for. The optimal MS/MS parameters are detailed in Table 1. The optimum fragmentor voltage was selected for each compound, which was 100 V for all basic compounds and 75 V for the acidic compounds. The collision energy was between

8 and 22 eV (basic compounds) and between 2 and 24 eV (acidic compounds). A precursor ion and two product ions were selected for each analyte, except for IBU for which only one transition was acquired. The most abundant transitions were measured for quantification, and the other transitions and the corresponding ion ratios were used for confirmation purposes in multiple reaction mode (MRM) mode (Table 1).

All compounds presented good linearity (r^2=0.998) in LC-MS/MS in the following ranges: 0.01 to 50 µg L^{-1} for ACE, MEP, MTP, PROP and TRI; from 0.05 to 50 µg L^{-1} for ATE, NAP, CLO ACID and IBP; 1 to 50 µg L^{-1} for SAC and FEN; and, 0.05 to 10 µg L^{-1} for RAN and DICLO.

3. Results and discussion

3.1. Preparation of sorbents

For the synthesis of the sorbents, precipitation polymerisation (PP) was the preferred method of polymerisation since this method delivers high quality polymer microspheres in the low micron size range. Accordingly, DVB and VBC were copolymerised by free radical polymerisation under PP conditions to give poly(DVB-co-VBC) microspheres that were lightly crosslinked, had diameters in the low micron size range and which contained reactive chloromethyl groups. Hypercrosslinking of the polymer microspheres was then conducted when the polymer microspheres were in a swollen state, to impart porosity into the polymer microspheres. The hypercrosslinking chemistry is extremely efficient and rapid, thus the hypercrosslinking reaction time was restricted to 10 minutes to leave a portion of the polymer-bound chloromethyl groups unreacted, as disclosed in previous studies [18], and available for subsequent polymer-analogous reactions with sarcosine and taurine.

The HXLPP-SAX/WCX sorbent was prepared by immobilising sarcosine residues on the hypercrosslinked polymer microspheres followed by N-alkylation. This sorbent was prepared in good yield and had a convenient beaded format thanks to the method of polymerisation (mean particle diameter = 4.3 µm); Figure 2a shows an SEM image of the HXLPP-SAX/WCX microspheres. Well-developed porous morphology is an important feature of high-performance, high capacity sorbents since specific surface area is related to capacity, so it is noteworthy that the HXLPP-SAX/WCX sorbent had a high specific surface area (Langmuir specific surface area = 860 m^2/g). The sarcosine loading level was 0.4 mmol/g.

The HXLPP-WAX/SCX sorbent was prepared by immobilising taurine residues on the hypercrosslinked polymer microspheres. This sorbent also had a beaded format (mean particle diameter = 6 µm) and was prepared in good yield; Figure 2b shows an SEM image of the HXLPP-WAX/SCX microspheres. The HXLPP-WAX/SCX sorbent had an even higher specific surface area than the HXLPP-SAX/WCX sorbent (Langmuir SSA = 1311 m^2/g). From the elemental microanalytical data, the functional group loading level (taurine content) of this sorbent was estimated to be 0.1 mmol/g.

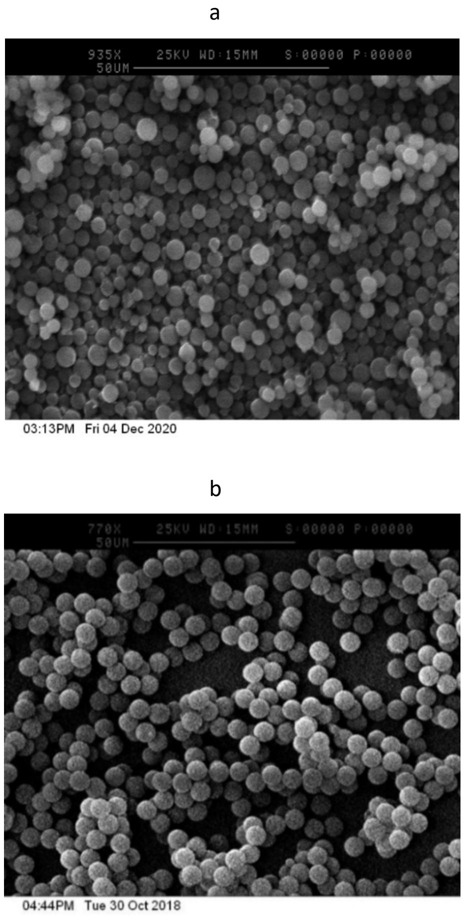


Figure 2. SEM micrographs of the HXLPP-SAX/WCX sorbent (a) and the HXLPP-WAX/SCX sorbent (b). The applied acceleration voltage of the incident electron beam was 25 kV.

3.2. Optimisation of the SPE protocol

For the evaluation of the HXLPP-SAX/WCX and HXLPP-WAX/SCX sorbents, model compounds with acidic and basic properties were selected. The SPE conditions should be carefully optimised to ensure the establishment of ionic interactions between the ionic groups of the sorbent and the ionisable groups in the compounds. The loading pH was selected based on the pK_a values of the model compounds (Table 1) and the sorbents, in order to promote ionic interactions between the model compounds and sorbents.

In a previous study where an HXLPP-WAX/WCX sorbent was evaluated [23], we concluded that pH 6 was a suitable pH for sample loading since this allowed ionisation of functional groups on the model compounds and sorbent. In the present study, the HXLPP-SAX/WCX sorbent has a similar WCX group to the HXLPP-WAX/WCX sorbent reported previously but has an SAX group instead of a WAX group (thus the SAX character will be turned on throughout the pH range). Hence, pH 6 should be a good loading pH for the HXLPP-SAX/WCX sorbent as well.

As for the HXLPP-WAX/SCX sorbent, it is expected to function as an anion- and cation-exchanger at low pH, since both the amine groups and the sulfonic acid groups will be ionised under these conditions. At pH values above the pK_a of the most weakly acidic model compound (IBP; $pK_a = 4.9$) and below the pK_a of the amine moieties on the HXLPP-WAX/SCX sorbent, the analytes and the sorbent are expected to be ionised, thus pH 6 was selected as the loading pH.

The SPE conditions under which the sorbents were evaluated initially were: 200 mg of sorbent (based on previous experience [15,17,26,27]) loaded with 100 mL of ultrapure water adjusted at pH 6 and spiked at 150 $\mu g\ L^{-1}$ with the analyte mixture. A washing step comprising of 1 mL MeOH was included to remove those compounds bound to the sorbents by reversed-phase interactions only and to improve the selectivity of the SPE. The elution conditions used were distinct for each sorbent. Based on previous studies [15,26], two different eluents were used in series for the HXLPP-SAX/WCX sorbent: an acidic eluent (5 mL 5% HCOOH in MeOH) followed by a basic eluent (5 mL 5% NH_4OH in MeOH). The elution recoveries were taken as the sum of the recoveries of the acidic and basic elution steps. For the HXLPP-WAX/SCX sorbent, a basic elution solvent (5 mL 5% NH_4OH in MeOH) was employed.

Upon applying the initial SPE conditions to the HXLPP-SAX/WCX sorbent, all of the compounds were extracted successfully with recoveries between 83 and 107%, except for RAN (64%) (Table 2). The basic compounds and the weakly acidic compounds were eluted from the sorbent in the acidic elution step, whereas the strongly acidic ACE ($pK_a = -0.3$) and SAC ($pK_a = -1.6$) were eluted in the basic elution step. Upon applying the initial SPE conditions to the HXLPP-WAX/SCX sorbent, the basic compounds were extracted with high recoveries from 70% to 112%. However, MET and PROP were highly retained and a higher elution volume (2 more fractions of 5 mL 5% NH_4OH in MeOH) was required to elute them from the sorbent. Regarding the acidic analytes, some weakly acidic analytes were partially lost in the washing step and so relatively poorly retained (recoveries from 20% to 61%), except CLO AC and FEN for which the recoveries were 94% and 78%, respectively. ACE and SAC were lost during the loading step because of repulsion of these highly polar acids by the sulfonate groups. The recoveries of analytes obtained in the initial SPE evaluation of both sorbents are presented in Table 2. Similar recovery levels were achieved for the basic compounds across both sorbents, whereas better recoveries were found for the acidic compounds when the HXLPP-SAX/WCX sorbent was used. The strongly acidic ACE and SAC were only retained by the HXLPP-SAX/WCX sorbent and efficiently eluted using a basic elution.

Table 2. Recovery (R, (%) obtained for both sorbents in initial experiments using the conditions indicated in the text.

				Recovery (%)	
				HXLPP-SAX/WCX[a]	**HXLPP-WAX/SCX**
			pK_a	**Elution**	**Elution**
Acidic	Strong	ACE	-0.3	96	-
		SAC	1.6	90	4
	Weak	CLO AC	3.4	95	94
		FEN	4.0	91	78
		DICLO	4.0	92	61
		NAP	4.2	83	20
		IBP	4.9	87	49
Basic		MEP	8.0	84	112
		RAN	8.2	64	70
		MET	9.1	97	99
		ATE	9.7	107	112
		MTP	9.7	97	102
		PROP	9.7	95	89
		TRI	10.8	98	95

[a] For HXLPP-SAX/WCX the elution recoveries were taken as the sum of the recoveries of the acidic and basic elution steps.

RSD (%) (n=5) < 9% when R (%) > 20%.

Moving forward from this initial evaluation, different parameters were optimised to improve the selectivity and capacity of the SPE method for both sorbents. The parameters investigated were the elution conditions, the washing volume and the sample volume.

3.2.1. Elution conditions

Firstly, the elution conditions were evaluated for both sorbents. To compare the results with the elution conditions used initially, for the HXLPP-SAX/WCX sorbent the order of eluents was switched, such that the basic elution (5 mL of 5% NH_4OH in MeOH) was performed before the acidic elution (5 mL of 5% HCOOH in MeOH). For the HXLPP-WAX/SCX sorbent, an acidic elution step comprising 5 mL of 5% HCOOH in MeOH was introduced before the basic elution step.

For the HXLPP-SAX/WCX sorbent, all analytes were eluted efficiently from the sorbent during the basic elution step (5 mL 5% NH_4OH in MeOH) with %R >75%, even the strongly acidic analytes ACE and SAC. Given these results, going forward the optimised elution step for the HXLPP-SAX/WCX sorbent comprised 5 mL of 5% NH_4OH in MeOH. For the HXLPP-WAX/SCX sorbent, the introduction of the acidic elution step before the basic elution step did not lead to any improvements in recovery (%R < 20%). Consequently, a basic elution step comprising 5 mL of 5% NH_4OH in MeOH was used to elute the analytes. However, it was necessary to use more 5 mL aliquots of basic MeOH to elute MET and PROP completely. For this reason, 5% NH_4OH in ACN was investigated as an alternative eluent in an effort to reduce the volume of eluent required; under these modified conditions, MET and PROP were eluted completely by the first two 5 mL aliquots of ACN containing 5% NH4OH, indicating that the elution strength is increased when MeOH is replaced by ACN.

Then, the elution volume had to be optimised and fixed with the goal being to use the minimum volume necessary to elute all the analytes completely from the sorbent. It was found that 7 mL of 5% NH_4OH in ACN was sufficient to elute MET and PROP completely. Moving forward, for all subsequent experiments with the HXLPP-WAX/SCX sorbent, 7 ml of 5% NH_4OH in ACN containing was the eluent used.

3.2.2. Volume of washing solvent

The next parameter optimised was the volume of MeOH used in the washing step. Since none of the analytes was lost during a washing step involving 1 mL of MeOH, the volume was increased to 3 x 2 mL fractions of MeOH in order to remove compounds bound to the sorbent by non-selective, hydrophobic interactions. Regarding the HXLPP-SAX/WCX sorbent, the acidic compounds were lost, either partially or completely, by the three-fold washing step, whereas the basic compounds were retained strongly and eluted subsequently with R (%) between 91% and 113%, except for RAN (48%). This is likely to be because the basic compounds establish stronger ionic interactions with the anionic moieties of the sorbent than the acidic compounds establish with the cationic moieties.

Given these observations, and to minimise losses during the washing step, the optimised washing volume of MeOH used in the washing step for all following experiments was 1 mL. It should be noted that when using volumes of washing solution greater than 1 mL, the sorbent still retains selectivity for the extraction of basic compounds (basic compounds remain bound to the sorbent even after 6 mL of MeOH is applied). Similar results were obtained for the HXLPP-WAX/SCX sorbent; when increasing the volume of MeOH used in the washing step the acidic compounds were lost.

In other studies, a washing step with MeOH has also been applied. For instance, in Prosen's study [28] different washing volumes (from 2-10 mL MeOH) were tested when extracting drugs from environmental waters by SPE/LC-HRMS with Oasis MCX cartridges (500 mg), aiming to get efficient removal of matrix interferences without compromising the recovery of the analytes. Use of 5 mL MeOH ensured the removal of the interferents with minimal losses of analytes, thereby enabling satisfactory recoveries (%R>75%) in ultrapure water. Higher volumes of MeOH were used as washing solvent since the amount of sorbent in the SPE cartridge was 500 mg.

In another study, Deeb et al. [19] used Oasis MAX and Oasis MCX sorbents in tandem to extract micropollutants and their transformation products from water and wastewater samples. They introduced two different washing solutions, one for each sorbent: 2 mL 5% NH_4OH in water to remove basic interferents from the Oasis MAX sorbent, and 2 mL 2% HCOOH in water to remove acidic interferents from the Oasis MCX sorbent. Satisfactory recoveries (≥90%) were achieved for all analytes in different water samples, and the matrix interferents were thoroughly removed. However, they only include an aqueous washing whose elution strength for effectively removing those compounds bound by reversed-phase interactions might be limited as compared to a washing step based on pure organic solvent.

3.2.3. Sample volume

The next parameter to optimise in order to improve the sensitivity of the method was the sample volume. The sample volume was increased from 100 mL to 250 mL and then to 500 mL. The data is presented in Table 3. For the HXLPP-SAX/WCX sorbent, the recoveries achieved when the sample volume was 250 mL were very similar to the recoveries obtained when the sample volume was 100 mL (successful retention of all compounds). When the sample volume was increased from 250 mL to 500 mL, the recoveries of the basic and strongly acidic compounds were maintained, whereas the recoveries of the weakly acidic compounds slightly decreased. Consequently, sample volumes greater than 500 mL were not evaluated. Going forward, the optimised sample volume used for the HXLPP-SAX/WCX sorbent was 500 mL.

Table 3. Recovery (%) obtained for both sorbents when different volumes of ultrapure water adjusted to pH 6 and spiked at 150 µg L^{-1} with the analyte mixture were percolated. See the text for the rest of SPE conditions.

			Recovery (%)					
			HXLPP-SAX/WCX			**HXLPP-WAX/SCX**		
			100 mL	**250 mL**	**500 mL**	**100 mL**	**250 mL**	**500 mL**
Acidic	Strong	ACE	106	103	111	3	1	-
		SAC	108	110	112	12	4	6
	Weak	CLO AC	94	96	87	97	94	91
		FEN	90	91	74	95	93	85
		DICLO	80	74	61	91	85	64
		NAP	99	98	98	25	19	30
		IBP	78	89	69	59	53	46
Basic		MEP	107	90	102	85	67	78
		RAN	75	74	74	69	73	67
		MET	104	100	94	95	82	90
		ATE	105	112	111	105	100	89
		MTP	100	105	105	102	99	100
		PROP	93	96	95	93	91	91
		TRI	95	96	97	95	93	98

RSD (%) (n=5) < 10% when R (%) > 25%.

As can be seen in Table 3, for the HXLPP-WAX/SCX sorbent the recoveries of analytes from a 250 mL sample were similar to those from a 100 mL sample. However, when the sample volume was increased to 500 mL, the recoveries of some compounds decreased slightly although the recoveries of others were maintained. For instance, the recoveries of DICLO decreased from 85% to 64%. The basic compounds showed excellent retention at all sample volumes tested, however ACE and SAC were always lost in the loading and washing steps. In view of these results, 250 mL was used as the sample volume in subsequent experiments.

As a comparison between sorbents, the HXLPP-SAX/WCX sorbent gave slightly better results for some of the basic compounds. Recoveries of 102% and 94% were achieved for MEP and MET with the HXLPP-SAX/WCX sorbent, respectively, whereas the analogous recoveries were 67% and 82% with the HXLPP-WAX/SCX sorbent. As for the weakly acidic compounds, HXLPP-SAX/WCX gave better recoveries except for FEN and DICLO (R (%) = 74% and 61%, respectively for HXLPP-SAX/WCX and 93% and 85%, respectively, for WAX/SCX sorbent). However, ACE and SAC were not retained by the HXLPP-WAX/SCX sorbent whereas they were retained essentially quantitatively by the HXLPP-SAX/WCX sorbent (%R>100%).

This new SPE data is in broad agreement with data from our previous study on an amphoteric sorbent, in which an HXLPP-WAX/WCX sorbent [23] was evaluated (recoveries were from 80% to 95% when the sample volume was 100 mL). However, a significant new finding relates to the ability of the HXLPP-SAX/WCX sorbent to retain strongly acidic analytes (neither the HXLPP-WAX/WCX sorbent nor the newly reported HXLPP-WAX/SCX sorbent are able to retain ACE and SAC in the loading step). We ascribe this to the fact that the HXLPP-SAX/WCX sorbent is the only material with strong anion-exchange character (the other two resins have weak anion-exchange character). Highly polar strong acids, such as the sweeteners ACE and SAC, are normally poorly or excessively retained in sorptive extraction techniques [29], thus it is very pleasing to see that the HXLPP-SAX/WCX sorbent enables the efficient extraction of both of the polar strong acids (with the basic eluent, the recoveries were 111% and 112%, respectively, when the sample volume was 500 mL).

For all other compounds investigated, results comparable to Segura’s study [30] were obtained. In Segura’s work, a group of organic contaminants was extracted from 250 mL of surface water by SPE using a number of different commercial mixed-mode cartridges: Strata-X-AW, Strata-X-CW and Strata-X (reversed phase materials with polar groups). Similarly to the recoveries reported in our study, extraction recoveries around 100% were obtained for DICLO, NAP, IBP and MET for the three cartridges tested, except for Strata-X-AW when the recovery of IBP was around 50%. It should be emphasised that in Segura’s study only one type of ion-exchange group (either cationic or anionic) was present on any given sorbent, whereas in our study we have two types of ion-exchange group coexisting on the same sorbent. In addition, the washing step used by Segura was aqueous-based only which may be less efficient at removing compounds (or interferences) bound by reversed-phase interactions.

Once the SPE procedure had been optimised, the optimal conditions for the SPE protocol were fixed as follows: 200 mg of sorbent; 500 mL of ultrapure water adjusted to pH 6 for the HXLPP-SAX/WCX sorbent and 250 mL of ultrapure water adjusted to pH 6 for the HXLPP-WAX/SCX sorbent; 1 mL MeOH as washing solution; elution with 5 mL of 5% NH_4OH in MeOH for the HXLPP-SAX/WCX sorbent and 7 mL of 5% NH_4OH in ACN for the HXLPP-WAX/SCX sorbent. In order to enhance the sensitivity of the method, the elution extracts were evaporated to dryness and reconstituted in 1 mL of mobile phase (no losses of analytes were observed during the evaporation step). Whilst both sorbents performed well, the best results overall were returned by the HXLPP-SAX/WCX sorbent, thus it was this sorbent which was used in the method validation work as applied to the SPE of environmental water samples.

3.3. Method validation

The SPE method for the HXLPP-SAX/WCX sorbent was validated using river and effluent wastewater samples using the optimised extraction parameters. The method validation was performed by LC-MS/MS to achieve lower detection and quantification limits and increase sensitivity. 0.1 µg L^{-1} and 0.25 µg L^{-1}were the concentration levels selected in river water and effluent wastewater samples, respectively, to calculate the apparent recovery (Rapp (%)) and the ME (%) for 250 mL river water samples and 100 mL effluent wastewater samples. Lower concentration levels were not considered due to the presence of some of the analytes in non-spiked samples. The method detection limits (MDLs) and method quantification limit (MQLs), repeatability and reproducibility between days were also evaluated for both samples. The Rapp (%), ME (%), MDLs and MQLs for river and effluent samples are shown in Table 4. Non-spiked samples of river water and effluent wastewater were analysed, and the signals obtained subtracted from the signal of the spiked sample in order to calculate the Rapp (%). Rapp (%) is the recovery of the whole method and considers the extraction efficiency and the ME (%). To calculate the ME (%), the formula ME (%) = (C_{exp}/C_{theo} x 100%) – 100% was used, where C_{theo} is the concentration of the standard solution and the C_{exp} is the concentration of the analytes when the sample was spiked with the analyte mixture just after the SPE procedure. Depending on the ME (%) obtained, it can increase or decrease the signal depending upon whether there is ion-enhancement or ion-suppression, respectively.

For river water, the Rapp (%) values ranged from 52% to 93% for all compounds, except MEP and MET (where Rapp (%) = 18% and 39%, respectively), as seen in Table 4. For effluent wastewater compared to river water, the Rapp (%) values were similar for all compounds, except for NAP and RAN where the Rapp (%) values were found to decrease to 41% and 25%, respectively.

The ME (%) values obtained were similar for both sample types and support ion-suppression for most of the compounds. Low ME (%) values were obtained due to the selectivity of the sorbent and the inclusion of a washing step (1 mL MeOH) to remove matrix interferences. MET and TRI were the compounds with the higher levels of ion-suppression, with ME (%) values of -30% and -20%, respectively, for river samples.

The MDLs and MQLs were determined for the river water samples and the effluent wastewater samples. As some compounds were present in the non-spiked samples, the MDLs and MQLs of the river and effluent samples were estimated from the limits of detection and quantification (LODs and LOQs), considering the Rapp (%) results and the preconcentration factor, expressed in ng L^{-1}. They ranged from 0.03 ng L^{-1} to 3 ng L^{-1} for the MDLs, and from 0.05 ng L^{-1} to 15 ng L^{-1} for the MQLs. Detailed information appears in Table 4.

Table 4. R_{app} (%), ME (%), when 250 mL of river water and 100 mL of effluent wastewater spiked at 0.1 µg L^{-1} and 0.25 µg L^{-1}, respectively, were extracted using the HXLPP-SAX/WCX sorbent and analysed by LC-MS/MS. MDLs and MQLs values obtained using this method.

	River water						Effluent wastewater					
Analyte	R_{app} (%)	Repe. (n=5)	Repr. (n=5)	ME(%)	MDLs (ng/L)	MQLs (ng/L)	R_{app} (%)	Repe. (n=5)	Repr. (n=5)	ME (%)	MDLs (ng/L)	MQLs (ng/L)
ACE	89	11	3	-5	0.03	0.1	103	5	3	-10	0.05	0.3
SAC	93	12	5	8	1.0	4.0	101	3	2	-12	2.0	10
CLO AC	80	7	2	3	0.1	0.3	71	5	4	-6	0.3	0.8
FEN	70	18	10	-10	1.0	6.0	69	2	4	-5	3.0	15
DICLO	81	6	5	17	0.05	0.3	61	7	5	-11	0.2	1.0
NAP	60	19	8	-9	0.2	0.4	41	10	10	-5	1.5	4.0
IBP	52	16	12	-9	0.4	0.8	59	11	6	13	0.8	2.0
MEP	18	17	15	-9	0.2	0.4	24	10	10	-12	0.30	1.0
RAN	69	10	5	5	0.1	0.3	25	8	10	-2	0.08	2.0
MET	39	18	11	-30	0.04	0.1	52	11	4	-5	0.05	0.3
ATE	76	6	4	2	0.1	0.5	105	6	8	-9	0.2	1.0
MTP	80	3	4	-4	0.04	0.1	75	9	6	4	0.1	0.3
PROP	60	19	10	-7	0.05	0.1	68	2	2	18	0.1	0.3
TRI	57	5	8	-20	0.05	0.1	92	2	4	-8	0.1	0.2

The method reproducibility between days and repeatability on the same day, expressed as the relative standard deviation (RSD, %) of five replicates of river and effluent samples spiked at a concentration level of 0.1 µg L^{-1} and 0.25 µg L^{-1}, respectively, were lower than 19% and 11%, respectively, and are shown in Table 4.

3.4. Analysis of real samples

The optimised SPE method was applied to river water and effluent wastewater samples to determine the acidic and basic compounds. The concentration levels obtained for the analytes present in three samples of river water and three samples of effluent wastewater are shown in Table 5. As a summary, the analytes were found in concentrations between 4-290 ng L^{-1} in river water samples and between <MQL-545 ng L^{-1} in effluent wastewater samples. These concentrations were calculated by taking into account the %Rapp using an external calibration curve. In both sample types, all analytes were detected. Figure 3 shows the chromatograms obtained when a non-spiked sample of river (A) and effluent wastewater (B) were analysed by SPE/LC-MS/MS. The concentration levels found in river water were lower than the levels found in effluent

wastewater samples, as was expected. NAP was found at similar concentration levels in effluent wastewater, but was below the MQL. The concentration levels of the majority of the compounds reported in this study are similar to those reported in other studies [22,31–36]. For instance, CLO AC, MET and ATE were reported in this study to be at concentration levels between 4 and 31 ng L^{-1}, which is the same as the concentration levels reported previously for these compounds present in Ebre River samples (1-50 ng L^{-1}) [22]. Regarding effluent wastewater, MET and ATE were found at concentration levels (17-370 ng L^{-1}) similar to other reports concerning effluent wastewater from the same treatment plant near Tarragona as used in this study [22]. IBP, MTP and PROP were also found at very similar concentrations to values reported for effluent wastewaters from different treatment plants [33,34,36]. ACE and SAC were found at slightly lower concentration levels than other studies on river water and effluent wastewater [31,32].

Table 5. Range of concentrations (in ng L^{-1}) when three different river water samples and three different effluent wastewater samples were analysed using the optimised SPE method with the HXLPP-SAX/WCX sorbent.

	Concentration (ng L^{-1})	
Analyte	**River water**	**Effluent wastewater**
ACE	10-199	332 - 487
SAC	12-112	33-343
CLO AC	4-16	1-10
FEN	7-30	<MQL-37
DICLO	12-70	40-177
NAP	4-27	<MQL-38
IBP	12-29	98-206
MEP	20-39	10-21
RAN	112-225	<MQL-361
MET	8-23	17-56
ATE	6-31	160-370
MTP	8-21	14-174
PROP	10-28	18-47
TRI	62-290	42-545

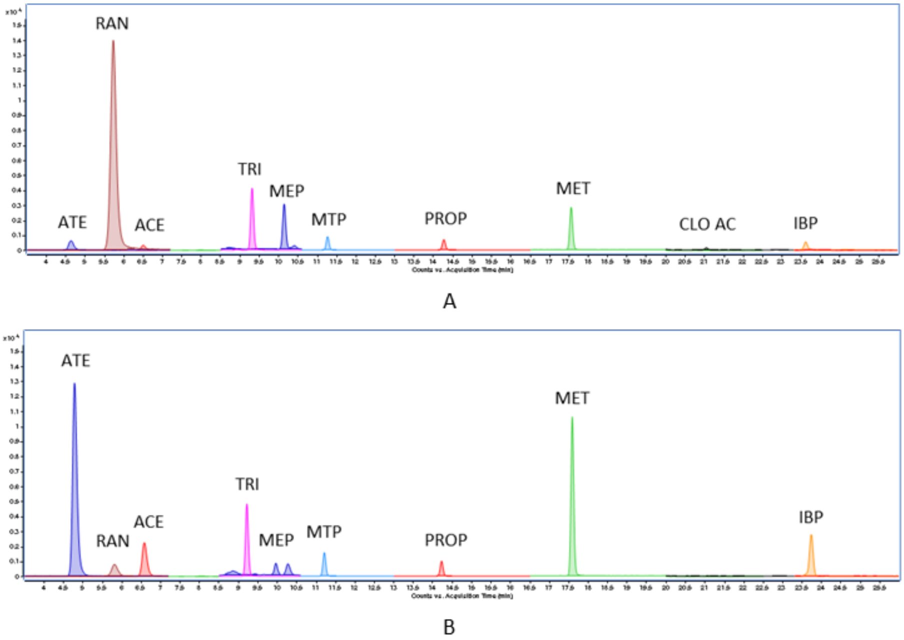


Figure 3. Chromatograms obtained when a non-spiked sample of river water (A) and effluent wastewater (B) was analysed by SPE/LC-MS/MS.

4. Conclusions

Two mixed-mode ion-exchange polymeric sorbents have been prepared by decorating hypercrosslinked polymer microspheres with two complementary ion-exchange motifs (one cation-exchange group and one anion-exchange group). More specifically, we have combined SAX character with WCX character in one of the novel sorbents (HXLPP-SAX/WCX) and WAX character with SCX character in the second sorbent (HXLPP-WAX/SCX). The polymers were prepared by polymer-analogous reactions on hypercrosslinked polymer microspheres, where the swellable precursors to the hypercrosslinked materials were prepared by precipitation polymerisation. The sorbents were produced in a convenient beaded format, had high specific surface areas, and were decorated with either sarcosine or taurine residues. It is the sarcosine and taurine motifs that confer the ion-exchange character onto the polymers.

The polymers were exploited as SPE sorbents for the retention of model compounds present in aqueous environments. Several SPE parameters were optimised (including the elution conditions, the washing volume and the sample volume), and the optimised methods enabled many acidic and basic analytes to be extracted efficiently from standard solutions. Both sorbents performed well, however the performance of the HXLPP-SAX/WCX sorbent was particularly impressive (highest recoveries and capacities) so this material was taken forward to method validation and the SPE of analytes from river water samples and effluent wastewater samples. Low %ME values were obtained for all compounds thanks to the inclusion of a washing step in the SPE protocol, and all analytes were quantified successfully at ng L^{-1} levels. The ability to capture anions and cations simultaneously using one single SPE sorbent has been demonstrated clearly in this work.

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Supplementary data

Additional polymer characterisation data

Poly(DVB-co-VBC)

FT-IR $\bar{\nu}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1266 (CH$_2$-Cl wag), 827 (1,3-disubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Expected C, 76.1 %; H, 6.5 %; N, 0.5 %; Cl, 17.0 %; Found for poly(DVB-co-VBC): C, 77.5 %; H, 6.7 %; N, Trace; SEM microscopy: mean particle diameter = 3.78 µm; C$_v$ = 18.75 %.

HXLPP

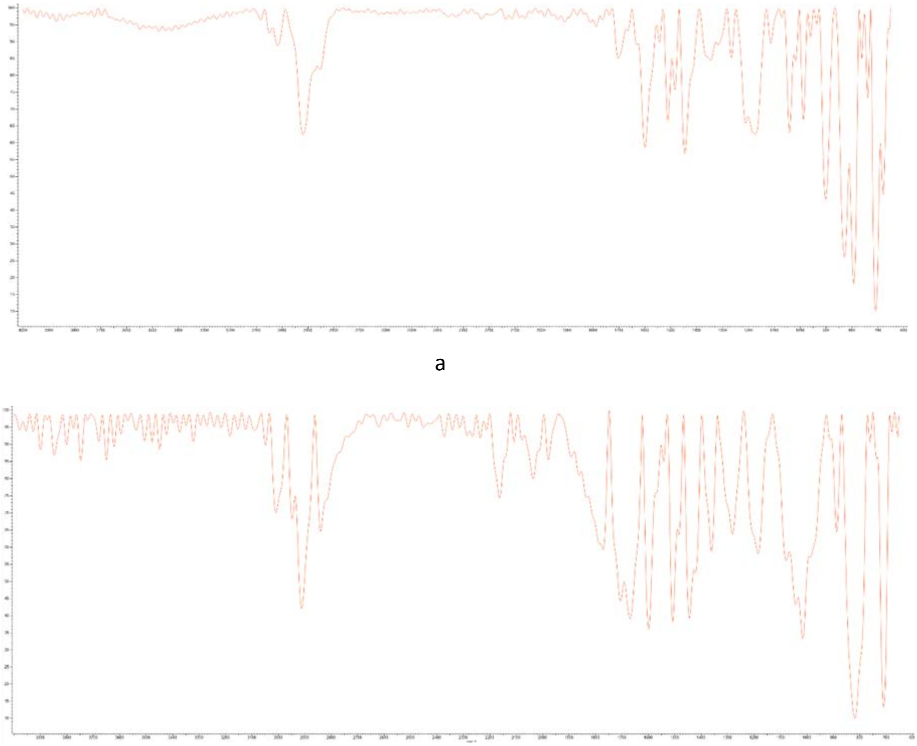
FT-IR $\bar{\nu}$/cm^{-1}: 3015 (aromatic C-H stretch), 2909 (aliphatic C-H stretch), 1264 (CH$_2$-Cl wag), 819 (1,3-disubstituted and 1,2,4 trisubstituted aromatic out-of-plane C-H bend), 677 (aromatic ring bend). Elemental microanalysis: Found for HXLPP: C, 77.4 %; H, 6.2 %; N, Trace; SEM microscopy: mean particle diameter = 4.22 µm; C$_v$ = 17.47 %.

HXLPP-WAX

FT-IR $\bar{\nu}$/cm^{-1}: 3013 (aromatic C-H stretch), 2906 (aliphatic C-H stretch), 1729 (ester C=O stretch), 1172 (ester C-O stretch), 1046 (amine C-N stretch), 817 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 707 (aromatic ring bend). Elemental microanalysis: Found for HXLPP-WAX: C, 79.7 %; H, 7.0 %; N, 1.8 %; SEM microscopy: mean particle diameter = 4.68 µm; C$_v$ = 15.66 %.

HXLPP-SAX

FT-IR $\bar{\nu}$/cm^{-1}: 3015 (aromatic C-H stretch), 2909 (aliphatic C-H stretch), 2850-2700 (broad, quaternary ammonium C-H stretch), 1727 (ester C=O stretch), 1187 (ester C-O stretch), 819 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 709 (aromatic ring bend). Elemental microanalysis: Found for HXLPP-SAX: C, 80.6 %; H, 6.9 %; N, 0.9 %; SEM microscopy: mean particle diameter = 4.58 µm; C$_v$ = 14.31 %.



a

b

Figure 1S. FT-IR spectra of HXLPP-WAX/SCX (a) and HXLPP-SAX/WCX (b).

3.1.3. Discussion of results

The following section discusses the most remarkable aspects of the SPE studies, even though the results and conclusions of each study are included in the scientific publication.

In this section, the potential of in-house mixed-mode ion-exchange polymeric materials as SPE sorbents to retain simultaneously ionizable analytes, such as acidic and basic compounds, was demonstrated. In these studies, three materials (HXLPP-WAX/WCX, HXLPP-SAX/WCX and HXLPP-WAX/SCX) were synthesised in collaboration with Prof. P.A.G. Cormack, from University of Strathclyde (Glasgow, Scotland). Different zwitterionic materials with the incorporation of different functional groups were synthesised during the stay, but the ones with the best characterization data (HXLPP-SAX/WCX and HXLPP-WAX/SCX) were further evaluated. The HXLPP-WAX/WCX was synthesised by a previous PhD student.

All three polymeric materials presented a well-developed porous morphology. Similar Langmuir specific surface area (SSA) values and mean pore diameter exhibit the three in-house materials, as shown in Table 1. HXLPP-WAX/WCX is the polymer with higher loading level (0.9 mmol/g) and lower mean particle diameter (3 µm). The hypercrosslinking process allowed to attain high SSA and low mean pore diameter to the polymers, causing an increase of the capacity as SPE sorbents. The characterization results obtained are coherent with these porous polymeric materials which contain a significant proportion of micropores.

Table 1. Characterization variables obtained for each in-house mixed-mode ion-exchange polymeric material.

Characterization parameters	HXLPP-WAX/WCX	HXLPP-SAX/WCX	HXLPP-WAX/SCX
Langmuir SSA	1140 m^2/g	860 m^2/g	1311 m^2/g
Mean particle diameter	3 µm	4.3 µm	6 µm
Mean pore diameter	2.7 nm	2.7 nm	2.8 nm
Loading level	0.9 mmol/g [a]	0.4 mmol/g [a]	0.1 mmol/g [b]

[a] sarcosine loading level; [b] taurine loading level

SPE parameters, such as loading, washing and elution were optimized before applying them in real samples. Table 2 compares the optimized ultrapure water SPE parameters for the three materials. As seen in Table 2, the ultrapure water optimal conditions were the same for all the sorbents, except for HXLPP-SAX/WCX, which allowed to use a higher loading volume, and for HXLPP-WAX/SCX, which needed the strength of the elution solvent to be increased, replacing MeOH by ACN, and to use higher elution volume (7 mL of elution solvent instead of 5 mL) to completely elute some basic analytes, MET and PROP. Comparable SPE conditions, such as sample pH loadings (pH 6-7), were used in other mixed-mode ion-exchange SPE sorbents reported in studies after the publication of these papers [1-4]. In Sorribes-Soriano study [1] a strong anion-weak cation-exchange mixed-mode sorbent (based on poly (vinylpyridine-co-methacrylic acid-coethylene glycol dimethacrylate) was in-house prepared to determine acidic and basic drugs in in oral fluid samples. A pH 7 loading solution followed by a washing of 1 mL 5% ACN aqueous phosphate buffer (0.1M, pH 7) and an elution solution made of 0.4 mL of 2-propanol was used to elute the analytes. In another study [2], similar SPE conditions (loading of 50 mL water sample adjusted to pH 6, followed by a washing step of 4 mL MeOH and an elution solution of 4 mL 4% TFA in MeOH) were applied when using a polystyrene-divinylbenzene sorbent with weak cation-exchange character for the extraction of illicit drugs in environmental water.

Table 2. Optimal SPE conditions used for each in in-house mixed-mode ion-exchange polymeric material.

SPE parameters	HXLPP-WAX/WCX	HXLPP-SAX/WCX	HXLPP-WAX/SCX
Conditioning	10 mL MeOH + 10 mL H_2O at pH 6	10 mL MeOH + 10 mL H_2O at pH 6	10 mL MeOH + 10 mL H_2O at pH 6
Loading	250 mL H_2O at pH 6	500 mL H_2O at pH 6	250 mL H_2O at pH 6
Washing	1 mL MeOH	1 mL MeOH	1 mL MeOH
Elution	5 mL 5% NH_4OH in MeOH	5 mL 5% NH_4OH in MeOH	7 mL 5% NH_4OH in ACN

The similarity of the results obtained for the three in-house mixed-mode ion-exchange polymeric materials are shown in Table 3, where the recoveries obtained when loading 250 mL (500 mL in the case of HXLPP-SAX/WCX) spiked with the mixture of the analytes in ultrapure water adjusted at pH 6 are detailed for the three different materials, HXLPP-WAX/WCX, HXLPP-SAX/WCX and HXLPP-WAX/SCX. Even though every material has different functional groups with strong and weak properties and therefore different ionic interactions with the analytes, the differences between the results obtained for the materials were minimal. The working pH ranges where both the analytes and the functional groups of the sorbents were in their charged form in each sorbent is different, being narrower for configurations containing groups with weak properties. The strong acidic compounds, ACE and SAC, were the only analytes which presented different results depending on the sorbent used. The HXLPP-SAX/WCX was the only sorbent able to efficiently retain them. Using the HXLPP-WAX/WCX, ACE and SAC were lost during the loading/washing steps when the loading pH was 5 or above since the amine moieties in the sorbent might not be in an ionic form. Regarding the HXLPP-WAX/SCX, ACE and SAC were lost during the loading step which might be attributed to the repulsion of these highly polar acids by the sulfonate groups.

Amphoteric analytes were only evaluated in the first study using HXLPP-WAX/WCX and they were discarded for the other two sorbent evaluations since they showed poor retention under all the conditions tested. Although alitame and neotame are dipeptides derived from α–amino acids and have ionizable groups that are chemically similar to the polymer-bound ionizable groups in sarcosine, they were always lost during the washing step. They were not retained probably due to charge repulsion effects.

The application of in-house mixed-mode sorbents as selective materials for the selected analytes was definitely demonstrated, which could be broadly applied to other ionizable analytes.

Table 3. %R obtained with HXLPP-WAX/WCX, HXLPP-SAX/WCX and HXLPP-WAX/SCX when using the optimal SPE conditions mentioned in Table 2.

			pK_a	**HXLPP-WAX/WCX**	**HXLPP-SAX/WCX**	**HXLPP-WAX/SCX**
Acidic	**Strong**	ACE	-0.3	3	111	1
		SAC	1.6	15	112	4
	Weak	CLO AC	3.4	94	87	94
		FEN	4.0	101	74	93
		DICLO	4.0	99	61	85
		NAP	4.19	n.e.	98	19
		IBP	4.85	n.e.	69	53
Amphoteric		ALI	3.4/8.2	10	n.e.	n.e.
		NEO	4.2/9.1	26	n.e.	n.e.
Basic		MEP	8.1	78	102	67
		RAN	8.2	n.e	74	73
		ATE	9.67	n.e.	94	82
		MTP	9.67	n.e.	111	100
		MET	9.1	94	105	99
		PROP	9.7	94	95	91
		TRI	10.8	97	97	93

Apart from being selective materials that at neutral pH can simultaneously retain a wide range on ionizable analytes, the ion-exchange mechanism could be tuned simply through control of pH. By controlling the loading pH, the materials get more selectivity. These results will lay the basis for the development of a new family of multifunctional sorbents, where two or more separation mechanisms can be embedded within one single material. For instance, employing the HXLPP-WAX/WCX when the samples were loaded at low pH, an anion-exchange mechanism dominated the retention, such that strongly acidic analytes could be fractionated with excellent recovery. When the samples were loaded at high pH the basic analytes could be fractionated through cation-exchange. At intermediate pH values, it was found that acidic and basic analytes could be captured, enabling a range of analytes to be recovered very effectively, even for the most complex environmental water samples tested.

After the publication of these papers, few studies with in-house zwitterionic mixed-mode ion-exchange sorbents have been published, in which silica-based functionalized with strong moieties sorbents were synthesized and evaluated through solid-phase extraction [5]. In this study, different drugs with ionic character were determined in river and effluent water samples, being 5 compounds in common with the previous works among them. The results obtained when loading 100 mL of ultrapure water spiked sample (recoveries between 84-101% for the compounds in common) demonstrated the success of the in-house materials, like the previous studies discussed in this section, in-house zwitterionic mixed-mode materials are postulated as an alternative to commercially available materials for the extraction of a broad range of ionizable compounds from environmental water samples. In addition, their applicability can be broadened to other types of analytes in different kinds of samples.

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3.2. Evaluation of mixed-mode ion-exchange materials to extract ionizable compounds by microextraction techniques in environmental waters

As described in the introduction of this Thesis, stir bar sorptive extraction (SBSE) is a microextraction sample treatment technique based on the principle of equilibrium distribution of interest analytes between aqueous solution and the extraction phase, the stir bar coating. It has the advantages of simple operation, good reproducibility, high recovery and low consumption of organic solvents. Currently, SBSE has been used owing to its versatility in both sampling and desorption modes and its great capacity for quantitative extraction [1] as alternative to the well-established solid-phase extraction technique.

The selection of a suitable coating material in the SBSE is critical when extracting target analytes with specific physical and chemical properties. Although different coating materials such as polydimethylsiloxane (PDMS) and ethyleneglycol-silicone (EG-Silicone) are available in SBSE, the broadly used one is PDMS which is mostly focused to extract non-polar compounds. EG silicone was later developed to extract the polar compounds, but it presents thermal stability and mechanical limitations. Stir bar coating with good mechanical and chemical stability are necessary during the extraction and desorption process. To solve these limitations, the preparation of in-house coated stir bars involving functional materials were introduced, either prepared by sol-gel or monolithic approach [2,3].

In-house mixed-mode ion-exchange materials were introduced to improve the selective extraction of ionic compounds. They integrate ionic moieties into a polymeric or silica backbone combining ionic with reversed-phase interactions. However, so far, only few mixed-mode materials are applied to SBSE or related technologies [4–7] as has been explained in chapter 1.1. For instance, a monolithic stir bar based on poly(methacrylic acid-3-sulfopropyl ester potassium salt-co-divinylbenzene) was synthesized for the simultaneous determination of nitroimidazole residues in honey by SBSE followed by LC-DAD [4]. In another study [5], perfluoroalkyl acids were determined in environmental water samples by SBSE followed by LC- MS/MS using in-house mixed-mode ion-exchange stir bars. The coated stir bars based on poly(1-vinylimidazole-ethyleneglycoldimethacrylate) (poly(VI-EDMA)) monolith were prepared by one-pot polymerization.

Among the different microextraction techniques evolved to improve the SBSE, a novel sorptive extraction technique is presented in this section. Capsule phase microextraction (CPME) has been recently introduced to overcome the limitations of other microextraction techniques. CPME is based on the principle of equilibrium extraction that uses a miniaturized microextraction capsule (MEC) as the extraction medium. The MEC involves two fused porous tubular polypropylene membranes, one to accommodate the sorbent through sol-gel technology, while the other encapsulates a magnetic metal rod. These MECs accommodate different coatings, such as PDMS, C_{18} or Carbowax 20M. The availability of different coatings in CPME is one of the main advantages of this technique over SBSE, since both techniques work under the same equilibrium principal and their extraction protocols are very similar.

CPME have been applied to extract personal care products from environmental water samples [8] and sulfonamides from milk [9]. Lakade's study [8] demonstrated the potential of CPME as sample preparation technique for the extraction of a group of personal care products (PCPs) from river water and effluent wastewater samples matrices analyzed by LC-MS/MS. Three different sol-gel sorbent materials (UCON, poly(caprolactone-dimethylsiloxane-caprolactone) (PCAP-DMS-CAP) and Carbowax 20M) were evaluated, being Carbowax 20M the coating which provided best extraction performance. In Georgiadis study [9], various sol-gel coatings were evaluated by CPME to determine four sulphonamide residues in milk samples using LC-DAD. Sol-gel polytetrahydrofuran, sol-gel PDMS, sol-gel Carbowax 20M, sol- gel PCAP-PDMS-PCAP and sol-gel PEG were the coatings evaluated. The CPME coating containing sol-gel PEG gave the best results of all the coatings tested.

In sections 3.2.1 and 3.2.2 the two developed studies focusing on preparation and evaluation of in-house mixed-mode ion-exchange coatings for SBSE and CPME, respectively, are presented in scientific article format. The results of these studies have been published articles have been published as scientific papers in the Journal of Chromatography A 1625 (2020) 461295, and Journal of Chromatography A 1663 (2022) 462748.

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

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.

Keywords: *weak anion-exchange materials; stir bar sorptive extraction; selectivity; liquid chromatography-mass spectrometry; acidic contaminants; environmental waters*

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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 (NH4OH) from Sigma-Aldrich and hydrochloric acid (HCl) from Scharlab (Barcelona, Spain) were used to adjust the mobile phase and the solutions for the SPE.

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%.

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. Both 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 (r2=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} × 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 ≥ 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.

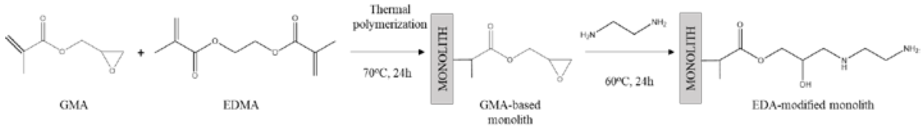


Figure 1. Scheme of preparation of GMA-based monoliths in PTFE magnets and their functionalization with EDA.

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.

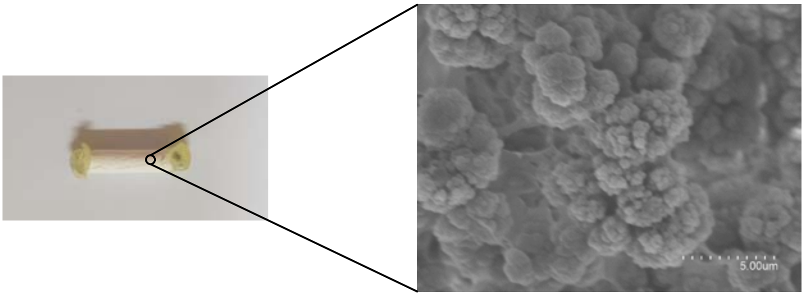


Figure 2. Photograph of PTFE magnet coated with EDA-modified monolith and its corresponding SEM micrograph (at 5000 × magnification).

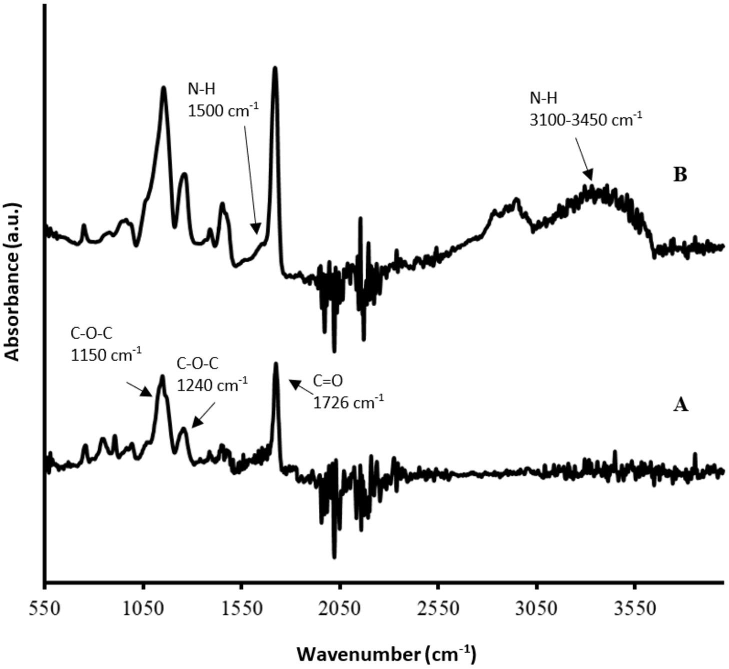


Figure 3. FTIR spectra of: (A) GMA-based monolith and (B) EDA-modified monolith immobilized onto treated PTFE magnets.

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 pKa 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.

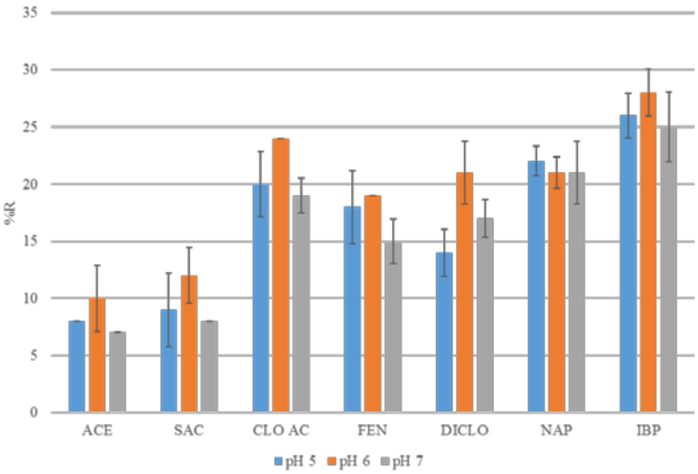


Figure 4. Effect of the pH on the acidic analytes when using the EDA2 material for SBSE.

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% NH4OH 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 NH4OH 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% NH4OH were selected as desorption solutions. It should be mentioned that similar elution conditions were used in studies [25,36] where coatings with strong anionic-exchange character were evaluated to retain a group of acidic compounds.

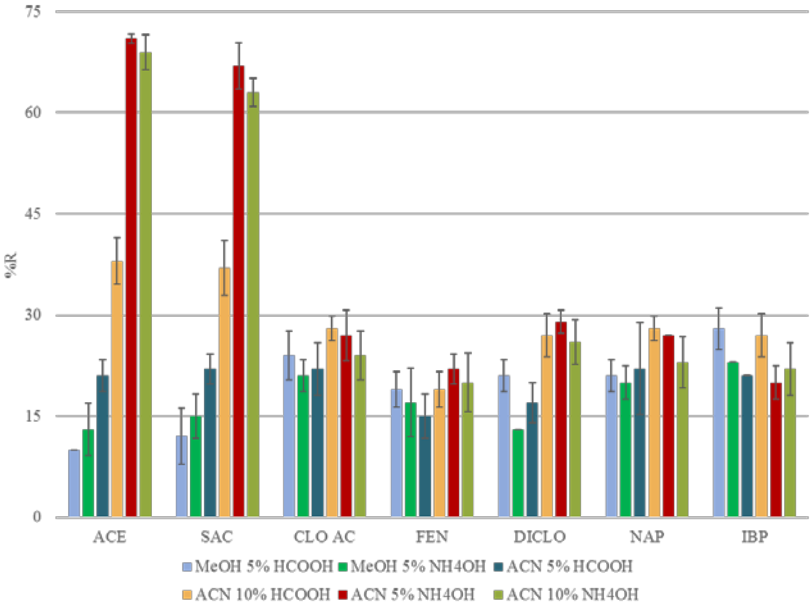


Figure 5. %R obtained with the EDA2 material when 2x3 mL elution solutions were tested.

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 %Rapp of the analytes in river water ranged between 30% and 47%, except for NAP, whose %Rapp was 20%. The %Rapp 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 (R2 ≥ 0.9902), except FEN (R2=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.

Table 2. %Rapp, %ME, MDLs and MQLs of the selected compounds in river water analyzed with the EDA2 material by SBSE.

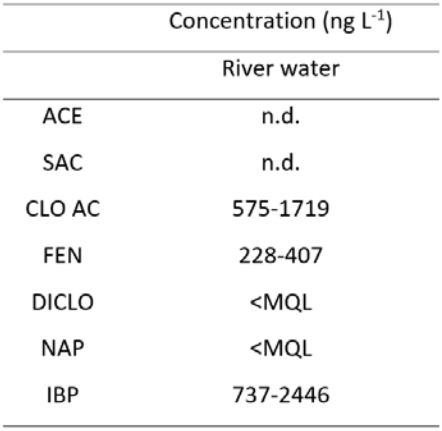
	River water				
	pK_a	$\%R_{app}{}^{a,\,b}$	$\%ME^a$	MDLs (ng L^{-1})	MQLs (ng L^{-1})
ACE	-0.3	43	-7	25	200
SAC	1.6	47	3	1000	2000
CLO AC	3.37	37	-3	25	500
FEN	3.96	31	-13	10	25
DICLO	4.00	47	17	5	25
NAP	4.19	20	-15	5	25
IBP	4.85	30	0	250	500

[a] spiked at 10 µg L^{-1}.
[b] % RSD (n=5) <9%.

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].

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

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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Supplementary data

Table S1. Analyte, pKa, ions and MS/MS conditions for the model compounds.

Analyte	pK_a	Ioniz. mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Product ion (m/z)	Collision energy (eV)
ACE	-0.3	NEG	162	**82**	11	78	15
SAC	1.6	NEG	182	**105**	22	62	24
CLO AC	3.37	NEG	213	**127**	8	85	5
FEN	3.96	NEG	241	**197**	2	93	5
DICLO	4.00	NEG	294	**250**	5	214	8
NAP	4.19	NEG	229	**185**	2	169	4
IBP	4.85	NEG	205.1	**161**	2		

* Bold denotes the quantification transition

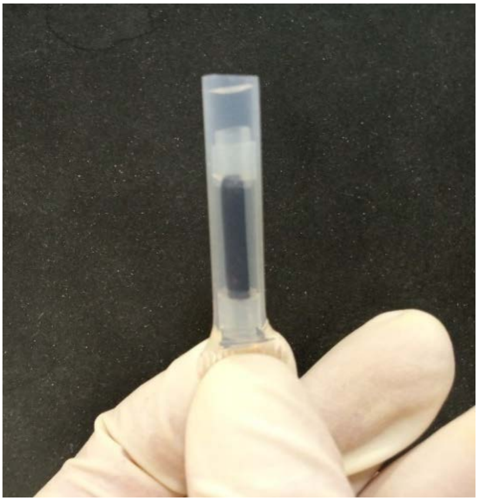

Figure S1. Experimental set-up used for the preparation of molded polymeric monoliths in PTFE coated magnets.

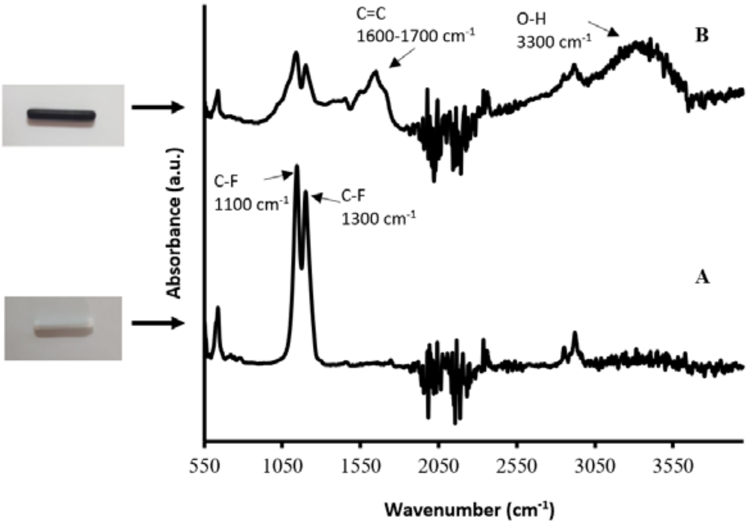

Figure S2. Photographs of PTFE magnets and their FTIR spectra: (A) bare PTFE magnet and (B) PTFE magnet treated with Fluoroetch and vinilyzed.

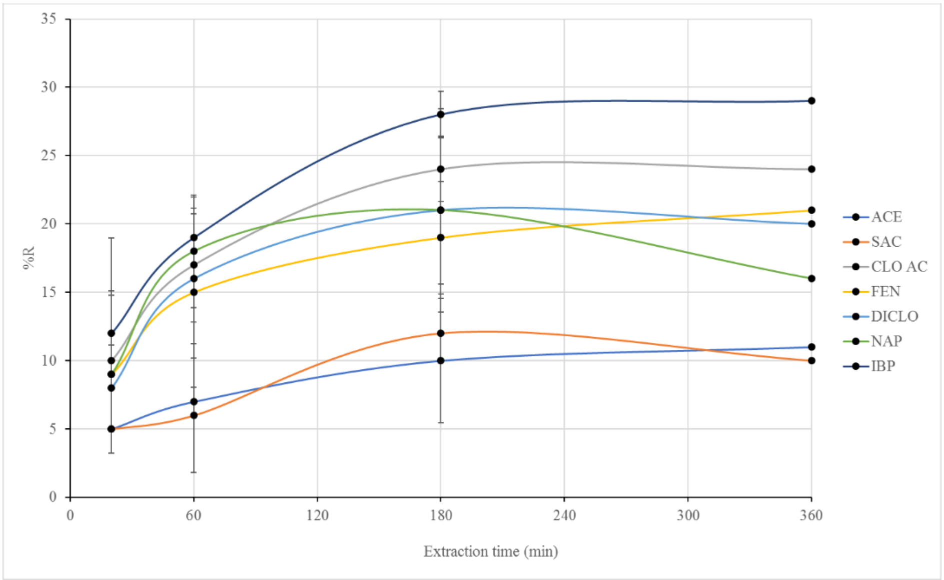

Figure S3. Effect of the extraction time on the acidic analytes when using EDA2 material for SBSE.

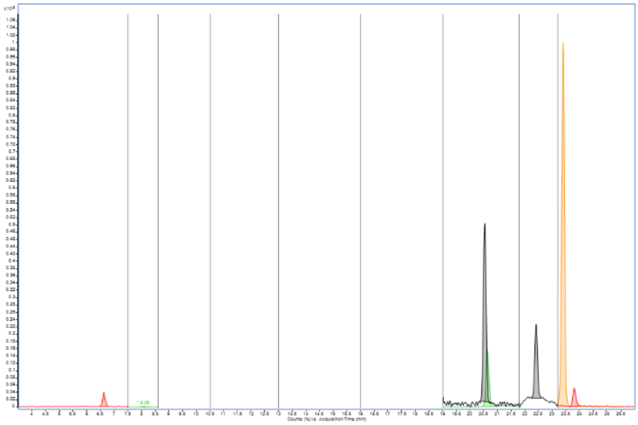

Figure S4. Chromatogram obtained when 10 mL of river water sample was spiked with the analyte mixture at 10 µg L^{-1} were analyzed using the SBSE/LC-MS/MS developed method.

3.2.2. Selective monitoring of acidic and basic compounds in environmental water by capsule phase microextraction using sol-gel mixed-mode sorbents followed by liquid chromatography-mass spectrometry in tandem

Selective monitoring of acidic and basic compounds in environmental water by capsule phase microextraction using sol-gel mixed-mode sorbents followed by liquid chromatography-mass spectrometry in tandem

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Abstract

In addition to the diverse extraction techniques available, capsule phase microextraction (CPME), which uses a microextraction capsule (MEC), has recently been introduced as a sorptive-based sample preparation technique. In this study, two different MECs (MEC-C18/SAX and MEC-C18/SCX) based on mixed-mode ion-exchange technology were synthesized and evaluated for the selective extraction of a group of ionizable compounds, including acidic and basic analytes. A sulfonic acid was used as the cation-exchange group in MEC-C18/SCX, and a quaternary amine as the anion-exchange group in MEC-C18/SAX. The extraction parameters optimized were sample pH, elution solvent, sample/elution volume and extraction/elution time. The optimized CPME method followed by LC-MS/MS was used to determine the ionizable compounds in environmental water samples, including river water and effluent wastewater, with excellent selectivity and matrix effect values below -30% (except -47% for methadone) and apparent recovery results ranging from 40% to 69%, except for ibuprofen (< 35%) and atenolol (< 25%). The analytical method was validated for environmental water samples, and used in the analysis of several samples in which some of the target compounds were found at ng L^{-1} concentration levels.

Keywords: *capsule phase microextraction; mixed-mode ion-exchange; sol-gel; acidic analytes; basic analytes; environmental samples*

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1. Introduction

Sample treatment and extraction techniques, especially those involving miniaturization like microextraction techniques, have recently become the focus of much attention. Microextraction techniques involve non-exhaustive miniaturized sample preparation techniques which, using high preconcentration factors, employ a small volume of the extracting phase relative to the sample volume, and offer multiple other advantages. They therefore represent an important contribution to the enhancement of sample preparation performance [1,2].

Microextraction techniques are classified depending on the type of extraction media used to extract the analytes: they can be liquid-based if the extraction media is a solvent, such as liquid-phase microextraction (LPME), dispersive liquid-liquid microextraction (DLLME), and hollow fibre liquid-phase microextraction (HF-LPME), among others; or sorptive-based, if the extraction media is a solid or semi-solid material, such as solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), rotating disk sorptive extraction (RDSE), fabric phase sorptive extraction (FPSE) and capillary microextraction (CME), among others [2–5]. Recently, Kabir and Furton introduced a new sample preparation technique known as capsule phase microextraction (CPME) to improve and simplify ongoing sample preparation [6]. CPME is based on the principle of equilibrium extraction and uses a microextraction capsule (MEC) as the extraction medium. The MEC is made up of two fused porous tubular polypropylene membranes, one to accommodate the sorbent through sol-gel technology and the other to encapsulate a magnetic metal rod. These MECs hold different sorbents, such as the non-polar sol-gel polydimethylsiloxane (PDMS) or sol-gel C18 and the polar sol-gel Carbowax 20M. CPME has been used to extract personal care products from environmental water samples [7] and sulfonamides from milk [8].

One of the key considerations in the discussion of sorptive extraction techniques is the range of different materials available. For instance, in SBSE, although PDMS (intended to determine non-polar compounds) is the most frequently used coating and the one that offers the best chemical and mechanical stability, other coatings, such as EG silicone (ethylene glycol-silicone) and PA (acrylate) for polar compounds, have been developed and discretely used to extract analytes from environmental samples [9–11]. However, these polar coatings present some mechanical and thermal stability problems. Aiming to increase the polarity as well as the stability of the coating, several new in-house synthesized materials have been developed [1,12]. Sol-gel technology has become one of the most popular methods for preparing novel coatings for microextraction techniques, and this approach has led to the development of many novel materials with large surface areas and high thermal and solvent stabilities [13–16]. For example, metal organic framework and PDMS coated stir bar were both prepared by means of sol-gel technology [16]. And, it was successfully applied to the extraction of a group of organophosphorus pesticides in environmental water samples. Moreover, other authors have presented strategies to develop polar coatings using the monolithic approach [17,18].

In addition, in the past few years mixed-mode ion-exchange materials using either silica or polymeric-based materials to which ionic moieties are introduced have been progressively applied to different microextraction techniques [19]. Different coatings have been developed using either the sol-gel or monolithic approach [20–23] in order to promote the selective extraction of the ionic compounds (through ionic interactions) while maintaining the reversed-phase interactions between the backbone and the remaining compounds. For instance, Huang et al. [21] designed a novel mixed-mode ion-exchange coating for the determination of quinolones using SBSE. It consisted of a monolithic material synthesized by means of the copolymerization of methacrylic acid-3-sulfopropyl ester potassium salt (MASE) and divinylbenzene (DVB). In the coating, the sulfonic groups promoted ionic interactions with the amino groups of the quinolones and DVB interacted through reversed-phase interactions with the ring alkyl groups and the benzene present in the structure of the quinolones.

Other strategies, involving nanomaterials such as metallic and carbon-based nanoparticles (magnetically modified graphene) and polymer-based nanocomposites combined with mixed-mode polymeric resins have been used as novel coatings for SBSE and have generated increasing interest among researchers exploring sample preparation [24,25].

In the present study, for the first time, two novel mixed-mode ion-exchange materials that combine C18 with quaternary amine moieties were developed to achieve strong anion exchange (MEC-C18/SAX) and, with sulfonic moieties, strong cation exchange (MEC-C18/SCX). These MECs were applied in CPME followed by liquid chromatography with mass spectrometry in tandem (LC-MS/MS) for the selective determination of a group of acidic or basic analytes in environmental samples. The CPME parameters including sample pH, sample/elution volume, extraction/elution time and washing/elution solvent were thoroughly optimized to promote selective interactions with the target compounds.

2. Experimental part

2.1. Materials, reagents and standards

For the preparation of the MECs, Accurel® porous capillary membranes were purchased from 3M Inc. (St. Paul, MN, USA). Cylindrical magnets (1/16" x 3/4") were from K&J Magnetics Inc. (Pipersville, PA, USA). Tetramethoxyorthosilicate (TMOS) and methyl trimethoxysilane (MTMOS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isopropanol, methylene chloride, hydrochloric acid (HCl), ammonium hydroxide (NH4OH), sulphuric acid (H2SO4) and hydrogen peroxide (H_2O_2) were obtained from Fisher Scientific (Milwaukee, WI, USA). N-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride and 3-mercaptopropyl trimethoxysilane (3-MPTMS) were purchased from Gelest Inc. (Morrisvile, PA, USA).

A 2510 Branson Ultrasonic Cleaner (Branson Inc., USA) was used to sonicate sol solutions. A Barnstead NANOPure Diamond (Model D11911) deionized water system (Dubuque, IA, USA) was used to collect deionized water for sol-gel synthesis.

The 14 model compounds selected for this study, including pharmaceuticals, illicit drugs and artificial sweeteners, were potassium acesulfame (ACE), atenolol (ATE), clofibric acid (CLO AC) (clofibrate metabolite), diclofenac (DICLO), fenoprofen (FEN), ibuprofen (IBP), methadone (MET), metoprolol tartrate salt (MTP), naproxen (NAP), propranolol (PROP), saccharin (SAC) and trimethoprim (TRI), and were all purchased as pure standards from Sigma-Aldrich. Mephedrone hydrochloride (MEP) was supplied by LGC Standards (Luckenwalde, Germany). All standards were of a purity exceeding 96%. The selected compounds and the pKa are shown in Table S1.

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

2.2. Preparation of sol-gel mixed-mode sorbents encapsulated in microextraction capsules

Creating sol-gel mixed mode sorbents in MECs involves several steps: (a) preparing the Accurel S6/2 tubular membranes; (b) preparing the sol solution for the sol-gel mixed-mode sorbents; (c) in situ creation of monolithic sorbent bed inside the membrane; (d) aging, conditioning and cleaning the microextraction capsules.

2.2.1. Preparing the Accurel S6/2 tubular membranes

The Accurel® polypropylene S6/2 capillary membranes were first cut into 3 cm pieces. The membranes were then rinsed with methylene chloride and subsequently air dried at room temperature for 30 min. The cleaned membranes were stored in an airtight glass bottle. A cylindrical magnet (2.54 cm) was inserted into one porous tubular membrane, and then the two tubular membranes (one empty and the other containing the cylindrical magnet) were impulse heat sealed (Pasco Inc., Rocky Mount, MO, USA) on both sides. The microextraction capsules were then ready for the in-situ creation of sol-gel mixed sorbents.

2.2.2. Preparing the sol solution

The sol solutions for sol-gel mixed-mode sorbents (C18/SAX and C18/SCX) were prepared separately. The sol solution for sol-gel C18/SAX was prepared by sequentially adding the following reagents at the proportion in molar ratio in parenthesis: TMOS(1), MTMOS (1), isopropanol (30), ODSTMS (0.2), N-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride (0.2), HCl (0.04) and deionised water (8), in an amber glass reaction bottle. The acid hydrolysis process took place at room temperature for 12 h.

The sol solution for sol-gel C18/SCX was prepared by sequentially adding TMOS (1), MTMOS (1), isopropanol (30), ODSTMS (0.2), 3-MPTMS (0.5), HCl (0.04) and deionized water (8), in an amber glass reaction bottle. The acid hydrolysis process took place at room temperature for 12 h.

2.2.3. In situ creation of the monolithic sorbent bed inside the membrane

Next, the hydrolyzed C18/SAX sol solution was transferred to a wide-mouth glass container and NH4OH was added to this solution in droplets with continuous stirring at a TMOS:NH4OH molar ratio of 1:0.18. Twenty pieces of MECs were immediately added to the sol solution and the solution was sonicated for 5 min. The sol solution formed a solid gel in 30 min.

The hydrolyzed sol solution for C18/SCX was transferred into another wide-mouth glass container and condensation was initiated by slowly adding NH4OH at a TMOS:NH_4OH molar ratio of 1:0.18. The sol solution was sonicated in an ultrasound water bath for 5 min. The sol solution formed a transparent gel in 1 h.

2.2.4. Conditioning and cleaning the microextraction capsules

Subsequent to the gelation of the sol-gel sorbents, the gels were aged and conditioned at 50ºC for 24 h. The MECs were then cleaned, and the gels adhered the microextraction capsules outside were removed by gently rubbing them against one another. The MECs were then cleaned using a mixture of MeOH:methylene chloride (50:50 v/v) under sonication for 30 min. Next, the MECs were then dried at 50ºC in a neutral environment under continuous helium gas flow. Finally, the MECs with encapsulated sorbents were ready for use.

2.3. Capsule phase microextraction conditions

Two different protocols were used: one for MEC-C18/SAX, which retained the acidic analytes and another for MEC-C18/SCX, which retained the basic analytes. Each MEC was first placed in a 50 mL glass vial and conditioned with 10 mL of MeOH for 10 min followed by 10 mL of ultrapure water adjusted to the same pH as the sample.

For MEC-C18/SAX, 25 mL of sample adjusted to pH 7 was extracted by stirring at 600 rpm for 180 min. Then, as the washing step, the MEC-C18/SAX was added to another glass vial containing 3 mL of ultrapure water, and placed in an ultrasonic bath for 1 min. After that, liquid desorption (LD) was performed in an ultrasonic bath using 3 mL MeOH containing 5% HCOOH for 5 min.

For MEC-C18/SCX, 25 mL of sample adjusted to pH 5 was extracted by stirring at 600 rpm. After that, the same washing procedure was applied with 3 mL ultrapure water. LD of MEC-C18/SCX was performed using 3 mL MeOH containing 5% NH_4OH and placed in an ultrasonic bath for 5 min. The elution solutions of each MEC were placed separately in a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to evaporate the extract until dry, and later it was reconstituted with 1 mL of mobile phase (ultrapure water adjusted to pH 2.8 with HCOOH/ACN, 90/10, v/v). All fractions were filtered with 0.45 µm PTFE syringe filters (Scharlab) before being injected into the chromatographic system. After each use, the MECs were cleaned twice with the corresponding elution solution and two more times with MeOH in the ultrasonic bath for 10 min each, dried and stored in a small glass vial until the next experiment. These MECs can be reused more than 30 times when analyzing environmental samples.

The methods developed were tested using two different types of water: river water from the Ebre River and effluent wastewater from a treatment plant near Tarragona, Spain. Both water samples were filtered through a 1.2-µm glass-fibre membrane filter and through a 0.45 µm nylon membrane filter (Fisherbrand, Loughborough, UK) prior to analysis.

2.4. Liquid chromatography-mass spectrometry analysis

An Agilent model 1200 series LC coupled with a 6460 QqQ mass spectrometer (MS/MS) detector with an electrospray ionization (ESI) interface was used. The LC system was equipped with an autosampler, a degasser, an oven and a quaternary pump (Waldbronn, Germany). The optimal mobile phase was a mixture of ultrapure water at pH

2.8 with HCOOH (solvent A) and ACN (solvent B). Two different gradient profiles were used depending on the compounds analyzed as one MEC selectively extracted the acidic compounds and the other selectively extracted the basic ones. For the acidic compounds, the gradient profile started with 15% of B, which was raised to 85% within 9 min and then to 100% within 4 min. It was subsequently held at 100% for 3 min before returning to the initial conditions after 2 min. The initial conditions were then maintained for another 2 min. For the basic compounds, the gradient profile started with 15% of B, which was raised to 30% within 6 min and then to 100% within 4 min. It was then was held at 100% for 3 min, and returned to the initial conditions after 2 min, which were maintained for another 2 min. The chromatographic column used in both separations was the Tracer Excel 120 C_8 (150 mm × 4.6 mm i.d., 5 µm particle size) supplied by Teknokroma (Sant Cugat del Vallès, Spain). The column was maintained at 30°C and the mobile phase flow rate was 600 µL min^{-1}. The injection volume was 20 µL.

In the MS/MS, the ESI negative mode was used for the acidic compounds (ACE, SAC, CLO AC, FEN, DICLO, IBP, NAP) and the positive mode for basic compounds (ATE, TRI, MEP, PROP, MET, MTP), as this configuration yielded better ionization in both cases. The optimal parameters for the acquisition of the acidic compounds were a fragmentor voltage of 75 V, a collision energy between 5 and 28 eV, 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. For the acquisition of the basic compounds, the optimal conditions were a fragmentor voltage of 100 V, a collision energy between 8 and 22 eV, a source gas temperature of 350ºC, a nitrogen flow rate of 13 L min^{-1}, a nebulizer pressure of 60 psi and a capillary voltage of 2500 V. A precursor ion and two product ions were selected for each analyte. The precursor ions were measured for quantification and the product ions and the corresponding ion ratios were used for confirmation purposes in MRM mode (Table S1). All selected compounds presented good linearity (r^2=0.997) in LC-MS/MS and the linear ranges were between 0.05 and 50 µg L^{-1}, except for ATE, MTP and PROP, which were between 0.01 and 50 µg L^{-1}, and SAC and IBP, which were between 1 and 50 µg L^{-1}.

3. Results and discussion

3.1. Development of mixed-mode microextraction capsules

CPME offers unique advantages in separation science by (a) protecting the sorbents from easy fouling by encapsulating them inside a microporous (0.2 µm pore size) polypropylene membrane, and (b) introducing a built-in bar magnet as an integral part of the device. Figure S1 shows a picture of microextraction tubes that were compared to a US penny (a), and SEM images showing the surface morphology of polypropylene tube at 100x (b) and 10,000x (c) magnifications. The sol-gel sorbents are located inside the tube.

Due to the inert nature of the polypropylene (PP) tube, sol solution does not interact with PP surface. Moreover, because of the encapsulation of the sorbent inside a microporous tubular membrane, the device can be directly introduced into the unmodified complex sample. The integration of the magnet into the device design also eliminates potential sample contamination via an external magnet. In addition, MECs can be used and reused many times, which substantially reduces overall sample preparation cost.

The synthesis of sol-gel C18/SCX sorbent involves three distinct steps: (a) the hydrolysis of sol-gel precursors in acidic medium, which is done in the absence of the MECs; (b) the condensation of hydrolysed sol-gel precursors in basic medium in the presence of the MECs, during which the hydrolysed sol-gel precursors rapidly condense to form a 3D network of sol-gel sorbent matrix; and (c) the oxidation of the MECs (after conditioning, cleaning and drying) by means of treatment with 30% H2O2 for 24 h and 0.05M H2SO4 for 2 h to oxidise the propyl mercapto ligand into the propyl sulfonic ligand. The sol-gel reaction steps are given in Fig. 1A.

The synthesis of sol-gel C18/SAX sorbent involves two steps: the hydrolysis of sol-gel precursors under acid catalyst and the subsequent polycondensation under basic catalyst. The reactions involved in the creation of sol-gel C18/SAX are provided in Fig. 1B.

3.2. Optimization of CPME procedures

To explore the mixed-mode ion-exchange properties of the developed MECs, we needed to carefully optimize the different parameters involved in the CPME procedure so that the MECs could establish both ion-exchange (specific) as well as reversed phase (non-specific) interactions with the ionized and neutral entities simultaneously, and these interactions could be turned on and off depending on the analytical focus during extraction.

The three different steps of the CPME procedure (i.e. the extraction, washing, and liquid desorption) were optimized in order to achieve the selective retention of the model compounds used. Both coatings, MEC-C18/SAX and MEC-C18/SCX, present strong ionic groups, a quaternary amine and a sulfonic group, respectively, which are charged across the range of the working pH. The model compounds have acidic and basic properties that should establish different interactions with the coating. For this reason, careful attention was paid to the elucidation of the retention mechanisms present during the extraction for each analyte, taking into account the pK_a values of the analytes selected, shown in Table S1. Monitoring the pH when loading the sample is crucial so that the compounds and the functional groups of the coating are in an ionic state. They can then interact by means of ionic interactions with the ionic groups attached to the coating, in addition to the

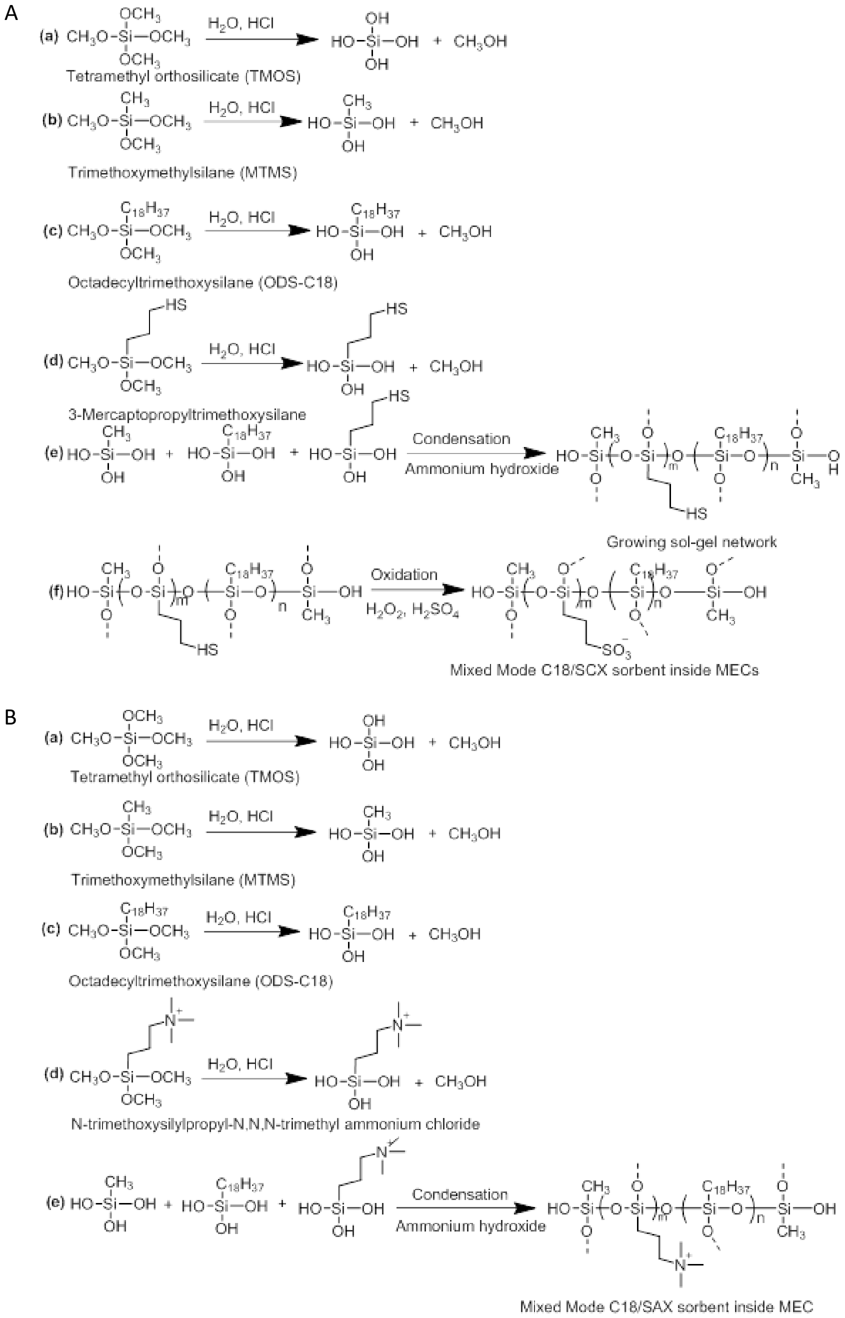

Figure 1. Reactions involved in different steps in synthesizing sol-gel C18/SCX (A) and sol-gel C18/SAX (B) within the MECs.

reversed-phase interactions of the coating. In contrast, in the elution step, the functional groups of the analytes must be in their neutral state using organic solutions at suitable pH conditions to disrupt retention and favour the elution of the analytes, since the coating has strong ion exchangers.

The starting conditions were 25 mL of ultrapure water spiked at 0.5 mg L^{-1} with the mixture of analytes and adjusted to pH 3 for the MEC-C18/SCX and at pH 7 for the MEC-C18/SAX, stirred at 600 rpm for 60 min. Based on previous studies [7,8,26,27], elution was performed with 5 mL of MeOH containing 5% NH4OH for MEC-C18/SCX and 5 mL MeOH containing 5% HCOOH for MEC-C18/SAX, with stirring for 10 min. During optimization, the elution solutions were not evaporated to dryness. Instead, the MeOH elution solution containing 5% HCOOH was diluted to 10 mL with ultrapure water and the MeOH solution containing 5% NH4OH was neutralized with HCOOH and diluted to 10 mL with ultrapure water to inject into the chromatographic system. These extracts from the optimization of the CPME parameters were injected into a LC-DAD using a gradient that separates all the compounds in the same analysis.

First, we evaluated the selectivity of all the analytes in both MECs. Under the initial conditions, MEC-C18/SAX showed selectivity towards the acidic analytes with recoveries of between 36% and 97%, while the basic analytes were lost in the loading step and recoveries were below 17%. MEC-C18/SCX, on the other hand, was selective towards the basic analytes with recoveries of 28-56%, and the acidic analytes were completely lost in the loading step. Therefore, MEC-C18/SAX was selected to extract the acidic analytes and MEC-C18/SCX was selected to extract the basic analytes. The acidic analytes established strong anionic interactions with the quaternary amine in MEC-C18/SAX, whereas the basic analytes established strong cationic interactions with the sulfonic acid in MEC-C18/SCX.

3.2.1. Sample pH

Once the compounds for each MEC had been established, the first parameter we investigated was sample pH. MEC-C18/SCX, which contains sulfonic moieties, is negatively charged in the entire pH range, as it is a strong cationic exchanger. At pH values below 6, the basic compounds should be in an ionic state according to their pK_a values (Table S1). Therefore, pH 3 and 5 were tested for MEC-C18/SCX. For all the (basic) compounds, recovery values of 28-56% were found when loading the sample at pH 3 and 42-81% at pH 5, except for MEP, the recovery values of which remained constant at both pH values. In view of these results, pH 5 was established as the optimal pH for loading samples in MEC-C18/SCX for the subsequent analyses.

For MEC-C18/SAX, which contains quaternary amine moieties and is positively charged in the entire pH range, pH 5 and 7 were tested because acidic compounds should be in an ionic state (pKa in Table S1) at pH values above 5. The extraction recoveries of all the (acidic) compounds increased from pH 5 to pH 7. For instance, CLO AC, FEN and DICLO attained extraction recoveries of 64%, 75%, and 82% at pH 5, while the recoveries at pH 7 were 70%, 85%, 97%, respectively. Therefore, pH 7 was designated the optimal pH for loading samples using MEC-C18/SAX for the subsequent analyses.

3.2.2. Liquid desorption conditions

Desorption parameters such as type and volume of desorption solvent and desorption time were evaluated to select those most effective for the desorption of the selected compounds. We tested different amounts (5% and 10%) of NH4OH in MeOH for MEC-C18/SCX and HCOOH in MeOH for MEC-C18/SAX and found that the extraction recoveries of all the compounds remained constant, suggesting that the difference between 5% and 10% of NH_4OH and HCOOH was not significant. Thus, MeOH containing 5% HCOOH for MEC-C18/SCX and MeOH containing 5% NH_4OH for MEC-C18/SAX were selected as desorption solutions.

We also tested an increase in desorption time to 15 min. No improvement was observed in either of the MECs when performing the desorption in the ultrasonic bath for 15 min compared to 10 min. Therefore, 10 min was established as the extraction time.

In addition to the starting volume of 5 mL, 3 mL was also tested as a desorption volume. The recoveries of the basic compounds in MEC-C18/SCX and the acidic compounds in MEC-C18/SAX were maintained when decreasing the desorption volume from 5 mL to 3 mL. Therefore, 3 mL was chosen as the optimal desorption volume for both coatings since the posterior evaporation time to dryness decreases as the desorption volume decreases.

3.2.3. Sample volume

The next parameter optimized was the extraction sample volume. We tested 50 mL of sample to explore extracting a higher volume. Using 50 mL as the loading volume, the extraction recoveries of the basic analytes in MEC-C18/SCX decreased from 40-65% to 25-53%. With MEC-C18/SAX, the acidic analytes showed a decrease in recoveries from 40-58% when using 25 mL of sample volume to 29-51% when using 50 mL. Consequently, 25 mL was selected as the loading volume for further experiments with both MECs.

3.2.4. Washing conditions

To remove interferents from the matrix and therefore increase selectivity, we included a washing step consisting of 3 mL of a washing solution. MeOH, ACN and ultrapure water were tested as solvents for the washing solution for both MECs. During the washing step, each MEC was placed in an ultrasonic bath for 1 min. As shown in Fig. 2. similar results were achieved for both MECs for all of the compounds when using ultrapure water as a washing solution and when no washing solution was used. For MEC-C18/SCX, the basic compounds attained similar results with ACN and ultrapure water. However, with MEC-C18/SAX, the acidic compounds were partially lost during the washing step with ACN. In addition, with both MECs, all the analytes were partially lost during the washing step when MeOH was used as a washing solvent, resulting in a decrease in extraction recoveries. Consequently, ACN and MeOH were ruled out as possible washing solvents. It should be mentioned that lower volumes of solvent were not tested because 3 mL is the minimum volume that ensures that the MEC is covered. Thus, in view of the results, 3 mL of ultrapure water was selected as the washing solution; nonetheless, the effectiveness of a water-based washing step was further studied when working with complex environmental samples (section 3.3).

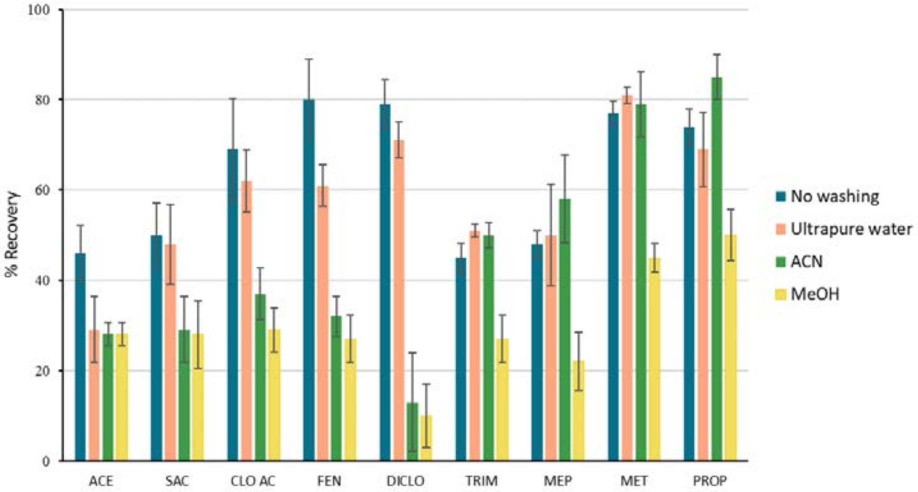

Figure 2. %R values obtained in the elution step for representative analytes when ultrapure water, ACN, MeOH were used in the washing step and no washing solution were applied.

3.2.5. Extraction time

We next studied the parameter of extraction time, examining 30, 60, 120, 180 and 240 min extraction times. The %R obtained in some of the selected compounds when increasing the extraction time from 30 to 180 min are shown in Fig. 3, and a similar trend was observed for the remaining compounds. The figure shows the increase of the recovery of the selected compounds when the extraction time increased.

In both MECs, no change in the recoveries of the analytes was detected when the extraction time was increased from 180 min to 240 min, except for ACE and SAC, which improved their recoveries at 240 min from 46% to 58% for ACE and 50% to 66% for SAC. As a compromise, 180 min was selected as the optimal extraction time for the subsequent analyses.

The CPME recoveries of all the selected compounds in both MECs are summarized in Table 1. The acidic compounds achieved recoveries from 60 to 80%, except the strong acidic compounds, ACE and SAC, which achieved recoveries of 46% and 50%, respectively. Among the basic analytes, MET, MTP and PROP attained good recoveries (77%, 59% and 74%, respectively), although lower recoveries were achieved for ATE and TRI (27% and 45%, respectively). In any case, these recoveries are satisfactory considering that CPME is an equilibrium technique, and not an exhaustive technique. Other equilibrium techniques such as SBSE and dynamic fabric phase sorptive extraction (DFPSE) have also been used to determine these compounds [28,29]. For instance, Aparicio et al. [28] developed a method based on SBSE to determine pollutants of environmental concern, including 14 pharmaceuticals. They tested two stir bar coatings: the classic PDMS coating and the novel EG-silicone coating. The novel EG-silicone coating yielded better results for pharmaceuticals, with recoveries of 55% for DICLO, 45% for CLO AC, 43% for PROP, and 15% for TRI. These recovery rates are lower than those achieved in our study, even though their extraction time was 24 h compared to the 180 min used in our study. In the study by Lakade et al. [29], ionizable compounds such as PROP and DICLO were extracted from environmental water samples by means of DFPSE using sol-gel Carbowax 20M (polar) as a coating. In that study, the recoveries obtained for PROP were around 30% and for DICLO approximately 85%, whereas in our study recoveries of 74% and 79% were achieved for these compounds, respectively.

3.3. Application to environmental water samples and method validation

After the optimization, CPME methods were applied to environmental water samples consisting of river and effluent wastewater samples. At this point, it is important to mention that in order to enhance the sensitivity of the method, the extracts from the LD

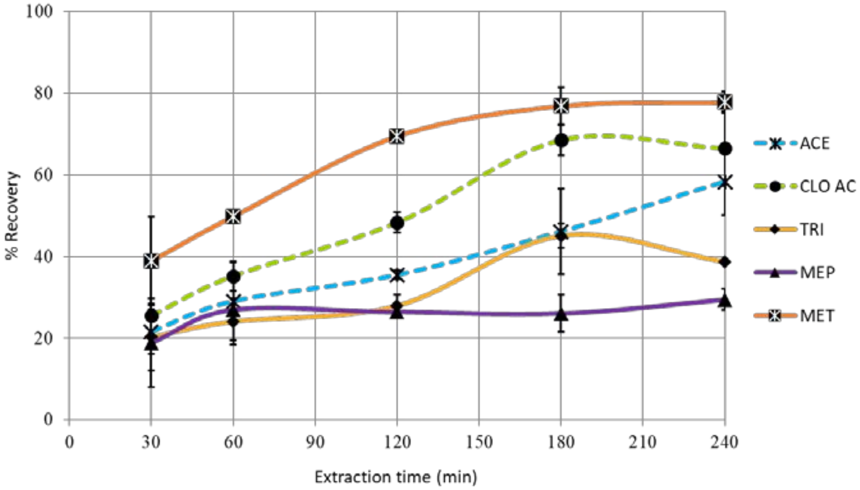

Figure 3. Effect of the extraction time on the recovery of some compounds in the MEC-C18/SCX and MEC-C18/SAX.

Table 1. %R obtained with the MEC-C18/SAX and MEC-C18/SCX for all the compounds when using the optimized CPME conditions in ultrapure water.

	Analyte	pK$_a$	%R
MEC-C18/SAX	ACE	-0.3	46
	SAC	1.6	50
	CLO AC	3.37	69
	FEN	3.96	80
	DICLO	4.00	79
	NAP	4.19	78
	IBP	4.85	60
MEC-C18/SCX	MEP	8.0	56
	MET	9.1	77
	ATE	9.67	27
	MTP	9.67	59
	PROP	9.7	74
	TRI	10.8	45

% RSD (n=5) <17%

were evaporated to dryness and reconstituted with 1 mL of mobile phase. No loss of analytes was observed during the evaporation step. In addition, two of the most polar compounds, namely ACE and SAC, were not included in the subsequent application to environmental samples because their recovery rate was low and they were present at high levels in most of the environmental samples analysed.

In a first step, as mentioned in section 3.2.4, the effectiveness of including a washing step using 3 mL of ultrapure water was assayed in effluent wastewater samples. The matrix effect (%ME) was compared after both procedures (including the washing step or not) in effluent wastewater. A blank sample of effluent wastewater was analyzed, and the signal obtained was subtracted from the signal of the spiked sample in order to calculate the %ME. The %ME was calculated using the formula %ME = (C_{exp}/C_{theo} x 100%) – 100%, where C_{exp} is the concentration of the analytes when the extract was spiked with the analyte mixture after the CPME procedure and C_{theo} is the concentration of the standard solution. The %ME obtained can be in form of enhancement if it constitutes an increase in the signal or suppression if it is a decrease in the signal.

The %ME values when the washing step was included or not in the extraction of effluent wastewater samples are listed in Table 2. As expected, the %ME decreased when a washing step was applied. For instance, a significant decrease ranging from -43% and -69% to -14% and -26% in the %ME was observed for the acidic compounds with MEC-C18/SAX and 3 mL of ultrapure water as washing solution. The same happened with MEC-C18/SCX, where the %ME of the basic compounds slightly decreased when a washing step was applied. For instance, %ME results of -34% for MET decreased to 23%, -39% for ATE decreased to -18%, and -38% for PROP decreased to -18%. Therefore, a washing step consisting of 3 mL of ultrapure water was included in the process.

We then calculated apparent recovery (%R_{app}) at two different concentration levels (0.2 µg L^{-1} and 2 µg L^{-1}) and %ME in 25 mL of river water and effluent wastewater samples. The %R_{app} is the recovery of the whole method, calculated as a ratio of the concentration obtained from a sample spiked before the extraction procedure and the concentration of the standard solution. The experimental concentrations were calculated using a calibration curve prepared with standard solutions. Table 2 shows the %R_{app} and %ME obtained at the lowest concentration level. The %R_{app} of the acidic compounds in river water ranged from 46% to 59%, except for IBP (%R_{app}, 35%). The basic compounds in the river sample achieved recoveries between 44 and 68%, except for ATE (25%). In effluent wastewater, the %R_{app} in all the compounds slightly decreased from those obtained in river water, except for DICLO. In addition, the %R_{app} obtained in both samples slightly decreased from the values obtained in ultrapure water due to the %ME present in the samples analyzed.

Table 2. %R_{app} and %ME obtained when 25 mL of in river and effluent water samples spiked at 0.2 µg L^{-1} was extracted using MEC-C18/SAX or MEC-C18/SCX by CPME.

		River		Effluent wastewater		
	Analyte	%R_{app}	%ME	%R_{app}	%ME	%ME[a]
		With washing				Without wash
MEC-C18/SAX	CLO AC	59	-18	46	-21	-47
	FEN	61	-19	47	-23	-50
	DICLO	60	-27	69	-26	-69
	NAP	46	-24	21	-14	-43
	IBP[b]	35	-12	15	-15	-50
MEC-C18/SCX	MEP	44	-10	24	-33	-36
	MET	53	-26	40	-23	-34
	ATE	25	-21	22	-18	-39
	MTP	51	8	42	-18	-21
	PROP	68	-13	55	-18	-38
	TRI	51	2	43	-29	-32

[a] Without applying the washing step based on 3 mL of ultrapure water
[b] Spiked at 2 µg L^{-1}
% RSD (n=5) <21%

In both samples, the %ME was acceptable and in the form of ion suppression in the majority of the compounds. The %ME values in MEC-C18/SCX were similar in the river and effluent samples. However, slightly higher ion suppression was observed in river water in the %ME of MEC-C18/SAX spiked at a lower concentration (Table 2) compared to when it was spiked at a higher concentration (ranging from -7% to -17%). With MEC-C18/SCX, the %ME at both concentrations showed higher ion suppression in the effluent sample than in the river sample, as expected. MTP and TRI presented ion enhancement at low and high concentration levels in the river sample, as did PROP at high concentration levels in the river sample. Similar %ME results were obtained in previous studies which determined these analytes in complex matrices using other techniques, such as SPE or DFPSE [29,30]. In a study [30] in which SPE with mixed-mode ion-exchange sorbents was used as an extraction technique, %MEs of -21% for CLO AC and -32% for DICLO were reported in river water, which are similar to the values obtained in our study (-18% and -27% for CLO AC and DICLO, respectively). Another study [29] using DFPSE with Carbowax 20M found %MEs of -7% for PROP in river water and -15% in effluent wastewater, comparable values to those observed in our study, which documented %MEs of -13% for this compound in river samples and -18% in effluent samples.

We also assessed the method limits (MDLs and MQLs), repeatability, and reproducibility between days in both samples. The MDLs and MQLs of the river and effluent samples were estimated from the instrumental limits (LODs and LOQs), considering the %Rapp results and the preconcentration factor, expressed in ng L^{-1}. In the river sample, the MDLs ranged between 0.3 ng L^{-1} and 2.2 ng L^{-1} and the MQLs ranged from 0.7 ng L^{-1} to 4.6 ng L^{-1}. The exception was IBP, whose MDL and MQL were 57 and 115 ng L-1, respectively, attributed to the high instrumental LODs and LOQs, but also to the low recoveries achieved. In effluent wastewater, MDLs ranged from 0.4 ng L^{-1} to 4.8 ng L^{-1}, and MQLs ranged from 0.7 ng L^{-1} to 9.5 ng L^{-1} (except for IBP, which were 133 ng L^{-1} and 267 ng L^{-1}). The repeatability of the method on the same day and reproducibility between days, expressed as relative standard deviation (%RSD) of five replicates of river and effluent samples spiked at a concentration level of 0.2 and 2 µg L^{-1}, were less than 21% and 17%, respectively.

3.4. Analysis of real samples

The optimized CPME method was employed to determine the model compounds in river and effluent water samples. Three different samples of river water and three of effluent wastewater were analyzed in triplicate. The concentration of the analytes present in the samples were calculated using the external calibration curve and taking into account the %Rapp and the concentration factor. Table 3 shows the concentration levels of the selected model compounds found in both samples. All the targeted compounds were detected in both types of sample, except NAP and IBP, due to its high MDLs. As expected, the concentration levels found in the river water samples were lower than those found in the effluent wastewater samples. For instance, MET and TRI, the concentrations of which were lower than the MQLs in the river samples, were quantified in effluent wastewater. However, MEP was present at below the MQLs in both types of sample, and for FEN it was even found at a slightly higher concentration in one of the river samples than in the effluent wastewater samples analysed.

The detection of the majority of the selected compounds reported in this work are consistent with those reported in other studies. In a previous study [30] conducted in our research group in which a similar group of compounds were determined in effluent water from the same sewage treatment plant as in this study, most of the compounds were found at slightly higher concentrations in effluent wastewater than in the present study. Similarly, TRI was found at similar concentration levels and FEN was found below the MQL. In other studies, where other river and wastewater samples were analyzed, NAP was found in quantities below the MQLs [31]. However, in our study NAP was not detected in either the river or the effluent samples. In addition, MTP and PROP were found at similar concentration levels, in both river water and effluent wastewater samples, while DICLO was found at slightly higher concentration levels in other studies [32–35].

Table 3. Range of concentration in ng L^{-1} when different river and effluent wastewater samples were analyzed using the CPME/LC-MS/MS developed method.

		Concentration (ng L^{-1})	
	Analyte	River water	Effluent wastewater
MEC-C18/SAX	CLO AC	7-15	10-16
	FEN	<MQL-17	<MQL
	DICLO	23-30	7-199
	NAP	n.d	n.d
	IBP	n.d	n.d
MEC-C18/SCX	MEP	<MQL	<MQL
	MET	<MQL-24	44-56
	ATE	8-45	52-847
	MTP	3-7	15-77
	PROP	3-9	59-63
	TRI	<MQL	321-536

4. Conclusions

In this study, we successfully pioneered the preparation of mixed-mode ion-exchange MECs using sol-gel technology to accommodate the coating in a porous polypropylene membrane.

The CPME parameters greatly influenced the extraction recoveries of the selected model compounds and a washing step was successfully introduced to the CPME method to reduce the presence of interferents in the matrix and consequently decrease the %ME. MEC-C18/SAX and MEC-C18/SCX were evaluated to selectively retain acidic or basic compounds, respectively, and provided satisfactory extraction efficiency for ionisable compounds.

The method presented here is simple and selective, with promising application for trace analyses in environmental samples such as river water and effluent wastewater samples. The proposed CPME technique using MEC-C18/SAX and MEC-C18/SCX could be expanded to extract other compounds in different samples in the future.

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Supplementary data

Table S1. Analyte, MRM transitions and MS/MS parameters for the model compounds.

		Analyte	Ionisation mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Product ion (m/z)	Collision energy (eV)
ACIDIC	STRONG	ACE	NEG	162	82	11	78	15
		SAC	NEG	182	105	22	62	24
	WEAK	CLO AC	NEG	213	127	8	85	5
		FEN	NEG	241	197	2	93	5
		DICLO	NEG	294	250	5	214	8
		NAP	NEG	229	185	2	169	4
		IBP	NEG	205.1	161	2		
BASIC		MEP	POS	178.1	160	8	145	12
		MET	POS	310.2	265	12	223	16
		ATE	POS	267.2	145	22	74.2	22
		MTP	POS	268.2	116	22	74	22
		PROP	POS	260.1	183	22	116	22
		TRI	POS	291.1	261	22	230	22

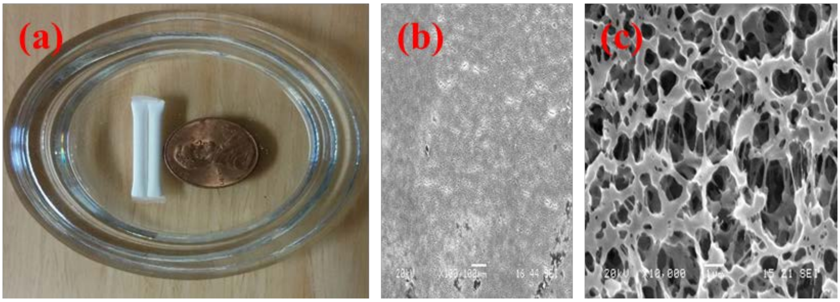

Figure S1. (a) An image of a microextraction capsule demonstrating its size relative to a US penny; Scanning Electron Microscopy images of the polypropylene (PP) tube at (b) 100x magnification; (c) 10,000x magnification, demonstrating porous surface morphology of PP tube.

3.2.3. Discussion of results

In this section the results obtained when using the SBSE and CPME techniques to evaluate the different mixed-mode ion-exchange coatings are discussed. Both studies are among the few ones that incorporate in-house mixed mode ion-exchange materials as coatings for SBSE and CPME.

In the first study, the covalent immobilization of polymer monoliths onto commercial PTFE-based magnets for SBSE was successfully achieved to further functionalize the epoxy-based monoliths with different content of EDA and create two different WAX materials, EDA1 and EDA2. The prepared covalently immobilized EDA-modified monoliths onto PTFE magnet exhibited good stability and reusability, although in EDA2 larger amine concentration (8.3 M in water) and high reaction temperature (80ºC for 24 h) were used in the preparation protocol employed [1]. Even though similar morphological structure was observed for both EDA1 and EDA2 monoliths, EDA 2 was chosen for further validation, as elemental analysis revealed EDA2 contained higher nitrogen content (i.e. ion-exchange capacity) present in the polymeric matrix (6.2 wt%), with respect to EDA1 (0.3 wt%) and provided extraction efficiency for the selected acidic compounds. Regarding, the CPME method, the incorporation of the coating in a porous polypropylene membrane to prepare the mixed-mode ion-exchange MECs using sol-gel technology was successfully achieved.

Comparing the dimensions and thickness of the coating in both techniques, we found, on one hand, that SBSE is 15 mm in length, while MEC is up to 20 mm in length. On the other hand, the thickness of SBSE is 1 mm, and the one for CPME is 0.5 mm. In summary, the coating volume for both techniques is very similar since SBSE had 118 mm^3 and CPME had 121 mm^3. Therefore, the retention capacity might be similar for both presented techniques.

As expected, it was proved that SBSE and CPME parameters had great influence in the extraction recoveries of the selected model compounds. From the optimized extraction conditions (Table 1) it can be observed that higher sample volumes can be extracted with the CPME method, with respect to the SBSE one. A stronger elution solvent and more elution time is needed when using the SBSE method, suggesting that stronger hydrophobic interactions are attained between the coating and the acidic analytes.

Table 1. Optimized extraction and elution conditions for both techniques.

Optimized conditions	**SBSE**	**CPME**
Sample volume (mL)	10	25
Sample pH	6	5
Extraction time (min)	180	180
Stirring rate (rpm)	600	600
Elution solvent	5% NH_4OH in ACN	5% NH_4OH in MeOH
Elution volume (mL)	3	3
Elution time (min)	20	5

A comparison of the recoveries obtained of each extraction technique in combination with LC-MS/MS in river water is detailed in Table 2, when applying the optimized conditions. Since basic analytes were not evaluated in the SBSE study, they were not included for comparison purposes. ACE and SAC were not included in the analysis of the environmental samples in the CPME study since they were hardly recovered, and they were present at high levels in the environmental samples analyzed. Regarding the SBSE study, the %R_{app} of the target analytes in river water ranged between 30% and 47%, except for NAP, whose %R_{app} was 20%, whereas for the CPME study the results ranged between 46% and 61%. Slightly better recoveries were attained when using the CPME protocol, demonstrating the good performance of this technique. CPME is successfully postulated as alternative sorptive extraction technique, with great results attained. In fact, the sorptive extraction technique field is constantly evolving. For instance, early this year the miniaturization of SBSE has been presented [2] as a suitable alternative to the emergent technique stir bar sorptive dispersive microextraction when the sample volume is limited.

Low %ME as ion suppression in most of the compounds (below 27%) was observed in the river water analyzed for both techniques, ranging from -3% to -27% for a spiked concentration of 10 µg/L and 2 µg/L for the SBSE and CPME technique, respectively. In the SBSE study, SAC and DICLO presented ion enhancement with %ME values of 3% and 17%, respectively and IBP presented no %ME. The low %ME demonstrated the selectivity of the prepared materials and therefore a washing step was not required.

Table 2. $\%R_{app}$ and %ME of the selected acidic compounds in river water analysed by SBSE and CPME with the corresponding coatings.

		SBSE		CPME	
	pKa	$\%R_{app}^{a}$	$\%ME^{a}$	$\%R_{app}^{b}$	$\%ME^{b}$
ACE	-0.3	43	-7	-	-
SAC	1.6	47	3	-	-
CLO AC	3.37	37	-3	59	-18
FEN	3.96	31	-13	61	-19
DICLO	4.00	47	17	60	-27
NAP	4.19	20	-15	46	-24
IBP	4.85	30	0	35	-12

[a] spiked at 10 µg/L

[b] spiked at 2 µg/L

Both techniques enable the selected acidic compounds to be quantified at low ng/L levels when applying to environmental samples. The most common compounds found in river water were CLO AC, FEN and DICLO. Some studies reported the presence of the same ionizable compounds in environmental samples, and were in line with these studies [3-5]. In CPME method, in the samples analysed the ranges of concentration obtained for the CLO AC (concentrations of 7-15 ng/L) were comparable to Sala's study [3], where the concentrations ranged from 1 to 50 ng/L. Regarding DICLO, similar concentrations were obtained in Garrido's study [4] (around 30 ng/L), with respect to CPME method (23-30 ng/L). IBP was found at high concentration levels when using the SBSE technique whereas in the CPME study was not found. Oliveira et al. [5] reported concentrations of 0.1-3110 ng/L in different river waters, in line with the concentrations obtained in the SBSE method, between 737-2446 ng/L.

The results derived from the studies in this section envisaged the successful application of mixed-mode ion-exchange materials in different kind of emerging sorptive extraction techniques.

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CHAPTER 4. CONCLUSIONS

The most relevant conclusions draw from the different studies included in this Thesis can be summarized as follows:

1. The different synthetic approaches investigated in this Thesis have provided successful mixed-mode ion-exchange materials for sorptive extraction techniques.

2. The potential of in house-synthetized zwitterionic sorbents to introduce positive and negative charges in the same SPE cartridge was confirmed and the optimal extraction conditions enable the best performance to be obtained.

3. The correct selection of the pH value when loading the samples and elute the analytes was very important in terms of favouring the ionic interactions between the analytes and the ionic groups attached on the sorbents.

4. The new mixed mode ion-exchange materials including strong and weak ionic groups enable the simultaneous determination of basic and acidic analytes.

5. An SPE method was successfully developed and validated for the simultaneous determination of artificial sweeteners, illicit drugs and pharmaceuticals in river and effluent wastewater samples employing liquid chromatography with tandem mass spectrometry.

6. The first example of a polytetrafluoroethylene (PTFE)-based magnet coated with weak anion-exchange (WAX) monolith as novel support for SBSE was successfully developed.

7. The preparation of ion-exchange microextraction capsules (MECs) using sol-gel technology to accommodate the coating in a porous polypropylene membrane was successfully developed for the first time.

8. Great selectivity was achieved when the MEC-C18/SAX and MEC-C18/SCX were evaluated to retain selectively acidic or basic compounds respectively.

9. The proposed SBSE and CPME methods are simple and very selective, with encouraging application to extract other acidic or basic compounds in different kinds of samples in the future.

10. High selective and sensitive methods were successfully developed to determine the target compounds at low concentration levels in different complex environmental samples.

APPENDIX

Appendix. List of publications

The results of the experimental part of this Thesis have been published in the following papers:

1. Selective monitoring of acidic and basic compounds in environmental water by capsule phase microextraction using sol-gel mixed-mode sorbents followed by liquid chromatography-mass spectrometry in tandem.
   Joan Carles Nadal, Francesc Borrull, Kenneth G. Furton, Abuzar Kabir, Núria Fontanals, Rosa Maria Marcé.
   Journal of Chromatography A 1625 (2020) 461295.

2. Microporous polymer microspheres with amphoteric character for the solid-phase extraction of acidic and basic analytes.
   Joan Carles Nadal, Kimberly L. Anderson, Stuart Dago, Irvin Joas, Daniela Salas, Francesc Borrull, Peter A.G. Cormack, Rosa Maria Marcé, Núria Fontanals.
   Journal of Chromatography A 1626 (2020) 461348.

3. Hypercrosslinked polymer microspheres decorated with anion- and cation-exchange groups for the simultaneous solid-phase extraction of acidic and basic analytes from environmental waters.
   Joan Carles Nadal, Stuart Dago, Irvin Joas, Francesc Borrull, Peter A.G. Cormack, Núria Fontanals, Rosa Maria Marcé.
   Journal of Chromatography A 1661 (2020) 462715.

4. Weak anion-exchange mixed-mode materials to selectively extract acidic compounds by stir bar sorptive extraction from environmental waters.
   Joan Carles Nadal, Mónica Català-Icardo, Francesc Borrull, José Manuel Herrero-Martínez, Rosa Maria Marcé, Núria Fontanals.
   Journal of Chromatography A 1663 (2022) 462748.

5. Novel in-house mixed mode ion-exchange materials for sportive phase extraction techniques.
   Joan Carles Nadal, Francesc Borrull, Rosa Maria Marcé, Núria Fontanals.
   Advances in Sample Preparation 1 (2022) 100008.

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