DEVELOPMENT AND APPLICATION OF NEW POLYMERIC MATERIALS FOR SORPTIVE EXTRACTION TECHNIQUES

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Development and application of new polymeric materials for sorptive extraction techniques

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La Prof. ROSA MARIA MARCÉ i RECASENS, Catedràtica del Departament de Química Analítica i Química Orgànica de la Facultat de Química de la Universitat Rovira i Virgili, i
La Dra. NÚRIA FONTANALS i TORROJA, Investigadora del Departament de Química Analítica i Química Orgànica de la Facultat de Química de la Universitat Rovira i Virgili,

FEM CONSTAR:
Que la present Tesi Doctoral, que porta per títol: "Development and application of new polymeric materials for sorptive extraction techniques", presentada per Dominika Bratkowska per optar al grau de Doctor per la Universitat Rovira i Virgili, ha estat realitzada sota la nostra direcció, a l'Àrea de Química Analítica del Departament de Química Analítica i Química Orgànica d'aquesta universitat, i que tots els resultats presentats són fruit d'experiències realitzades per l'esmentada doctoranda.

I, per a que consti, expedim aquest certificat a Tarragona, 25 de setembre de 2011.

Prof. Rosa Maria Marcé i Recasens Dra. Núria Fontanals i Torroja

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To see a world in a grain of sand,
And a heaven in a wild flower
Hold infinity in the palm of your hand
And eternity in an hour.

Wiliam Blake

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CHAPTER 1. INTRODUCTION

In spite of the efforts that have been undertaken over the last few years to clean up the environment, contamination remains a significant problem and is still associated with an ongoing risk to human health. This problem is unquestionably largest in the developing world due to the traditional sources of pollution such as industry, urbanisation and insufficient waste management, which create pollution that may be released into the environment. Even in recent years, a wide range of new contaminants have come to the light, such as pollutants related to transport and applications of modern chemicals in personal care products and detergents, in food, for water treatment and so on [1-3]. The majority of these contaminants usually occur at low concentrations so effects on health are often far from immediate.

In relation to this issue, the task of analytical chemists is to provide qualitative or quantitative information on the analytes present in different types of samples (biological, food and environmental, among others). The analysis is usually challenging due to the complexity of matrices, which contain a wide range of interferences. Many of the analytes are difficult to determine due to their physicochemical characteristics. With regard to the complex nature of different matrices, sample preparation is key to providing reliable analytical methodology and may also be used as a clean-up step when the sample or the extract of the sample cannot be directly injected into a chromatographic system, or for eliminating interfering compounds to decrease the complexity of the sample. Considerable efforts have been made to develop sensitive instrumental techniques for the identification and determination of target analytes, as well as significant attempts to find suitable sample preparation techniques. The analytes of interest usually occur at concentrations lower than the limits of detection of instrumental techniques and so a preconcentration step is necessary [4]. Consequently, the first and most crucial step of the analytical procedure is usually pretreatment of the sample to enhance both the selectivity and sensitivity of the method. Thus, the selection of suitable sample preparation techniques is performed by first considering the physical state of the sample (solid, liquid or gas) and then its other properties (e.g. solubility or polarity)[5].

Several extraction techniques have been used for the extraction of organic contaminants in solid and liquid samples. The classic techniques most

commonly used to extract contaminants from solid samples are Soxhlet or ultrasonic extraction. However, they have been replaced by techniques that use less solvent and are less time-consuming, such as microwave-assisted extraction (MAE) and pressurised liquid extraction (PLE), among others. With respect to extracting contaminants from liquid samples, different extraction techniques can be used and among them, solid-phase extraction (SPE) has been the most widely-used extraction and preconcentration technique for determining organic pollutants due to the wide range of sorbents commercially available that cover a variety of analytes. However, recent trends in sample preparation include the use of more environmentally-friendly techniques that reduce the use of organic solvents, such as solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE). Techniques employed in the analytical field should be robust, fast and provide reliable data. They must also be tailored to the final analysis, bearing in mind the instrumentation and the required accuracy [6-8]. Currently, a wide spectrum of techniques is available and the selection depends on different parameters, including the characteristics of the compounds under study, their possible concentrations, the kind of matrix, as well as the cost of the instruments [1]. The most frequently-used techniques for the separation and determination of target analytes are gas chromatography (GC) and liquid chromatography (LC), but other separation techniques, such as capillary electrophoresis (CE), are also used. Although several detection systems are available for these techniques, the quantification of the analytes at low concentration levels is not always possible, therefore the current trends are the use of more powerful detection systems such as mass spectrometry (MS), alone or in tandem, due to its high sensitivity and selectivity. Thanks to this powerful combination, it is possible to determine trace levels of contaminants in the environment, such as industrial chemicals, polar pesticides, pharmaceuticals and their metabolites, personal care products, hormones, bioactive compounds and many others [9,10].

In light of the above, the introduction of this Thesis highlights some of the most important materials for sorptive extraction techniques reported in recent decades that have been applied to the determination of organic contaminants. In the following section the widely-used liquid sample extraction techniques for the preconcentration of organic pollutants will be discussed briefly.

As regards the different extraction techniques for liquid samples, liquid-liquid extraction (LLE) has been one of the most useful sample preconcentration techniques. The LLE technique is labour-intensive, time-consuming and a multi-step process. Each step may introduce losses and errors, mainly in the extraction of volatile compounds. Another problem is the waste disposal of solvents, which is an additional cost of the analytical procedure and a possible health hazard to laboratory workers, as well as having a further impact on the environment. Therefore, LLE is being replaced by less labour-intensive techniques, such as solid-phase extraction (SPE) and others [11,12].

Currently, SPE is one of the most widespread sample preparation techniques for liquid samples. In contrast to LLE, SPE benefits from a shorter procedure time, low solvent consumption and a simple procedure. The fundamental principle of the SPE technique is based on the sorption of analytes on a solid phase. SPE is used to isolate analytes from different type of samples (gas, fluid or liquid) *via* their transfer to and retention on a solid-phase sorbent. The analytes are then usually eluted with a small volume of suitable organic solvent prior to the instrumental analysis. The applicability of SPE in environmental [11,13], forensic and clinical analyses [14,15] has been described and reviewed extensively in numerous books and publications. More detailed information about SPE and its applications will be further described in Section 1.1.

Microextraction by packed sorbent (MEPS) is a new development in the field of sample preparation, introduced by Abdel-Rehim in 2004 [16]. The MEPS device has been miniaturised to work with small sample volumes. The main difference between MEPS and SPE is that the packing is integrated directly into a syringe not in a cartridge. The sample preparation, extraction and injection steps are carried out on-line using the same syringe [16]. In MEPS technique, a small amount of sorbent is located in the syringe and retained by two frits. The sorbent is manually preconditioned and the syringe is combined with the autosampler. The sample is percolated very slowly through the cartridge and analytes are retained on the sorbent. Any interferences are removed at the washing step, and finally, the target analytes are eluted with an organic solvent directly into the injector. MEPS can be connected on-line with GC or LC and fully automated. The MEPS can be used for a range of different samples. It has

been used on biological samples and human hair [17] to extract a range of various pharmaceuticals, such as anti-cancer drugs and β-blockers, as well as drugs of abuse, such as cocaine and its metabolites or amphetamines. The main advantage of MEPS is the small volume of sample needed for the sample preparation. However, experience in the use of MEPS in combination with commercially available instrument for routine analysis is limited. Future research should focus on exploiting the advantages of MEPS, for instance, the use of more selective sorbents, such as molecularly imprinted polymers (MIPs) or restricted access materials (RAMs).

Another interesting sorptive technique is solid-phase microextraction (SPME) that was introduced by Pawliszyn and co-workers in 1989 to address the demand for faster sample preparation. SPME is a simple, efficient and solventless sampling technique which integrates extraction, preconcentration and sample introduction into one step. SPME is based on the immobilisation of the extracting phase and it has mainly been used for the direct extraction of organic compounds from samples [18-20]. SPME is usually performed using fibres, but capillary tubes coated with a suitable stationary phase may also be used. Initially, SPME was designed to be coupled to GC because of the ease of introducing SPME fibres to the GC injector. Nevertheless, SPME can also be coupled to LC or CE by means of the interface. SPME has been widely used for many years in various applications, such as environmental samples [21,22], food and fragrance analysis [23,24], biological fluids [25] and so on. Among the numerous advantages of SPME, there are also several drawbacks, such as fragility and limited lifetime of the polymer coating and relatively high cost (of commercially available fibres). And being the main disadvantage of SPME the reduced capacity due to the small volume of polymer coating on the fibre [6,26].

To overcome this limitation, an alternative to SPME is stir bar sorptive extraction (SBSE), which was introduced by Pat Sandra's research group in 1999 [27]. One of the greatest advantages of SBSE over the SPME is the larger extracting phase, which means that a larger amount of analyte can be extracted and the overall sensitivity is improved. In SBSE, a magnetic stir bar is encapsulated in a polymeric coat, and the compounds are extracted from the matrix into a non-miscible liquid phase. After a certain stirring time, the stir bar is removed from

the aqueous sample and then subjected to thermal or liquid desorption of the analytes. Due to the much higher volume of the polymeric phase, the extraction efficiency is better than for SPME. The SBSE technique based on the principle of sorptive extraction has become a widely-used analytical technique for the preconcentration of organic compounds from liquid samples [28-30]. In recent years, many applications in the environmental, food and biomedical fields can be found [29,31]. However, until very recently, only PDMS-coated stir bars (Twister) are commercially available which decreases the applicability of SBSE to the extraction of the non-polar compounds due to the limited extractability of more polar analytes. To overcome this drawback, several research groups are working on the development of more polar coatings applicable for the enrichment of hydrophilic pollutants by SBSE [31,32]. More detailed information relating to SBSE techniques and their applications will be discussed in later sections.

To reduce the consumption of organic solvents some liquid-microextraction techniques have been developed. One of the first techniques was single-drop microextraction (SDME), which was developed by Dasgupta's group in 1995 [33]. SDME is based on the rule of distribution of the analytes between a microdrop of extraction solvent at the tip of the microsyringe, and a liquid phase [34]. In SDME, the syringe needle is inserted into a closed vial. When the tip of the needle is placed in the desired position (immersed in the liquid phase or in the headspace), a hanging droplet of solvent is exposed to the sample by pressing the plunger of the syringe. After extraction, the droplet is withdrawn into the syringe barrel by lifting the plunger. The extract can be directly submitted to GC and LC analysis. Different modes of SDME have been developed and combined with various analytical applications, such as direct immersion (DI-SDME) [35], headspace (HS-SDME) [36] or continuous-flow microextraction (CFME) [37]. Typically, organic solvents such as octanol, cyclohexane, ethyl acetate or toluene, have been used as acceptor phases in SDME. However, in recent years, ionic liquids (ILs) have been investigated as suitable extracting phases for SDME that may enhance analyte selectivity [34,38]. SDME is a fast, simple and environmentally-friendly technique, because of the low amounts of organic solvents used [39]. However, SDME still has some limitations, such as the small volume of the extractant microdrop, which restricts the amount of analytes extracted and affects

extraction efficiency. Other issues include drop instability, limited choice of extractant, poor sensitivity and low reproducibility [40].

In an attempt to enhance the extraction process, Lee's research group [41] developed dynamic liquid-phase microextraction (LPME). It should be pointed out that dynamic LPME is not strictly a SDME since the drop configuration is not included. In this technique, the commonly-used microsyringe acts as a microseparatory funnel for extraction, which allows the mass transfer of analyte between the solvent microfilm, formed on the inner surface of the microsyringe, and the sample solution by the repeated movement of the plunger. Dynamic LPME demonstrated a greater enrichment factor within a shorter time than SDME, in which extraction was passively carried out into an organic solvent drop [42]. This technique can easily be performed in a microsyringe without any modification and the sensitivity can be readily adjusted by varying the number of samples and the sample volume. Dynamic LPME was employed in different applications, such as in determination of phthalate esters [43] or polycyclic aromatic hydrocarbons [44] from environmental water samples.

A further step in the LPME technique, hollow-fibre LPME (HF-LPME), was introduced in 1996 by Pedersen-Bjergaard and Rasmussen [45]. Compared to SDME, the goals of HF-LPME are to increase the volume of extracting solvent and its protection, as well as the enhancement of the stability of the organic solvent. In HF-LPME, a water immiscible organic solvent is immobilised as a thin supported liquid membrane in the pores of the wall of a porous hollow-fibre (HF). It is easily achieved by dipping the HF for a short time in the organic solvent, which is directly transferred to the pores by capillary forces. Next, the lumen of the HF is filled with a small volume of an acceptor solution and then placed in the sample for extraction of target compounds. The acceptor phase can be an organic solvent, resulting in a two-phase extraction system compatible with GC. Alternatively, the acceptor phase can be aqueous providing a three-phase system suitable for LC or CE [46,47]. The advantages of HF-LPME over SDME are higher robustness and sensitivity, as well as higher reproducibility.

Membrane extraction techniques have been used for several years and their use has been reviewed with respect to the advances and developments in implementation of membrane extraction techniques [48,49]. Membrane extraction can be varied depending on the type of membrane involved: porous, nonporous or semi-permeable. In porous membrane extraction, separation is the result of the sieving effect and is influenced by pore diameter. However, with a nonporous membrane, separation depends on the solubility and diffusion rates of the analytes in the membrane [50]. In both of these cases, the properties of the membrane material can influence the selectivity and the duration of the process. With respect to semipermeable membranes, these physical barriers only allow certain particles across them, based on their size or charge. The most commonly-used porous membrane extraction techniques are microporous membrane liquid-liquid extraction (MMLLE) and supported liquid membrane extraction (SLM). These are analogous to the aforementioned HF-LPME, two-phase and three phase, respectively. Meanwhile, the most popular nonporous membrane technique used in extraction is membrane-assisted solvent extraction (MASE) [42]. In MASE, the analytes from the donor aqueous sample are extracted into the organic solvent which is retained by capillary forces in the micropores of the hydrophobic polymer. Then, similarly to the MMLLE or SLM techniques, the analytes are transported to the acceptor phase either by back-extraction or diffusion. The membrane is usually made of low-density polyethylene, dense polypropylene, PDMS silicone rubbers or other porous hydrophobic material.

The main advantages of MASE are high selectivity and clean-up from complex matrices, the minimal solvent consumption and the possibility for automation and on-line coupling to analytical technique [51]. The disadvantage is that before the first extraction with the new membrane, a preconditioning step must be performed in order to eliminate interfering compounds (mainly alkanes and phthalates) from the membrane material. Numerous applications of membrane-based extraction techniques in environmental and clinical analysis have been reviewed [49,51].

New extraction tools for sample preparation are continually being developed and adapted. Among the extraction techniques for liquid samples, SPE continues to be widely used, but SPME and SBSE have fast been gaining ground. Many

sorptive extraction techniques have become excellent tools for the trace determination of organic pollutants in different types of samples. The research and development of new sorptive materials for use in sorptive extraction techniques is a growing and promising area. The technology has evolved, through changes in format and materials, in order to simplify the sampling process and/or automation. It has been broadly demonstrated through a wide range of commercialised materials and an even wider range of in-house prepared materials. The most important of them will be discussed in the following sections.

1.1. Solid-phase extraction

Solid-phase extraction (SPE) is a widely-used sample preparation technique based on the partitioning of the analytes between two phases, one of which is a solid sorbent [52,53]. The analytes are extracted from the liquid sample and retained on the solid material and can be removed at a later step by eluting with a solvent with a greater affinity for the analytes (elution or desorption step). SPE can be used directly as an extraction technique for liquid matrices or as a clean-up method for solvent extracts [14,53].

As is well known, the SPE process basically consists of four steps. The first step is the conditioning of the sorbent, which promotes good surface contact between the sample and sorbent. Second, the loading of the sample that passes through the sorbent (by gravity or the application of vacuum or pressure) and the analytes are retained on the sorbent. Third, the optional washing step which involves passing a solvent that eliminates retained interferences while the remaining analytes are retained on the sorbent. Finally, the elution of the target analytes retained on the sorbent using an appropriate solvent or mixture of solvents.

The sorptive materials used in SPE can often be applied in various formats, such as cartridges, syringes or disks [53]. The selection of format depends on the application as well as availability. Cartridges and syringes can be easily prepared in the laboratory and a number of different sorbents is available while disks are produced by manufacturers and the number of sorbents available in this format is limited. These SPE formats are suitable for environmental analysis, due to the high capacity and large amount of sorbent (150-500 mg), where large volumes of samples can be percolated. They are also suitable for biological applications in smaller amounts (10-50 mg), where small sample volumes can be preconcentrated. When comparing cartridges and disks in SPE, the cartridges are definitely more convenient, since the disks are mostly used in large-volume sample preparation.

Other SPE formats, such as the 96-well disk SPE plate or pipette tips containing small amounts of sorbent (1 mg or less) and thus lower capacity, are designed for analysis of biological samples. The semi-automated 96-well disk SPE plate format is based on the standard 96-well microtitre plate format. Automated multi-well plate technology has been implemented in quantitative bioanalysis

with a number of applications [54,55], since it effectively replaces manual operations and reduces handling errors.

SPE can be performed off-line or on-line mode connected to chromatographic techniques. Off-line SPE is the most frequently-used mode [52,56]. A typical SPE cartridge contains the sorbent, which is placed between two frits. The cartridge is then connected to a SPE manifold and the sample is percolated through. The benefit of the off-line SPE system is that it allows sequential extraction and uses different combinations of cartridges connected in series, as well as the percolation of high sample volumes. An additional advantage is that the extract can be stored and different measurements can be performed using the same extract. A drawback of off-line SPE procedures, due to the manual action, is that they can be time consuming, often requiring many steps before reaching a concentrated extract suitable for instrumental analysis. To minimise or replace manual tasks, on-line SPE can be employed.

On-line SPE using column-switching techniques is quickly gaining acceptance in analytical applications [57-59]. In on-line SPE, the sorbent is placed in a precolumn, which is coupled to the chromatographic system. The range of advantages of automated systems includes analyte trace enrichment, unattended on-line sample preparation and analysis and reduced losses. Moreover, the analysis of all the eluate from the SPE extract increases sensitivity.

As mentioned above, one aspect of SPE where research has focused in recent years is the intensive development of different sorbents. Several important developments will be commented briefly in the following paragraphs. The first application of SPE was the use of charcoal-filled columns in the 1950s to isolate organic pollutants from surface water [60]. In the mid 1960s a crosslinked polystyrene-divinylbenzene (PS-DVB) resin Amberlite XAD-1 was introduced by Rohm and Haas Company [61]. Apart from XAD-1, a series of styrene-divinylbenzene and ethylene-dimethacrylate resins were introduced for extracting organic contaminants from waters. In further developments, Musty *et al.* [62] introduced porous polyurethanes and polyurethane foams that had been used for extraction of polychlorinated biphenyls (PCBs) from water samples.

The use of different sorbents in SPE was derived them from phases used previously in chromatography. In the early 1970s, the introduction of macroreticular porous polymers resulted in increased interest in SPE [63], and extended its applications to air samples and broaden the application field in aqueous samples. Simultaneously, a new generation of carbon sorbents, such as carbonaceous molecular sieves or graphitised carbon blacks (GCB), appeared in analytical applications due to their higher affinity for more polar compounds [56]. Sorbents such as GCB and porous graphitic carbon (PGC) are examples of carbonaceous sorbents that can be used for trapping polar compounds from diverse matrices such as sediment samples, water and food [64,65]. Moreover, carbonaceous sorbents present excellent adsorption capacity, as well as their chemical, mechanical and thermal properties. In contrast, the main drawback is that they have excessive or even irreversible retention for some compounds.

A significant development, that stimulated intensive interest of analysts in SPE, was the introduction of bonded phases. In the 1980s, the growth in use of bonded phases [66] was encouraged by fast developments in chromatography. As well as this, chemically bonded silicas were employed in a number of applications. For many years, the silica-based sorbents have been the most frequently-used phases for SPE. Silica-based sorbents can be obtained by chemically immobilising different groups on silica, and depending on the groups attached, they can be used for the extraction from liquid samples of a wide range of analytes with different characteristics. Silica-bonded sorbents can be classified as reverse-phase sorbents modified with octadecyl (C_{18}), octacyl (C_8), ethyl (C_2), cyclohexyl (CH) and phenyl (Ph), which can be used in the extraction of analytes from organic samples. Their interaction mechanisms are mostly based on Van der Waals forces (hydrophobic interactions), thus these materials were designed for extraction of non-polar analytes. Normal phase silica-based sorbents modified with aminopropyl (NH_2), cyanopropyl (CN) and diol functional groups are suitable for the extraction of polar analytes from aqueous matrices. The main drawbacks of silica-based sorbents are instability at extreme pHs and the content of silanols, which can irreversibly bind some groups of compounds such as tetracyclines [67]. They also show low extraction efficiency of polar analytes and relatively low capacity [68].

Through the search for the ideal SPE sorbent, it becomes evident that there is no universal multi-application sorbent suitable for different target analytes. Each sorbent is successfully applied for certain purposes but drawbacks are found when used with different groups of compounds or matrices.

Nowadays, SPE can be said to hold a well-established position. One of the greatest advantages of the use of SPE is the wide range of sorbents available. Many years of research and investigation have resulted in a great development of sophisticated materials for SPE, in particular, the polymeric materials that overcome the drawbacks of conventional materials, aiming to enhance capacity and selectivity. The most interesting examples of recently investigated sorbents will be described in detail in the following sections.

1.1.1. Polymeric sorbents for solid-phase extraction

Porous polymeric sorbents are a class of SPE materials that overcomes some of the disadvantages of the aforementioned sorbents. Due to the chemical stability and wide range of physico-chemical properties, polymeric sorbents have become the most commonly-used materials in SPE.

In recent years, much attention has been paid to the preparation techniques of polymers in an effort to optimise synthetic approaches for preparing sorptive materials to suit SPE requirements. It has been demonstrated that the nature of the polymeric sorbents has an impact on the ability of the SPE device to perform consistently. Also, a variety of porous structures can be obtained during or after the crosslinking process by varying the parameters of the polymer synthesis, such as the amount of crosslinking agent and diluent, as well as the initiator concentration or the polymerisation temperature. The variety of bonding chemistries provides distinct surface characteristics and the capacity of the material will be affected by the structure of the polymer. In the following sections, the characteristic of polymeric sorbents and their applications will be discussed as well as these of some specific sorbents presently used in SPE.

1.1.1.1. Hydrophobic polymeric sorbents

The conventional polymeric sorbents in SPE are macroporous resins based on PS-DVB. These resins have a hydrophobic structure with a specific surface area up to 500 $m^2 g^{-1}$. The structure of PS-DVB is shown in Figure 1.

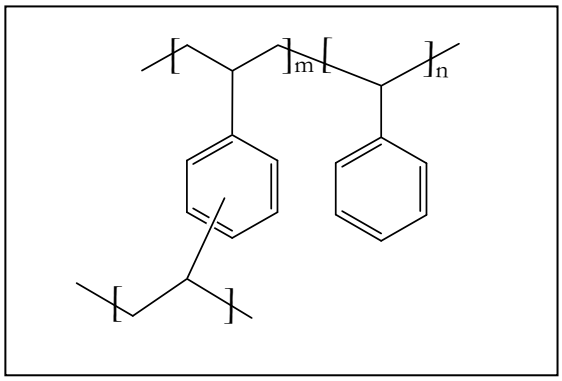

Figure 1. Structure of PS-DVB skeleton.

With the use of hydrophobic sorbents for extracting analytes, the specific surface area of the sorbent should be taken into account, as the larger the specific surface area, the higher the number of π-π sites capable of interacting with the analytes.

Highly-crosslinked polymers were developed to enhance the capacity of polymeric sorbents. Highly-crosslinked PS-DVB resins, obtained by suspension polymerisation, have a hydrophobic structure and have a specific surface area up to 800 $m^2 g^{-1}$, resulting from the high content of crosslinking agent during the synthesis. Their interaction mechanisms with the analytes are mainly composed of Van der Waals forces and π-π interactions of the aromatic rings incorporated in the sorbent structure. Their capacity can be improved by increasing the ratio of DVB, and thereby increasing the number of π-π interactions. However, it should be taken into consideration that exceeding certain levels of DVB concentration results in a decrease in crosslinking efficiency (due to steric hindrance) and there is no increase in the specific surface area of the polymer [69].

Typical examples of commercially available polymeric resins are: Amberlite XAD-2 (~300 $m^2 g^{-1}$) and Amberlite XAD-4 (~900 $m^2 g^{-1}$) from Rohm and Haas, PLRP-S-10 (~500 $m^2 g^{-1}$) and PLRP-S-30 (~350 $m^2 g^{-1}$), both from Polymer Labs, or more recently SampliQ PS-DVB (~600 $m^2 g^{-1}$) from Agilent Technologies [68]. Initially, Amberlite XAD-2 and XAD-4 were widely used for the off-line SPE of organic micropollutants from water, but later, they were replaced by more efficient materials. However, some applications of functionalised XAD copolymers have been recently reported for the SPE of trace metals from biological and environmental samples [70].

Apart from the PS-DVB macroporous copolymers, which have been widely used and their synthesis was well-established, other hydrophobic monomers, such as divinylbiphenyl (DVPh) or vinylnaphtalene (VN) have also attracted attention of some research groups. Trochimczuk *et al.* [71,72] investigated the sorption properties of two crosslinked resins based on vinylnaphtalene-divinyl-naphtalene (VN-DVN) [71] and vinylbiphenyl-divinylbiphenyl (VPh-DVPh) [72]. The results showed higher retention of phenol using resins with higher specific

surface areas, which indicates that the enhancement in specific surface area of these hydrophobic sorbents increased the hydrophobic interactions.

As previously mentioned, the specific surface area is key to enhancing extraction capacity. Hypercrosslinked polymers were introduced in the 1970s by Davankov [73] and over the past few years, they have attracted the interest of many research groups. In an attempt to overcome the limitations of highly-crosslinked polymers, Davankov and Tsyurupa developed a novel polymerisation technique, which is based on the crosslinking of preformed linear polystyrene through the Friedel-Craft reaction, so that methylene bridges are formed between aromatic rings. The polymers obtained have microporous structure with a specific surface area usually higher than 1000 $m^2 g^{-1}$ and the sorbent capacities are significantly high [74].

Syntheses of hypercrosslinked resins were developed further by Jerabek *et al.* [75]. The hypercrosslinked resins were obtained by an extensive post-crosslinking of vinylbenzyl chloride-divinylbenzene (VBC-DVB) suspension polymerised resin, with the generation of internal electrophiles as the crosslinking mechanism using a Friedel-Crafts catalyst. Hypercrosslinked resins were further modified by Sherrington group [76,77] by employing gel-type and permanently porous VBC-DVB resin with Friedel-Crafts catalyst [76,78]. This generated resins with a bimodal distribution of pore sizes. The hypercrosslinking reaction allows the introduction of a very fine pore structure into a wide range of polymers and networks of small molecules. As shown in Figure 2, firstly the precursor particles are generated by suspension, non-aqueous dispersion or precipitation polymerisation. Then, the precursor is swollen or dissolved in a thermodynamically good solvent, for instance dichloromethane (DCE). This step introduces space between monomers or polymer chains. Subsequently, the precursor is crosslinked using the Friedel-Crafts reaction, such that the overwhelming majority of repeat units are linked to their neighbours. Fast hypercrosslinking leaves the polymer chains locked in the expanded form. After withdrawing the solvent, the previously occupied space generates pores. Moreover, this procedure was applied to preformed beads of sizes ranging from nanoparticles to microparticles and macroparticles. Thus, using porous resins as precursors, products can be obtained that contain additional pores (generated

during hypercrosslinking reaction). This approach allowed better control over the porous morphology of the hypercrosslinked products. All the hypercrosslinked resins exhibit an extremely high specific surface area and excellent sorption properties [74,77,79]. These properties of the sorbents are determined by specific physical structure of the polymers and make hypercrosslinked sorbents more retentive than conventional macroporous sorbents.

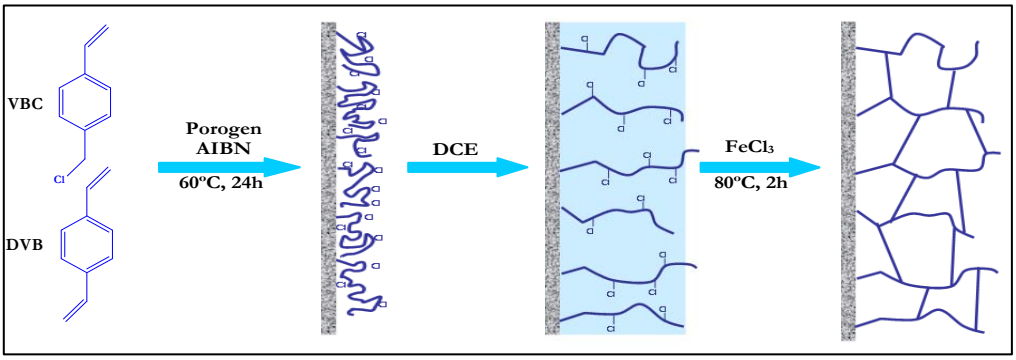

Figure 2. Schematic representation of hypercrosslinking reaction.

Hypercrosslinked polymers are commercially available and manufactured by several companies, such as LiChrolut EN (Merck, Germany) and Isolute (International Sorbent Technology, UK). They came into use on a large scale as great sorptive materials for the SPE of organic pollutants from air and aquatic environments. Other commercial hypercrosslinked resins are Hypersol-Macronet, Styrosorb 2, Styrosorb MN-150 or Styrosorb MT-430 (Purolite Int. UK), HySphere-SH (Spark Holland, Netherlands), Supelclean ENVI-Chrom P (Supelco, Bellefonte, PA, USA), Bakerbond SBD1 (J.T. Baker, Netherlands), and Amberchrom CG161 (Supelco). All of these were used in a number of applications from different matrices. As regards, LiChrolut EN has been used to extract a variety of compounds from different water samples [83-85]. For example, Carabias-Martínez *et al.* [80] used LiChrolut EN for the extraction of herbicides and metabolites from surface water and groundwater with good recovery of target analytes. LiChrolut EN was also used for extraction in multiresidue methods to screen low levels of nicotinoid insecticides [81] and the determination of *N*-methylcarbamate pesticides [82] in drinking water samples, achieving high recoveries of 85-104% for all target analytes, following a preconcentration of 1 L of the water sample. It was also used for extraction of

sulphonamides from milk [83], providing promising results for the determination of these veterinary drugs in biological matrices. The Supelclean ENVI-Chrom P sorbent has also been successfully used for the determination of carbohydrates in biomass samples, achieving good analytical performance and high recoveries [84]. In other research, the SPE with Amberchrom C 161 followed by derivatisation and GC was used for the determination of chlorophenols in tap water and lake water samples [85]. When extracting 10 mL of the sample, it was found that the recoveries of chlorophenols in the water samples exceeded 90%.

1.1.1.2. Hydrophilic polymeric sorbents

The exclusivity of hydrophobic interactions between the sorbent and the analytes often leads to poor retention of polar analytes. To overcome this drawback, the research in new SPE materials focuses on the development of hydrophilic materials. The hydrophilic polymeric sorbents can be obtained by chemical modification of the existing hydrophobic materials with polar functional groups or by copolymerisation of monomers containing suitable moieties [68].

Chemically Modified Sorbents

In the first approach, PS-DVB polymers were chemically modified with polar moieties. The major advantage of this approach is that the precursor particles can be selected previously based on their morphological characteristics (*i.e.* particle size, specific surface area), which are maintained during the functionalisation process. To ensure proper contact between the reagents and the crosslinked precursor, the conditions of the functionalisation reaction should be carefully selected. The Friedel-Craft reaction is the most frequently-used reaction for chemically-modified sorbents preparation and it has been adapted by several research groups to enhance the properties of the commonly-used hydrophobic sorbents [86-89]. The first chemically-modified sorbents were obtained by Fritz *et al.* , with the introduction into the polymeric skeleton acetyl, hydroxymethyl [86] and sulphonic functionalities [87].

A few years later, Masqué *et al.* introduced various hydrophilic groups such as o-carboxybenzoyl [88], 2,4-dicarboxybenzoyl and 2-carboxy-3/4-nitrobenzoyl

groups [89] onto the surface of PS-DVB copolymers. The modified materials were used for the extraction of polar compounds. It was found that increased surface polarity resulted in a marked increase of the recovery values of polar analytes, such as resorcinol and phenol. It was demonstrated that the modified resins yield higher recoveries compared to their unmodified analogues. However, it should be noted that the extent of the modifications was lower than 20% in all cases.

In recent years, several chemically-modified sorbents have been commercialised. One frequently-used commercial sorbent is Isolute ENV+, developed by International Sorbent Technology (IST), which is a PS-DVB with a specific surface area of around 1100 $m^2 g^{-1}$ and chemically modified with hydroxylated groups. For instance, Isolute ENV+ was applied for the enrichment of neutral, volatile polyfluorinated alkyl substances in air samples [90] and organophosphate flame retardant metabolites in urine [91], providing good retention capacity for the target analytes. With regard to the extraction performance of pharmaceuticals [92] and pesticides [93], Isolute ENV+ provided results comparable to other commercially available materials, such as LiChrolut EN.

Another commercial type of material worth mentioning is Strata-X, which is PS-DVB chemically modified with pyrrolidone groups and has a specific surface area of ~800 $m^2 g^{-1}$. As with all chemically modified sorbents, Strata-X exhibits hydrophilic, hydrophobic and π-π retention mechanisms, which allow the screening of a broad range of acidic, basic and neutral analytes. Strata-X was used in SPE for the determination of various organic contaminants in environmental water samples [94-96]. Strata-X has also been employed in SPE to clean-up biological samples [97,98]. For example, Babic *et al.* [95] used Strata-X for the extraction of multi-class pharmaceuticals of different polarities from wastewater. Recoveries obtained for most target pharmaceuticals, after percolating 100 mL of the sample using Strata-X cartridges, were higher than 50%. However, it was observed that less polar compounds were better retained on the sorbent than those more polar.

Another commercialised sorbent from Varian and Polymer Laboratories is Bond Elut Plexa, which is also chemically modified. However, no additional

information about its structure and properties is available. Bond Elut Plexa has been used for the determination of (R,R)-fenoterol in rat plasma [99] providing promising results.

Spe-ed Advanta is another chemically-modified polymeric sorbent with a carboxyl moiety, commercialised by Applied Separations. Spe-ed Advanta was evaluated by Sirvent *et al.* [100] for the extraction of phenolic compounds from water, providing good SPE performance, which can compete with other commercially available sorbents.

Supel-Select HLB is other chemically-modified styrene-based sorbent with a specific surface area around 400 $m^2 g^{-1}$, commercialised by Supelco. According to the information provided by the supplier, Supel-Select HLB has multiple hydrophobic and polar retention mechanisms thanks to its hydrophilic modification being suitable for the extraction of polar compounds, but due to the fact that the patent is pending, the functional group that modifies the sorbent is unknown. However, to the best of our knowledge and due to its novelty, no studies have been reported on Supel-Select HLB as an SPE sorbent.

Chemically-modified sorbents are very versatile for extracting a wide range of analytes or performing analysis under different matrix conditions. In order to improve the retention of polar analytes and develop further new sorptive materials, apart from the enlargement of the specific surface area, another possibility is to increase the degree of hydrophilicity during the synthesis by incorporating a polar monomer into the polymer skeleton.

Copolymers containing hydrophilic monomers

One of the first commercially available and the most widely-used hydrophilic sorbents is Oasis HLB (Waters), which has a macroporous poly(N-vinylpyrrolidone-divinylbenzene) copolymer and has a specific surface area of ~800 $m^2 g^{-1}$. Oasis HLB has been extensively used in SPE techniques for a variety of applications, mainly for the extraction and clean-up of a broad spectrum of organic compounds, such as pharmaceuticals, from a variety of matrices [101-103]. For instance, Oasis HLB has been used by Pedrouzo *et al.* [101] for the

extraction of pharmaceuticals (including anti-inflammatories, lipid regulators or antiepileptic drugs, among others) from wastewater. Recoveries were between 33% and 91% in wastewater treatment plant (WWTP) effluents and 33% to 72% in influents, when preconcentrating 250 mL and 100 mL of the sample respectively, except for very polar analytes. Oasis HLB has also been used by Gómez *et al.* [103] for determining various therapeutic pharmaceuticals in hospital effluent wastewaters. Recoveries found after the SPE of 100 mL of the sample ranged from 76% to 113%, for most target analytes. However, for the most polar analyte studied, the recovery obtained was only 45%.

In comparative studies [93,94], when extracting several pharmaceuticals from water samples, Oasis HLB and Strata-X provided comparable results, which indicates that their similarities in structure generate similar retention, although interferences were present to a lesser extent when Strata-X was used [94]. In another study, Barron *et al.* [104] reported a multiresidue determination of pharmaceuticals from sludge and sludge-enriched soils using pressurised liquid extraction with the methanol-water (50:50, v/v) solution as an extraction solvent, followed by SPE and comparison with 6 different commercially available sorbents (including LiChrolut EN, Oasis HLB or Strata-X). It was found that Oasis HLB, in comparison to other sorbents, offered considerably higher recoveries than Strata-X sorbent and even higher recoveries of polar analytes than LiChrolut EN.

Another example of a hydrophilic sorbent is Abselut Nexus, commercialised by Varian which is based on a methacrylate-divinylbenzene (MA-DVB) copolymer and has a specific surface area of ~600 $m^2 g^{-1}$. It was applied to the analysis of biological samples rather than environmental. In most applications, Abselut Nexus was used to clean up complex matrices including urine [105,106], plasma [106,107] or tissue [108].

When different sorbents (including LiChrolut EN, Isolute ENV+, Oasis HLB and Abselut Nexus) were compared in the SPE of acidic, neutral and basic analytes from aqueous samples [109], it was found that the best results, when preconcentrating 1 L of water sample, were obtained with Oasis HLB. It

provided quantitative recoveries of 83-102% for most polar pharmaceuticals under study. Oasis HLB was followed in performance by the Abselut Nexus, with recoveries of 70-90% for most analytes. However, it showed very low extraction efficiency for hydrophilic analytes, such as paracetamol, caffeine or clofibric acid. The other sorbents, such as LiChrolut EN and Isolute ENV+, showed a comparable performance in the extraction of basic analytes. However, the recoveries of acidic analytes were significantly lower.

Bakerbond Speedisk H_2O-Philic DVB is another commercial hydrophilic material, supplied by J.T. Baker. However, information available describing its physical characteristics is very limited. Another example of a commercialised hydrophilic sorbent is Discovery DPA-6S from Supelco, which is based on polyamide and has a very low specific surface area (a few square metres per gram) due to its linear structure. Another commercially available hydrophilic sorbent based on polyamide is SampliQ OPT from Agilent Technologies. However, there is no data available about its sorbent characteristics. Since these sorbents are new, no applications have yet been reported.

Apart from a range of commercially available sorbents, some research groups have synthesised hydrophilic polymers to be applied as SPE sorbents over the past few years. Some examples of these in-house synthesised sorbents will be presented below.

In this regard, Bagheri's research group synthesised a series of conductive resins based on polyaniline (PANI)[110], poly-N-methylaniline (PNMA), polydiphenyl-aniline (PDPA) [111] and polypyrrole (PPy) [112]. All these polymers are hydrophilic and have low specific surface areas, which results from their linear structure. These sorbents were successfully applied for the SPE of phenolic compounds from water samples and compared to commercially available sorbents, such as LiChrolut EN (1200 $m^2 g^{-1}$) or Oasis HLB (800 $m^2 g^{-1}$). The results indicated that in-house synthesised sorbents provided extraction efficiencies comparable to those obtained with commercial materials, except phenol, which was much better recovered with LiChrolut EN and Oasis HLB, due to their large specific surface area [110-112].

Another example was the series of sorbents that contain hydrophilic monomers prepared by Trochimczuk *et al.* that synthesised materials based on acrylonitrile (AN), methacrylonitrile (MAN) [113,114] and cyanomethylstyrene (CMSt) [115]. All were crosslinked with DVB. The resins were prepared using different degrees of each monomer (both hydrophilic and crosslinking agents), so their properties, such as specific surface area and hydrophilicity, were tailored to the initial content of each monomer. The copolymers obtained have a large specific surface area and nitrile groups distributed evenly throughout the polymeric network. Then, the sorptive properties were investigated and compared to the conventional PS-DVB copolymers using phenol and its derivatives as model compounds. It was found that both features (hydrophilicity and specific area) have an influence on sorptive properties, since the best results were obtained using materials with 50:50 (hydrophilic monomer/crosslinker monomer) ratio. It was also demonstrated that materials with a high proportion of strongly polar nitrile groups performed significantly better than the PS-DVB copolymers [113,114].

Bielicka-Daszkiewicz *et al.* [116] prepared different porous copolymers using di(methacryloyloxymethyl)naphthalene-divinylbenzene with ester functional groups (DMN-DVB), 4,4´-bis(maleimido)diphenylmethane-divinylbenzene with imide functional groups (BM-DVB), p,p´-dihydroxydiphenylmethane diglycidyl methacrylic ester-divinylbenzene (MEMDE-DVB) and p,p´-dihydroxydiphenyl-propane diglycidyl methacrylic ester-divinylbenzene (MEDDE-DVB). The synthesised sorbents were found to be polar, with a specific surface area close to 100 m^2 g^{-1}. Subsequently, these sorbents were applied for SPE of phenol and hydroquinone from water samples and their extractability was compared to commercially available polymeric sorbents. The results indicated that low specific surface area could be responsible for low recovery values of target analytes in comparison to commercial materials.

Other in-house synthesised sorbents containing 1-vinyl-2-pyrrolidone and divinylbenzene (VP-DVB) were prepared by Gawdzik's group. The synthesised materials provided specific surface areas close to 800 m^2 g^{-1} [117]. Next, they were used as a sorbent in the extraction of phenol and the results were slightly better than those obtained with commercially available Strata-X under identical conditions.

In order to enhance the capacity of the sorbents and reinforce their hydrophilic interactions, our research group contributed to the development of sorbents with the presence of different hydrophilic moieties. To this end, a series of hydrophilic sorbents based on 4-vinylpyridine-divinylbenzene (4VP-DVB) [118], N-vinylimidazole-divinylbenzene (NVIm-DVB) [119,120] and 4-vinylimidazole-divinylbenzene (4VIm-DVB) [121], that have different nitrogen content and specific surface area in the range of 600-800 m^2 g^{-1}, were developed and evaluated for the extraction of several polar contaminants from environmental water samples. Once again, the results showed that the retention ability of hydrophilic sorbents depended on balance of specific surface area and polarity. It was also demonstrated that the performance of hydrophilic sorbents provided higher recoveries than the use of hydrophobic materials. The results obtained clearly indicated that the polar nature of the sorbent is a crucial parameter in the extraction of polar analytes.

Although the results indicated great potential, when large volumes of the sample were passed through the sorbent, the most polar compounds were not retained, indicating that the morphology of the polymers must be enhanced. Therefore, to improve the morphology of the polymer while maintaining the hydrophilicity, a hypercrosslinked polymer (HXLGp) which contains some hydroxyl moieties in the skeleton was synthesized and its performance for the on-line SPE of several polar analytes was evaluated [122], providing good recoveries. The efforts have also been directed to develop hypercrosslinked sorbents with hydrophilic characteristics. Our research group continues the development of hypercrosslinked SPE sorbents with different hydrophilic moieties [76]. The efforts have been undertaken to introduce new hypercrosslinked sorbents, and they seem to be very promising materials for SPE. However, more research is still required to allow achieving the full potential of hypercrosslinked sorbents in the future.

The materials described so far aims to enhance the capacity. However, in spite of the demand for efficient sorptive material for the extraction of target analytes from complex samples, an important goal is to obtain clean extracts from the extraction, eliminating interferences, and thus improving both sensitivity and selectivity.

1.1.1.3. Mixed-mode ion-exchange sorbents

The first ion-exchange materials were silica-based resins chemically modified with ion-exchange moieties, and their retention mechanism was based exclusively on ionic interactions. However, because of the drawbacks of conventional silica-based materials, there was a need to replace them with porous polymeric ion-exchange resins, which have a higher exchange capacity and a wider pH operating range. Porous crosslinked polymers were demonstrated to be efficient materials for many separation processes [123]. However, their capacity was still limited. Therefore, attention has recently been devoted to the mixed-mode polymeric sorbents, which are based on polymeric skeleton with ionic moieties, and therefore, they can interact with ionic species while also effectively retaining non-charged species through hydrophobic and hydrophilic interactions. The concept of mixed-mode sorbent technology is based on the selection of an adequate sorbent, then careful choice of the pH and suitable solvent in each SPE step that allows the matrix components and interferences to be eluted separately from the target analytes, in the washing and elution steps, respectively. Mixed-mode sorbents for extraction of ionic or ionisable compounds from complex environmental, biological and food matrices have been extensively reviewed [124].

Mixed-mode sorbents are classified as being either cationic or anionic, and also as either strong or weak, depending on the nature of the ionic group attached to the polymeric resin. In applications of strong ion-exchange sorbents, the chargeability of the analytes or interferences (not of the sorbent) can be tuned in the different SPE steps. In the case of weak ion-exchange sorbents, the purpose is to switch the chargeability of the sorbent (not of the analytes or the interferences, if possible).

With regard to the strong ion-exchangers, strong cation-exchange (SCX) sorbents are modified with sulphonic acid while strong anion-exchange (SAX) sorbents are modified with quaternary amines. A general example of a mixed-mode polymer structure is depicted in Figure 3. In contrast, weak ion-exchangers, such as weak cation-exchange (WCX) sorbents, usually contain carboxylic acid, whereas weak anion-exchangers (WAX) can be modified primary, secondary or tertiary amines for weak anion-exchange, respectively [124].

Over the past few years, the number of commercially available mixed-mode polymeric sorbents has increased significantly. The first commercially available mixed-mode polymeric sorbents were supplied by Waters and were based on the previously described Oasis HLB. Figure 3 presents the structure of Oasis mixed-mode ion-exchange sorbent. As can be seen, all these sorbents are based on Oasis HLB skeleton and are modified with different functional groups.

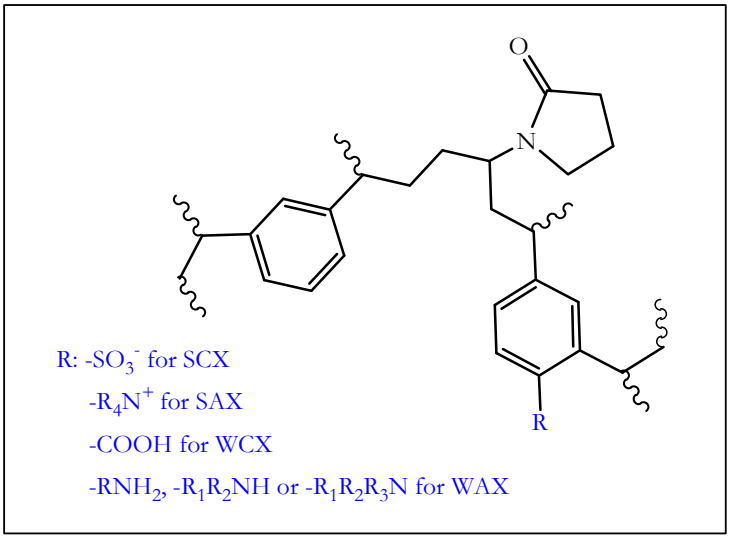

Figure 3. Structure of Oasis mixed-mode polymeric sorbent.

The first commercialised mixed-mode sorbent was the strong cationic Oasis MCX. This sorbent was obtained by the introduction of sulphonic acid groups (pK_a<1) on Oasis HLB polymeric skeleton, combining the properties of the hydrophilic sorbent with the characteristics of a strong cation exchanger [124]. The combination of RP and cation exchanger features of the Oasis MCX material allows the separation of samples into an acidic-neutral fraction as well as the extraction of basic drugs. These mixed-mode sorbents are widely used in each kind of application. Several applications of these sorbents in different matrices are presented in Table 1. The extraction of analytes with SCX sorbent requires a sample with an acidic pH in order to ionise basic analytes and to minimise the dissociation of acidic analytes. Therefore, the acidic, basic and neutral compounds can be extracted at low pH, since the cation-exchanger binds the basic compounds, which are in the ionised form, retained by RP interactions

acidic and neutral compounds. For instance, Gheorghe *et al.* [125] reported a method for the determination of cocaine and its metabolites in surface water, using Oasis MCX, since it allows improved selectivity towards basic analytes due to the pH and polarity changes during the loading, washing and elution steps. After a usual conditioning step, the sample was adjusted to acidic pH and loaded, in order to protonate the basic analytes and to establish ionic interactions with the sulphonic group in the sorbent. Subsequently, the washing step with MeOH was performed to disrupt all the RP interactions and elute all the non-protonated compounds (including neutral and acidic). Finally, the target analytes were eluted with a medium basic solution, such as 5% NH_4OH in MeOH, that ensured deprotonation of the basic analytes, while the sorbent was still in the ionic form thank to its acidic strength. This carefully selected SPE protocol resulted in clean extracts and delivered high recoveries of target analytes.

As can be seen in Table 1, Oasis MCX has been also used for the general unknown screening of drugs and toxic compounds in clinical research [126]. The related applications show the general use of SCX conditions. However, in some cases they can be modified to reduce the matrix effects. As an example, Al-Odaini *et al.* [127] reported a multiresidue method for determination of different pharmaceuticals and synthetic hormones in complex water samples, using Oasis MCX following LC-(ESI)MS/MS analysis. Under optimised SPE conditions, the recoveries obtained were higher than 70% for most of target analytes, when 100-150 mL (depending on the matrix) of complex water sample was preconcentrated. In another study, Bijlsma *et al.* [128] developed a method for the determination of drugs of abuse in environmental waters. The preconcentration with Oasis MCX was performed under typical SCX SPE conditions, but the washing step was performed with aqueous basic solution (2% NH_4OH in water) instead of organic solution, to eliminate interferences, such as inorganic salts. The matrix effects were considerably lower and the results showed satisfactory recoveries ranging from 70% to 120% for most target analytes, when 50 mL of surface water and urban wastewater were preconcentrated.

The other commercialised mixed-mode sorbent was the strong anionic Oasis MAX modified with quaternary amine (pK_a>18), that combines the hydrophilic nature of Oasis HLB with strong anion-exchange character, providing high

selectivity and sensitivity for acidic compounds and metabolites. In typical SAX protocol, the sample is loaded at neutral pH or above to ensure that the acidic analytes are ionised and the ionic interactions are established with the quaternary amine group in the sorbent. The washing step usually consists of an optional basic aqueous wash for the favoured retention of acids, and a wash with pure organic solvent, such as MeOH or ACN, that eliminates the basic and neutral analytes and/or interferences bounded to the sorbent by RP interactions. Finally, the acidic analytes are eluted with acidic solution, usually 2-5% HCOOH in organic solvent, which ensures the protonation of acids (in their neutral form) while the sorbent is still protonated. As an example, Oasis MAX was applied for extraction of steroids [129]. It was demonstrated that Oasis MAX can effectively retain acidic compounds, such as estradiol, through an anion-exchange mechanism, whereas other neutral steroids (e.g. testosterone) could not be retained. Oasis MAX has also been recently used in on-line SPE applications. For instance, Ling Lai *et al.* [130] developed a method for the determination of antiparasitic drugs in animal tissues, using on-line anionic mixed-mode SPE. It was found that employment of Oasis MAX for the extraction of target analytes provided good selectivity and high recoveries. For more applications see Table 1.

The same supplier also commercialise the weak ion-exchange sorbents Oasis WCX and Oasis WAX. These resins, also based on Oasis HLB skeleton, have been chemically modified with carboxylic acid (WCX) and piperazine (WAX) groups, respectively. Oasis WCX has been used for the extraction of psychostimulants [131] and fluoroquinolone drugs [132], among others from different matrices. For instance, it was reported that recoveries found were greater than 80% for all target analytes and the matrix effects were significantly reduced, when extracting fluoroquinolones from 250 mL of effluent wastewater, using Oasis WCX. These positive results were obtained due to the inclusion of the methanol clean-up step which selectively removed the acidic and neutral interferences in sewage samples, thereby producing a cleaner extract that displayed much less signal suppression in the quantification step [132]. In other research, Oasis WAX has been employed in SPE for determining fluorescent whitening agents in environmental waters [133]. Its performance was also compared to the unmodified analogue (Oasis HLB). However, the results indicated that Oasis HLB did not efficiently extract the target analytes due to the interaction of Oasis HLB sorbent with anionic analytes.

Table 1. Commercially available and in-house synthesised mixed-mode polymeric sorbents and their applications.

	Sorbent	Analytes	Matrix	Technique	Ref.
SCX	Oasis MCX	Pharmaceuticals	Environ. waters	LC-MS/MS	[127]
		Illicit drugs	Surface water & wastewater	UPLC-MS/MS	[128]
		Pharmaceuticals & illicit drugs	Surface water	UPLC-MS/MS	[134]
		Isosteroidal alkaloids	Rat plasma	LC-MS	[135]
		Pharmaceuticals	Biological Samples	LC-MS	[126]
	Strata-X-C	Pharmaceuticals	Environ. waters	GC-MS	[136]
		Drugs of abuse	Surface water & treated wastewater	LC-MS/MS	[137]
		Herbicides	Serum	LC-MS/MS	[138]
	Bond Elut Plexa PCX	Cationic metabolites	Soil	CE-MS	[139]
		β-agonists	Animal feed	LC-MS/MS	[140]
	SampliQ-SCX	Benzimidazole fungicides	Tomato puree	LC	[141]
		Melamine & cyromazine	Animal Feeds	GC-MS	[142]
SAX	Oasis MAX	Steroids	Tissue	LC-MS/MS	[129]
		Antiparasitic drugs	Animal tissues	LC-MS/MS	[130]
		β-lactam antibiotics	Wastewater	LC	[143]
		Fungicides	Wine	LC-MS/MS	[144]
	N-VIm-DVB	Pharmaceuticals	Environ. waters	LC	[145]
WCX	Oasis WCX	Psychostimulants &their metabolites	Rat plasma	LC-MS	[131]
		Fluoroquinolones	Wastewater	LC-Fl & LC-MS/MS	[132]
	Strata-X-WC	Pharmaceuticals	Plasma & blood	LC-MS	[146]
		Antibiotics	Honey	LC-MS/MS	[147]
WAX	Oasis WAX	Whitening agents	Environ. waters	LC-MS/MS	[133]
	Strata-X-AW	Sulphaes, sulphoates & phosphates	Urine	CE-MS	[148]
		Estrogens	River sediments	LC-MS	[149]
	HXLPP-WAX-EDA	Pharmaceuticals	Environ. waters	LC	[150]
	HXLPP-WAX-piperazine	Pharmaceuticals	Environ. waters	LC	[58,150]

In contrast, recovery values using Oasis WAX were significantly higher thanks to the ionic binding as well as the hydrophobic interactions.

To improve the clean-up of complex samples, a common approach is to use tandem sorbents in series (one or two of which present mixed-mode characteristics) [124]. As an example, Oasis WCX has been also combined with Oasis MAX to determine ofloxacin enantiomers in sewage, using two-step SPE [151]. The enantiomers were first extracted by Oasis WCX, and further purified by Oasis MAX, resulting in high recoveries of target analytes. Similarly, Lavén *et al.* [152] reported a method for the determination of basic, neutral and acidic pharmaceuticals in wastewater, using Oasis MCX and MAX in tandem. It was found that use of these sorbents in tandem provided a significant improvement in recoveries of target analytes, and reduced ion suppression, compared to the results obtained with Oasis MCX alone.

Other commercially available mixed-mode materials are Strata-X based sorbents, supplied by Phenomenex. These sorbents are obtained by chemically modifying Strata-X skeleton (containing polar vinylpyrrolidone) with sulphonic acid groups, quaternary amine groups, namely Strata-X-C and Strata-X-A as strong ion exchangers, or carboxylic acid groups and ethylene diamine groups, namely Strata-X-CW and Strata-X-AW as weak ion exchangers. Several examples of their applications for the extraction of various drugs of abuse, pharmaceuticals and hormones from complex matrices have been reported (see Table 1). For example, Bones *et al.* [137] reported a SPE-LC-(ESI)MS/MS method for the determination of illicit drugs and abused pharmaceuticals in treated wastewater and surface water samples at the ng L^{-1} level. Due to the expectation that the most of the selected analytes would be in their protonated cationic form in solution mixed-mode cation-exchange sorbents, both Strata-X-C and strong Strata-X-CW, were investigated. It was found that the highest degree of analyte recovery was achieved when using the Strata-X-C sorbent with recoveries of 50-65% for most analytes. Strata-X-C was also used in the SPE procedure for the screening of highly polar, water-soluble, and less-volatile herbicides in serum, providing high recoveries of target analytes [138]. Recently, Liao *et al.* [153] reported the application of Strata-X-AW and Strata-X-C for the measurement of acid metabolites of tryptophan-NAD pathway and related acids in urine samples.

However, despite the many efforts being made, neither Strata-X-C nor Strata-X-AW alone could retain all five target acids effectively. Therefore, to extract effectively all target analytes, the use of two sorbents separately was required. Moreover, Strata-X-C sorbent has also been used in tandem with the Strata-X cartridge for the determination of anti-infectives in raw sewage and WWTP effluents [154]. It was demonstrated that the tandem SPE method improved anti-infective recoveries compared to single cartridge methods. When 250 mL of complex primary effluent wastewater was preconcentrated, the recoveries using tandem SPE cartridges were higher than 75% for most of the target analytes.

Other mixed-mode sorbents known as Speedisk sorbents were introduced by J.T. Baker. These sorbents were obtained by modifying the precursors (Speedisk H_2O-Philic & Speedisk H_2O-Phobic) with ion-exchange groups, such as Speedisk H_2O-Phobic SC-DVB, which is DVB sorbent modified with sulphonic acid or Speedisk H_2O-Philic SA-DVB, which is hydrophilic sorbent modified with quaternary amine. There is also a weak anion-exchange version of Speedisk sorbent available - Speedisk H_2O-Phobic WA, which is DVB resin modified with secondary amine. Another example of a chemically modified PS-DVB sorbent with weak anion-exchange group is Chromabond Easy. However there is very little literature available on its applications. As an example, Jezewska *et al.* [155] tested different sorbents, such as Oasis HLB, Speedisk H_2O-Phobic SA-DVB, Chromabond Easy and two silica-based sorbents for extraction of 4-biphenylamine from workplace air (prior to extraction the air samples passed through a glass fibre filter with sulphuric acid and the compounds retained on the filter were subsequently washed out with suitable solvents). It was found that the best results were provided by Oasis HLB (89%) following Speedisk H_2O-Phobic SA-DVB (83%), whereas the recoveries obtained using Chromabond Easy and silica-based sorbents were significantly lower (54-69%).

Bond Elut Plexa PCX from Varian is a mixed-mode sorbent, based on a chemically modified resin with a polar moiety, which has been modified with a strong anionic group (no details available) to extract charged compounds (see Table 1).

Another group of commercially available ion-exchange sorbents from Agilent Technologies includes SampliQ SCX, SampliQ SAX, SampliQ WCX and SampliQ WAX, which are DVB modified resins with sulphonic acid (SampliQ SCX), quarternary amine (SampliQ SAX), carboxylic acid (SampliQ WCX) and primary amine (SampliQ WAX). It should be mentioned that these sorbents are based on DVB, unlike the SampliQ OPT sorbents, which is based on polyamide. However, due to the recent introduction of these sorbents, there are very few applications reported of their SPE performance.

Mixed-mode ion-exchange sorbents have attracted much interest in recent years and their excellent properties as well as possible applications encouraged efforts to synthesise new mixed-mode sorbents. Some examples of these new materials are discussed below, and their applications are also summarised in Table 1. The first study on in-house synthesised mixed-mode material applied in SPE was reported by our group [145], where the NVIm-DVB sorbent, originally designed as a hydrophilic sorbent, also showed an ion-exchange feature, since its imidazole group can be protonated depending on pH. Therefore, the NVIm-DVB sorbent was classified as an anion-exchange sorbent and evaluated under RP, WAX, and SAX conditions in the selective extraction of acidic pharmaceuticals, and was further compared to the commercially available Oasis HLB, Oasis WAX, and Oasis MAX. It was demonstrated that NVIm-DVB act as a SAX sorbent providing quantitative recoveries of target analytes, comparable to those obtained with commercially available Oasis MAX.

Later, due to their previous experience with in-house synthesised hypercrosslinked sorbents, our group prepared two weak anion-exchange sorbents [150]. The hypercrosslinked polymers in the form of microspheres were further modified with 1,2-ethylenediamine (HXLPP-WAX-EDA) and piperazine moieties (HXLPP-WAX-piperazine) to impart WAX features. Both HXLPP-WAX sorbents were evaluated successfully and their performance was compared to commercially available WAX sorbents (Oasis WAX and Strata-X-AW). The HXLPP-WAX resins performed better than the macroporous commercial sorbents, due to their selectivity imparted by the amine moieties and stronger RP mechanisms of the HXLPP structures. Then, the sorbents were applied to the selective extractions of acidic analytes from complex environmental samples

(including river and effluent WWTP), providing recoveries of 90-100% for most of target analytes. The HXLPP-WAX-EDA sorbent [58] was also successfully applied in the on-line SPE-LC method to percolate a large volume of different water samples. In all cases, the HXLPP-WAX-EDA resin provided near-total recoveries of the most acidic compounds under study and clean chromatograms.

As mentioned above, the SPE technique is the field where most efforts have been undertaken to develop new sorptive materials to enhance capacity in the extraction of polar compounds, but also to improve selectivity. One of the principal purposes is the development of polymer-based materials that can be readily packed and used as SPE sorbents with enhanced chemical and morphological properties. Although mixed-mode sorbents have been successfully applied for the selective extraction of polar compounds, further improvements in materials to simplify the sample preparation step are still expected.

1.1.1.4. Supported ionic liquid phases

Supported ionic liquid phase (SILP) is a new class of advanced materials, which combine the characteristics of ionic liquids (ILs) and the features of a solid support. The search for new, environmentally-friendly media has drawn attention to ILs due to their interesting and promising properties. ILs form a class of non-molecular solvents that are composed entirely of ions. Typical cations are based on the imidazolium, pyridinium, or phosphonium group, whereas anions are usually inorganic, such as halides, tetrafluoroborate [BF_4^-] or hexafluorophosphate [PF_6^-], but also organic anions, such as trifluoroacetate [CF_3COO^-] or trifluoromethanesulphonate [$CF_3SO_3^-$] are used. Given the numerous combinations of cations and anions, ILs can be tuned to provide the desired chemical and physical properties.

An increasing interest has been paid lately in the applications of ILs in the analytical area [156-162], such as extraction solvents [159], mobile phase additives [160] as well as supported stationary phases in chromatographic separations [161]. Another interesting application of ILs in the analytical field are IL membranes for the selective transport of organic compounds [162].

To take advantage of the excellent chemical and physical properties of ILs, they have been immobilised onto solid support. The properties of SILPs can be modified by the variation of cations and anions. Various kinds of IL-immobilised materials were synthesised and used as LC or GC stationary phase to separate some inorganic ions and organic compounds [163,164].

Primarily, SILPs have been used to improve SPME performance, due to their excellent properties as extracting phases. Therefore, to face the demands of SPME, and to overcome some drawbacks of commercially available fibres [165], ILs were immobilised to be adapted for SPME. The applications of SILPs in SPME will be further discussed in the following section.

The first applications of SILPs in SPE techniques were performed using silica-based SILPs. An example of preparation of IL-modified silica is shown in Figure 4. Firstly, silica was activated with nitric acid/water to increase the content of silanol groups on the silica surface, then the precursor reacted with the excess of 3-chloropropyltriethoxysilane. In the next stage, the chemically-bonded chloropropyl group on the silica surface reacted with 2-ethyl-4-methylimidazole providing SILPs.

For instance, Tian *et al.* [166,167] applied IL-modified silica sorbents as a special sorbent in SPE. The SILP obtained was successfully applied as a special sorbent to isolate bioactive compounds, such as tanshinones [166] or liquiritin and glycyrrhizic acid [167] from natural plants. In comparison to other silica sorbent (C_{18}), IL-modified silica provided better extraction efficiency and showed higher affinity for the analytes.

More recently, Zhou *et al.* [168] reported development of IL-based silicas applied for SPE of astaxanthin from *Saccharina japonica* algae. Another good example of SILP sorbent was presented by Fang *et al.* [169], where IL-functionalised silica were successfully applied for selective SPE of sulphoyl-urea herbicides from environmental water and soil samples. The optimised SPE protocol included cleaning step with water following further elution with methylene chloride/methanol. Concerning the surface water samples, the recoveries obtained for all target analytes, when 250 mL of the sample was percolated through IL-functionalised silica, ranged from 74% to 118%.

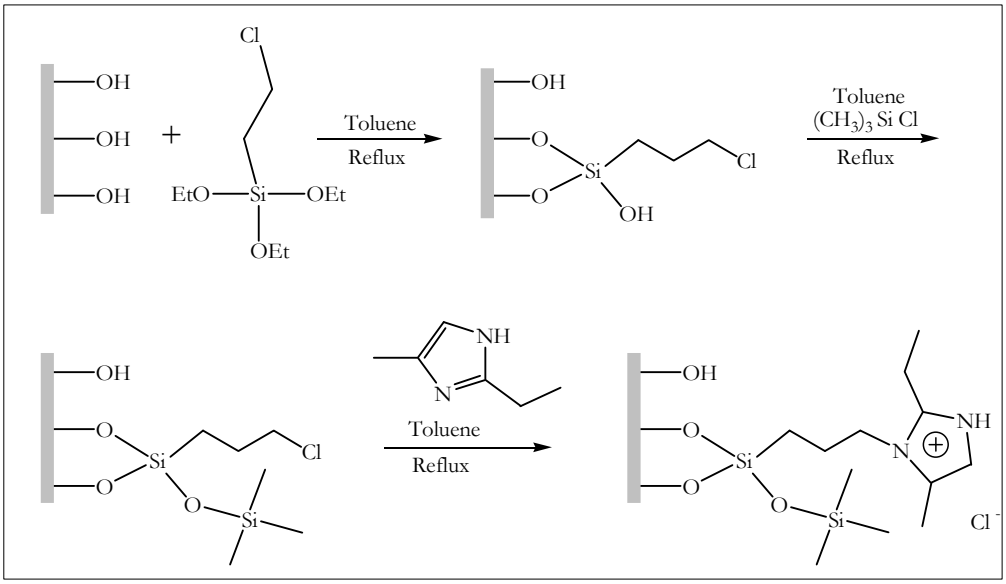

Figure 4. The preparation scheme of ionic liquid-modified silica [167].

In another study, Li *et al.* [170] immobilised IL onto mesoporous SBA-15 adsorbent and applied it for the extraction of α-tocopherol. The results exhibited very high adsorption selectivity for target analyte as well as good reusability of the functionalised IL-modified mesoporous sorbent.

Although in most of applications SILPs are silica-based, there are also a few polymeric SILPs that have been employed in different applications. One of the considerable advantages of using SILPs with polymeric supports lies in the relatively easy functionalisation of ILs onto the polymeric support, making them tunable to provide the desired affinity and capacity towards target analytes.

One of the first application of polymeric SILP, $[MI^+][CF_3COO^-]$, as a selective sorbent for SPE, was reported by our group [171]. The VBC/DVB copolymer, obtained *via* typical suspension polymerisation, was modified in reaction with N-methylimidazole, followed by further substitution of Cl^- groups of the polymer for $[CF_3COO^-]$. In the evaluation of $[MI^+][CF_3COO^-]$ material as a SPE sorbent, it was demonstrated that $[MI^+][CF_3COO^-]$ acted as SAX sorbent, allowing selective and quantitative extraction of a group of acidic target analytes. Even, when 1000 mL of sample, such as tap or river water was preconcentrated, following an efficient washing step that eliminated basic analytes and

interferences, there was a significant increase in selectivity, exhibiting high recoveries of acidic analytes. In addition, the method delivered a clean chromatogram and the results obtained using SILPs were comparable to those obtained with the commercially available mixed-mode polymeric Oasis MAX.

More recently, Bi *et al.* [172] reported the application of amino-imidazolium polymer in the SPE process to isolate matrine and oxymatrine from *Sophora Flavescens* plants. The proposed method showed a higher affinity for the target analytes and was successfully applied for their extraction. Comparing different SPE cartridges (blank polymer, C_{18} and NH_2 sorbents), it was found that IL-based polymers sorbent exhibited higher selectivity than other sorbents. Subsequently, the same group [173] developed another IL-modified polymeric sorbent that was employed for isolation of caffeine and theophylline from green tea.

As mentioned above, SILPs have been applied as an SPE sorbent to enrich or isolate analytes from complex biological or environmental samples. However, there are relatively few reports on the application of IL-modified polymers as a selective sorbent in SPE. Hence, further investigation on the improvement of selectivity in sorptive materials for extraction of polar analytes should be carried out in the future.

1.1.1.5. Other materials for SPE

Apart from aforementioned sorbents, to meet demand for improved selectivity and capacity, the innovations in material science for SPE have paved the way for many materials developed in recent years, both polymeric and non-polymeric. Many of these sorbents have been applied for the extraction of a variety of compounds from different matrices. The following section is an attempt to describe briefly these materials and their possible applications. To that end, firstly the selective well-established materials will be discussed, and later, the newly-developed materials and their applications will be presented.

Restricted access materials

Restricted access materials (RAMs) have been designed for the isolation of low molecular mass analytes, mainly drugs, directly from biological matrices with

minimal sample pretreatment [174,175]. RAMs represents a group of "semi-selective" sorbents that are based on the limited access of analytes to the sorbent rather than selective analyte-sorbent interactions. The RAMs allow the separation of analytes through a combination of size exclusion and hydrophobic or ion-exchange interactions [176]. They work in such a way that macromolecules are excluded from accessing those sites of the sorbent where retention takes place. Thus only small particles are able to penetrate into the pores and interact with the sorbent. The exclusion of macromolecules can occur as a result of a chemical diffusion barrier created by a polymer (or protein) network covering the exterior surface of the support or with a physical barrier based on the pore diameter.

RAMs have been applied, mainly as a clean-up step, in the extraction of drugs in biological matrices (plasma, saliva, urine or hair) [174-178] and the purification of plasmid DNA [178]. There have also been a few applications in environmental analysis, such as wastewater and soil [179]. More recently, RAMs were applied for the determination of contaminants in food samples (e.g. honey, wines, fruits and vegetables) [174,180]. Currently, RAMs are mainly used as clean-up materials, prior to the determination of target analytes.

Immunosorbents

Another class of selective SPE sorbents is immunosorbents (ISs). They are efficient materials due to their high selectivity, which allows extraction, concentration and clean-up from complex matrices in a single step [181]. ISs are based on reversible and selective antigen-antibody interactions. They are obtained by covalently bonding the desired antibody onto a suitable sorbent [182]. The antibody is immobilised onto a solid support and used as an affinity ligand to extract the target analyte and structurally-similar compounds from liquid samples.

ISs have been widely used for sample enrichment from biological fluids and tissues [183,184]. ISs have been also employed in environmental analysis providing cost-effective and fast analytical methodology for the determination of estrone in complex water samples at low ng L^{-1} concentration levels, with a minimum sample preparation [185]. Although ISs have high selectivity, their

main drawbacks are instability (in most cases), the use of biological material, the strict conditions required for their appropriate use, the limited number of applications in which IS can be reused, and the high cost. In order to overcome these disadvantages molecular recognition has been used for the synthesis of new polymers.

Molecularly imprinted polymers

In order to maintain the high selectivity provided by ISs and simultaneously overcome their limitations, a trend for using molecularly imprinted polymers (MIPs) appeared in the mid-1990s. MIPs are highly crosslinked polymeric materials, which are based on molecular recognition elements engineered to bind one target analyte or a group of structurally-related analytes with high selectivity [186-188]. The choice of the template molecule and the selection of monomers are the most important factors in the development of an MIP [186]. The synthesis of the MIPs offers three different approaches: covalent, noncovalent and semi-covalent [189]. In the covalent approach [186], reversible covalent bonds between the template and monomers are formed before polymerisation and then the template is removed from the polymer matrix by the cleavage of covalent bonds, which should be re-formed upon rebinding of the target analyte. However, the problem of designing a suitable template-monomer complex, where covalent bond formation and cleavage will be easily reversible, makes this approach quite limited. Of the three approaches, the non-covalent approach is by far the most commonly used for the synthesis of MIPs [186,189]. This approach is based on the formation of relatively weak non-covalent interactions, such as hydrogen bonding or ionic interactions, between template molecule and selected monomers prior to the polymerisation. Subsequently, the rebinding step of the template to the MIP is achieved through non-covalent interactions. The semi-covalent approach is a kind of midway alternative, where the template is covalently bound to a functional monomer, but the rebinding step is based on non-covalent interactions [189].

Molecularly imprinted solid-phase extraction (MISPE) has been widely applied as a selective sorption technique in environmental, biological and food analysis [186-188]. One of the main advantages of MIPs is their effective sample clean-up abilities, which aim to eliminate all the compounds retained by non-selective

interactions, while retaining the target analytes onto the sorbent. This purpose can be accomplished by the careful selection of a suitable solvent to break down all the non-selective interactions. Some examples of the application MIPs for the extraction of analytes from environmental and biological samples are presented here. For instance, Beltran *et al.* [190] reported the synthesis of the MIP and its application to SPE for the extraction of carbamazepine. The optimal conditions involved the conditioning of the cartridge, loading the sample under basic aqueous conditions, clean-up using ACN and elution with MeOH, which provided recoveries of 80% analysing 100 mL of effluent wastewater. In further studies, other in-house prepared MIPs were used for extraction of cephalexin [191] and parabens [192] from environmental water matrices. In another study, Demeestere *et al.* [193] reported that MIPs were applied in the analysis of antidepressants in environmental waters. They presented extraction recovery levels greater than 70%, similar to those obtained using HLB sorbents. These results showed that some analytes, such as paroxetine, fluoxetine, and citalopram, had a high tendency to be retained on the MIP (%R > 68%. Different MIPs were also prepared and applied for extraction of triazine pesticides such as propazine [194], among others. Regarding the biological analysis, a number of various applications of MISPE have been tested for different samples, such as human plasma [195,196] and urine [197]. For example, Dzygiel *et al.* [195] evaluated MIPs as selective SPE sorbents for the determination of sildenafil and related compounds in plasma. In another study, Caro *et al.* [197] developed and applied MIP as a selective sorbent in a two-step SPE method for the extraction of fluorinated quinolones from human urine and tissue samples. The samples were previously percolated through an Oasis HLB sorbent and subsequently passed through the MIP in order to reduce matrix interferences. The developed method provided cleaner extracts, suppressing the interfering peaks arising from the complex biological matrices. MISPE has been also used for the extraction of amoxicillin [198] and barbiturates [199] from urine, or ketamine [200] and diazepam [201] from hair samples.

Although most of researchers synthesise their own MIPs at laboratory scale, several MIPs are commercially available. At least two companies, including Aspira Biosystems, Inc. (San Francisco, California, USA) and MIP Technologies AB/Biotage (Uppsala, Sweden), currently produce MIPs for various uses.

However, they are intended for the extraction a class of compounds rather than being analyte-specific. Currently, in analytical applications, the most widely used are Affinilute MIPs (Biotage) and SupelMIPs (MIP Technologies AB/Supelco). Among the range of SPE phases currently available, there are sorbents optimised for nonsteroidal anti-inflammatories, β-agonists, β-blockers, polycyclic aromatic hydrocarbons (PAHs), nitrosamines, triazines, antibiotics and others. For instance, Zorita *et al.* [202] used commercially available Supel MIP NSAIDs to enrich and clean up anti-inflammatories and clofibric acid from sewage water, achieving recoveries for all compounds in all matrices ranging between 84% and 116%, when preconcentrating 25 mL of the sample. In addition, no matrix effects were observed when these types of samples were analysed.

The main feature of MISPE is that, being highly selective in nature, detection and quantification can be performed with simple analytical techniques such as LC-UV as well as more complex systems such as LC-MS, or even LC-MS/MS. In some studies, MISPE procedure allowed very effective clean-up and the complete removal of matrix components when dealing with relatively dirty samples such as wastewater, and therefore a significant reduction in matrix effects. For instance, Sun *et al.* [203] reported no matrix effects when these types of samples were analysed.

Carbon nanotubes

Apart from the previously mentioned SPE materials, other non-polymeric materials have also been applied as SPE sorbents in recent years. The first class is represented by carbon nanotubes (CNTs) and carbon nanofibres (CNFs) that have been introduced as a novel SPE material. CNTs is a nanomaterial with great characteristics such as higher specific surface area (100-3000 $m^2 g^{-1}$), significantly high mechanical strength and chemical stability as well as electrical and thermal conductivities [64,204].

The structural characteristics and wide range of properties of CNTs let them interact strongly with organic molecules, *via* non-covalent forces, such as hydrogen bonding, electrostatic forces, π-π stacking and hydrophobic interactions [205]. The surface, made up of a carbon atom hexagonal network in graphene sheets, interacts especially with the benzene rings of aromatic analytes.

A hollow and layered nanosized structure and possible interactions make CNTs a good candidate for application as an SPE sorbent. CNTs have proved to be an efficient sorptive material for conventional SPE. The use of CNTs as SPE sorbents has been reported in numerous reviews [204-207]. CNTs and CNFs were successfully applied for the extraction of a wide range of organic and inorganic analytes from different complex samples [205-208]. For instance, multi-walled CNTs used as packed materials for sensitive determination of pesticides in environmental water samples provided good recoveries [208].

Although CNTs presented great potential as SPE sorbents, most applications have been performed on hydrophobic compounds. There are only a few applications of CNTs in the extraction of polar analytes. For instance, Niu *et al.* [209] applied single and multi-walled CNTs for the SPE of several highly polar compounds, such as cephalosporin antibiotics, sulphonamides and phenolic compounds. It was found that the analytes were strongly retained by CNTs, exhibiting stronger retention than graphitised carbon blacks (GCBs), especially for cephalosporin's and sulphonamide antibiotics. However, the phenolic compounds were better retained by GCBs. The majority of these applications have been developed in laboratory-made cartridges, since there are only very few commercialised CNTs available. From a detailed revision of recent literature, it can be seen that CNTs are considered to be excellent material for extraction. However, it has been found that it is highly necessary to dry CNTs at least at 80-120 ºC for a few hours [210] prior to their use as SPE sorbents. With respect to reuse, only some studies reported this issue [208], stating that the cartridge can be reused up to 200 times without efficiency loss and that good reproducibility between cartridges is achieved, which is comparable to other polymeric sorbents.

Electrospun nanofibers

Based on the theory that the large surface areas of nanocomposites promote interaction between nanocomposites and analytes, in recent years, attention has been paid to the applicability of fibres with microscale structures as sorbents [211]. Similarly, materials obtained using electrospinning can easily produce fibres with nanoscale fibrous structures. Moreover, electrospinning has the ability to control the diameter, morphology, and spatial alignment of electrospun nanofibers [212]. Thus, electrospun nanofibres, which have larger specific

surface area than the microfibres, present analytical capability as an effective SPE material. Recently, electrospun nanofibers [213] were successfully employed as SPE sorbents for the preconcentration of several trace pollutants in environmental water samples (2 mL) providing recoveries in the range of 87% to 93% for most aromatic hydrocarbons studied, except for benzene (only 40%). Although the authors claimed that this kind of material provide promising results, several questions relating to the sorptive capacity and ability to extract polar compounds still need to be answered. Electrospun nanofibres were also applied in the extraction of trazodone [214] in plasma samples (0.1 mL) providing extraction recoveries of 58% to 75%. The effective interaction of the sample with the nanofibre sorbents further enabled miniaturisation in a nanoscale sample-preconcentration device and simplicity of sample preparation.

Aptamers and oligosorbents

Aptamers represent another trend in materials for sample preparation which was first reported in 1990 [215,216]. Aptamers are oligonucleotides (DNA or RNA) that contain a specific sequence able to bind target molecules, such as drugs, proteins or other organic compounds, with the same specificity and affinity as antibodies. Aptamers are generated through the systematic evolution of ligands by exponential enrichment (SELEX), which is an in vitro selection process that allows the identification of unique DNA/RNA from large populations of random sequence oligomers [217]. Generally, the affinities of aptamers for hydrophobic compounds are lower than that of hydrophilic molecules. Aptamers have been applied for the selective isolation of drugs (e.g. ibuprofen) [218]. However, the use of aptamers for SPE is still in its infancy.

Selective supports called oligosorbents (OS) have been recently developed using aptamers immobilised onto a solid support. Different supports, such as sepharose, silica or agarose with cyanogen bromide-activated (CNBr), thiol, glutaraldehyde or streptavidin, generating desired properties can be used to obtain a stable and efficient binding of aptamers [219].

The supports used for the preparation of OS should be hydrophilic to avoid the non-specific retention of unwanted analytes during the percolation of liquid samples containing target analyte. In addition, they must contain reactive

functional groups suitable for the terminal group of the aptamer that allow efficient and oriented immobilisation, as well as guaranteeing a strong retention of the target analyte. OS based on aptamers was applied for the determination of drugs of abuse from biological fluids [219]. The use of these selective SPE sorbents for extraction of cocaine from blood provided better purification than a protein precipitation treatment widely used for blood analysis. The samples were directly loaded onto the sorbent and the eluted fractions were analysed by LC without any additional treatment. The high selectivity of the aptamers allowed the efficient suppression of interfering compounds. They were also applied for SPE of mycotoxins in oenological analysis [220], demonstrating selective clean-up, high extraction recoveries (~100%) and high affinity of the aptamer for the target analyte. This approach seems to be a promising alternative to IS (higher stability) and MIPs, especially for expensive analytes, since its production requires lower amounts of the target molecule.

Admicelles and hemimicelles

Admicelles and hemimicelles are classes of materials introduced to analytical science in the last decade [221,222]. These supramolecular sorbents are prepared by the adsorption of ionic surfactants on the surface of mineral oxides. Admicelles are roughly spherical aggregates formed by adsorption of an additional surfactant on hemimicelles through hydrocarbon chain interactions.

An attractive feature of hemimicelle/admicelle-based SPE is its versatility. In respect of the amphiphilic nature of surfactants, admicelles and hemimicelles have regions of disparate polarity that allow for the solubilisation of solutes with different characteristics. Therefore, surfactant hydrocarbon chains solubilise hydrophobic contaminants. Surfactant polar groups may retain solutes *via* hydrogen bonding, π-cation or electrostatic interactions, while amphiphilic analytes will be strongly retained through the formation of mixed aggregates [221,223]. Hemimicelle/admicelle-based sorbents have significant advantages, such as high extraction efficiencies, easy elution of analytes or high breakthrough volume and they have been recently proposed for the preconcentration of a variety of pollutants with a wide range of polarities from complex environmental matrices [223-228].

Analytical applications of hemimicelle/admicelle-based sorbents in SPE have recently been reviewed [222,224]. Hemimicelles and admicelles have proved to be suitable for the extraction of carcinogenic PAHs [223], chlorophenols [225], oestrogens [226] or perfluorinated compounds [227] from wastewater, surface and underground water. For instance, the results obtained, when 350 mL of surface water was preconcentrated by magnetic SPE using magnetic nanoparticles coated with hemimicelles of alkyl carboxylates, provided recoveries of target analytes ranging from 85% to 94% [223]. Moreover, no clean-up of the extracts or solvent evaporation was required, and the sorbent proved to be compatible with the presence of stabilising agents for preservation of the analytes during storage of samples. Admicellar sorbents were also applied for extraction of pesticide multiresidues from natural waters [228] providing quantitative recoveries for most of the pesticides tested. Most of the applications developed have mainly used sodium dodecyl sulphate adsorbed on alumina as a sorbent, but other approaches have also been investigated. For example, Lunar *et al.* [229] used cetyltrimethyl ammonium bromide (CTBA) and cetylpyridinium chloride adsorbed onto silica as sorbents for SPE of linear alkylbenzene sulphonate homologues, exhibiting recoveries of analytes from wastewater samples ranging between 86% and 110%, using sample volumes between 10 mL and 25 mL. In another study, Sun *et al.* [230] used magnetic mixed hemimicelles SPE for the preconcentration of several sulphonamides from environmental water samples, reporting satisfactory recoveries ranged from 70% to 102%, enriching 500 mL of the sample. More recently, Zhu *et al.* [231] developed mixed hemimicelles SPE based on CTBA-coated Fe_3O_4/SiO_2 core-shell nanoparticles, for the determination of herbal bioactive constituents from biological samples, achieving recoveries higher than 93%. In comparison to the classical protein precipitation method, the amount of the analytes found in the biological samples using mixed hemimicelles SPE was definitely superior.

Although the wide variety of available sorbents allows the clean-up, retention and preconcentration of analytes with a broad range of physico-chemical properties and polarities, the SPE process strongly depends on the careful choice of extraction conditions. Several crucial variables in SPE optimisation will be discussed in the following section.

1.1.2. Parameters affecting SPE

As mentioned previously, the selection of a suitable SPE sorbent is crucial for the efficient recovery of analytes from the sample. The wide spectrum of available materials for SPE permits the clean-up of interferences, retention and preconcentration of analytes with different polarities and physico-chemical properties.

SPE is a flexible process whose optimisation should take into account different factors that can influence its efficiency. A fundamental understanding of this process leads to better strategies for optimisation of the procedure. The selection of the sorbent is the key point in SPE because it can control parameters such as selectivity and capacity. This selection depends strongly on the target analytes and their interactions of the selected sorbent and the analytes that directly influence the extraction efficiency. The sorbent selected should ideally be porous and allow sufficient interaction between analytes and active groups. Furthermore, ideal SPE sorbents should be selective towards the target analytes, have large surface areas, exhibit good surface contact with the sample and maintain stability in the sample matrix and the solvents used. Apart from extraction mechanism and a specific sorbent chemistry, the selection of the sorbent mass also affects SPE procedure. This factor is extremely important, since an insufficient amount of sorbent leads to column overload and poor or irreproducible recovery values, while an excess of sorbent multiplies solvent requirements and might also decrease recovery. Therefore, the suitable sorbent mass should deliver a sufficient capacity to retain the analytes and other compounds that can also be retained during the loading [232].

Depending on the target analytes, selected sorbent and sample matrix, SPE procedures can vary a great deal, from simple loading and elution to more complicated work-up routines, such as selective extraction with a clean-up step. Therefore, several parameters that affect extraction process including loading conditions, such as sample volume, pH, ionic strength as well as solvents for both the washing and elution steps, should be optimised.

One of the principle factors that influences the extraction efficiency of analytes is the sample pH. The pH of the sample is important for weakly acid or basic compounds and depends on their pK_a. The pH of the sample plays a critical role

in the SPE procedure, because its value determines the existing state of analytes and thus influences the extraction efficiency of the target analytes [53]. Depending on sample pH, the analytes can take the neutral or charged form, and consequently the way that they are retained. For example, Stafiej *et al.* [96] found that recoveries of a group of anti-inflammatory drugs and oestrogens, extracted at low pH from water samples using different sorbents (silica-based, PS-DVB and Strata-X), were significantly higher than recoveries obtained at neutral pH (where the analytes were in the ionic form). Similarly, Weigel *et al.* [109] reported that an increase in sample pH using Oasis HLB led to a reduction in the extraction efficiency of acidic analytes, such as ibuprofen. Furthermore, to retain the analytes by ion-exchange from aqueous solution, the pH of the sample matrix must be tuned in such a way that both the analytes and the functional group on the sorbent are charged. Negatively charged analytes are extracted using mixed-mode sorbents, such as SAX or WAX, analogically positively charged analytes can be extracted on SCX or WCX materials [233]. For instance, when the WAX mixed-mode sorbent was used for selective extraction of acidic pharmaceuticals [150], the loading step was fixed at pH 7, in order to ensure protonation of the WAX sorbent and compounds with $pK_a > 5$ and, at the same time, deprotonation of the most acidic compounds of the group $pK_a < 5$, in order to enhance the retention of these analytes on the sorbent through ion-exchange interactions.

The ionic strength of the sample also influences the SPE of polar compounds. When extracting compounds which are relatively soluble in water, the addition of salts is often used in order to increase extraction efficiency. Addition of salts, such as NaCl, may also increase retention of polar analytes due to the balance between the analyte and the sorbent [234]. However, currently, this extra step can be avoided by the selection of a suitable sorbent or careful optimisation of SPE procedure.

Another approach that can influence the extraction process is the addition of an organic solvent to the sample in order to avoid wall effect. However, this approach is only used for very specific applications.

Regarding sample volume, this factor obviously depends on the analytes, the sample matrix, as well as the SPE mode, size and the format of SPE devices that

will be employed. For instance, an SPE disk is recommended for larger volumes of environmental samples containing high amounts of particulates, or when a high flow rate is required during sample loading. In contrast, 96-well plates will be suitable for small volumes of biological samples. With respect to the sample volume depending on SPE mode, off-line SPE of target analytes from environmental water samples typically involves the concentration of samples ranging in volume typically from 50 mL to 1000 mL [132,145,152], while in on-line mode samples, volume is significantly lower, ranging from 5 mL or even less to 300 mL [11]. When selecting the sample volume it should be ensured that their volume is lower than the breakthrough volume of the analytes. The breakthrough volume is an important parameter in determining the suitability of an SPE procedure for preconcentrating the target analytes. The breakthrough volume is defined as the maximum volume of sample that can be loaded onto an SPE bed, providing a given ratio of outlet to inlet analyte concentration without significant losses of analytes for elution. It is different for each analyte and depends mainly on the analyte's polarity and on the amount and affinity for a particular type of sorbent. In order to extract large volumes of sample and achieve lower detection limits, the breakthrough volume under experimental conditions should be as high as possible [52].

Regarding the sample complexity, preconcentration of analytes from biological and environmental matrices often provides wide range of interferences. Generally speaking, when considering and choosing the sample clean-up, there are two strategies for isolating the target analytes. In the first, matrix interferences are retained on the sorbent, while target analytes pass through the cartridge unretained. This approach is typically used when the desired analytes are present in high concentration. In the second approach, the analytes of interest are retained while matrix interferences pass through the cartridge unretained or, if retained, they can be removed with simple washing. This strategy is especially useful when target analytes are present at low levels, or analytes with different polarities need to be isolated. The clean-up step should be suitable for the particular sorbent used for application. This step is usually performed using mixed-mode sorbents or MIPs. In addition, the selectivity of the extraction can be increased by choosing a suitable washing step. This step usually includes rinsing with a pure organic solvent, such as MeOH or ACN among others, which

are able to disrupt all the RP interactions between sorbent and non-protonated compounds. The best approach towards using SPE is to search for a solvent mixture that will wash the interferences from the sorbent without the loss of target analytes [11]. For instance, when the sample is loaded on the MIP sorbent in a low polarity solvent, a selective loading step can be performed, in which only the target analyte is selectively retained on the MIP while the sample matrix is left behind [235]. However, if the target analyte is presented in an aqueous medium, the analyte and other interfering compounds can be retained non-specifically on the polymer. Consequently, to achieve a selective extraction, a clean-up step with an organic solvent, such as a low-polarity organic solvent (e.g. dichloromethane, toluene or chloroform), is introduced prior to the elution step. The clean-up solvent should minimise the non-specific interactions without disrupting the selective interactions between the MIP and the analyte of interest. As an example, Chapuis *et al.* [196] reported a biological application that dealt with the extraction of α-blockers from plasma and soil samples using a alfuzosin-imprinted polymer. An aliquot of 500 μL of plasma was percolated and, after a clean-up step with dichloromethane, the matrix components were removed whereas the target analyte was selectively retained and eluted using MeOH/CH_3COOH (95/5, v/v) mixture with recoveries close to 100%. Also bearing in mind that the pH of the washing step may greatly affect the clean-up step and recovery, the pK_a values of analytes and sorbent should be taken into account. As an example, in the study reported by Strahm *et al.* [236], the glucuronide and sulphate forms of steroids were selectively extracted from urine in the two steps, using Oasis WAX. After sample loading, the cartridge was first washed using 4 mL of 10% HCOOH in MeOH/H_2O (95/5, v/v) to neutralise the glucuronides and wash them out at low pH, retaining target analytes onto the sorbent.

Some other important parameters that directly affect the recoveries of the target analytes are the type and volume of the elution solvent. The correct choice of elution solvents and sequence of their application to the sorbent is dependent on the target analytes to be eluted from the cartridges and the elution strength of the solvent. The elution should be performed with solvent strong enough to allow complete elution of target analytes in a relatively small volume. The selection of elution solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups. Whatever solvent is

employed, it must demonstrate adequate performance for the target analytes, at the levels of interest. The elution is usually performed with an organic solvent or a mixture of two or more solvents in combination with a pH change to disrupt binding [52,234]. MeOH, ethyl acetate, ACN, which have different elution strengths and polarities, are most commonly used [14,53]. However, the basic or acidic solutions in MeOH, ACN or H_2O, can also be used for the elution in mixed-mode sorbents applications. For instance, Baker *et al.* [237], for the extraction of drugs of abuse from wastewater and surface water retained by Oasis MCX, used 0.6% HCOOH in MeOH to wash out interferences retained by RP, followed by elution with 7% NH_4OH in MeOH, achieving high recoveries for most target analytes. In the previously mentioned study [236], when extracting glucuronide and sulphate steroids from urine, to elute completely sulphate conjugates from Oasis WAX sorbent after the previous washing step, the authors used 5% NH_4OH in MeOH/H_2O (90/10, v/v). The increase in pH allowed the neutralisation of the piperazine-anion-exchange moiety of the sorbent and the elution of the sulphate conjugates, providing clean extract suitable for further LC-(ESI)MS/MS analysis. Moreover, the elution solvent sequence and composition may be varied to achieve the best analytical data when seeking to isolate the analytes or clean the column of interferences.

In order to reduce matrix interferences, some authors suggest the addition of certain quantity of a chemical reagent, such as sodium sulphite, which through different mechanisms transforms interferences and prevent its appearance in chromatograms [89,238]. In the case of complex matrices, such as biological samples or influent wastewater, a preliminary sample treatment is recommended before the SPE process, such as a deproteinisation of biological samples or simple filtration to avoid clogging problems [239]. With particularly complex samples, an additional clean-up step such as a simple wash and back-extractions can be used to render the sample clean enough to reach satisfactory results [14].

During the SPE process, the flow rate is also an important factor that should be considered, since it not only affects the recovery of the analytes, but also controls the analysis time. The adjustment of flow rate depends on the format used as well as the SPE step. Regarding sample loading in off-line mode, most authors used the typical flow rate, ranging from 3 to 10 mL min^{-1}, whereas

during the washing and elution step, the flow rate should be slower, to allow the effective elution of the target analytes.

As stated above, several factors have an influence on the SPE process, so in order to provide quantitative results, the SPE conditions should be carefully selected. SPE is a well-established and routinely used technique and the use of suitable SPE media as well as the lower number of sample preparation steps is also particularly advantageous. The trends in sample preparation have also introduced other microextraction techniques, such as SPME and SBSE, which are good alternatives to SPE. Application of polymeric materials in these techniques will be discussed in following sections.

1.2. Solid-phase microextraction

Solid-phase microextraction (SPME) was introduced by Pawliszyn as a solvent-free and time-efficient sampling technique which integrated extraction, preconcentration and sample introduction into one step [240]. The development of SPME, mainly when coupled to GC, has addressed the need for the simplification of the sample preparation process, both in laboratory and on-site, where the investigation is located [241]. SPME reduced significantly basic disadvantages of the sample preparation step in LLE or SPE techniques, such as long sample preparation time, multi-step procedures or even the consumption of toxic solvents [19].

SPME is based on the partition equilibrium of the analyte between the samples and a sorbent. The idea of SPME is to use a small amount of extracting phase (e.g. coated fused-silica fibre) dispersed on a solid support, which is placed in contact with the sample for a certain time. After the extraction step, the fibres are transferred (with a syringe-like handling device) to the analytical instrument, for separation and quantification of the analytes. SPME devices are available either in fibre or capillary column form. However, the fibres are most commonly used. SPME has been used in a wide range of applications including environmental, clinical, forensic and food analysis. The details of SPME and its application are summarised in a number of well-documented reviews and books [18,21,25]. SPME has been routinely used in combination with thermal desorption (TD) and GC for analysis volatile and semivolatile compounds [22,23], but is also widely used in combination with liquid desorption and LC to determine weakly volatile or thermally labile compounds [242]. Among other uses, SPME was also successfully coupled with CE [243,244] in pesticide and pharmaceutical analysis.

Three basic modes of SPME sampling include direct immersion (DI), headspace extraction (HS-SPME) and membrane protected extraction, and can be applied to different kinds of samples (aqueous or gaseous). The extraction modes are illustrated in Figure 5. In direct immersion DI, the coated fibre is immersed in the sample and the analytes are transferred directly from the matrix to the extracting phase. In headspace (HS) extraction, the analytes are transported from the gas phase equilibrated with the sample. The main reason for this modification is to protect the fibre coating from damage by high molecular mass

or others [19,21,245]. In the extraction with membrane protection, the fibre is separated from the sample with a selective membrane, which allows the analytes to pass through while interferences of high molecular weight are blocked. In the membrane protected mode, the principal purpose is to protect the fibre against adverse effects caused by high molecular weight compounds from dirty samples In addition, an HS trapping membrane protection was found to be very useful for determination of analytes with volatility too low for the headspace approach [241].

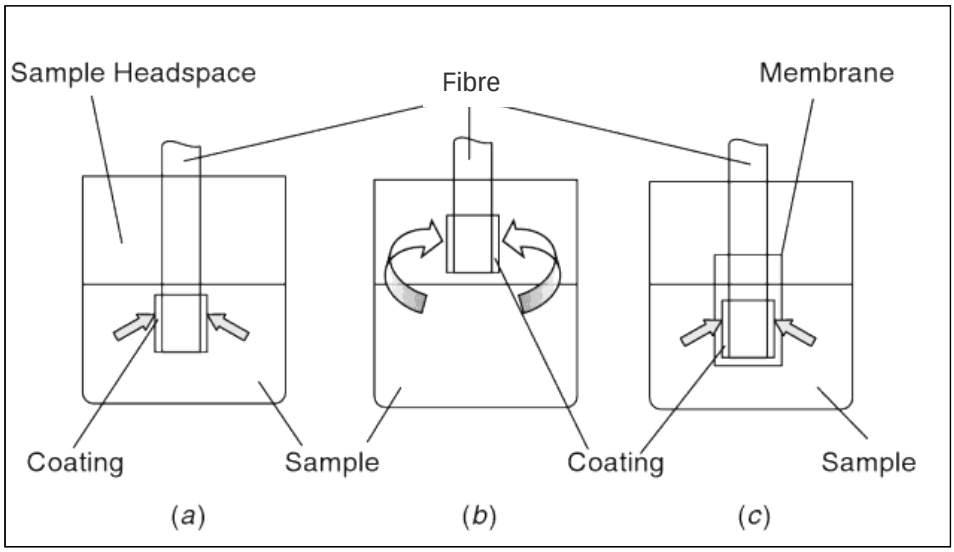

Figure 5. Mode of SPME operations: (a) DI-SPME, (b) HS-SPME, (c) membrane-protected SPME [241].

Although SPME fibre is commonly used, there is also another format available, namely in-tube SPME.

In-tube SPME uses an open tubular capillary column, into which sample is introduced by the flow (dynamic in-tube SPME) or by repeated draw/eject cycles of the sample solution (static in-tube SPME). In-tube SPME is usually employed in combination with LC. Using in-tube SPME, organic compounds in aqueous samples are directly extracted and concentrated into the stationary phase of the capillary column and then desorbed by mobile phase flow or static desorption solvent. Although fibre and in-tube SPME techniques share a similar

theoretical background, the basic difference between them is that the extraction of compounds is carried out on the exterior of the fibre and on the interior of the capillary column, respectively.

The SPME process, similarly to SPE, can be influenced by different variables. Most of the factors affecting sorptive extraction techniques have been discussed in previous sections. Therefore, here other parameters that have impact on the extraction efficiency of SPME will be described.

One of the most important parameters that influences the SPME process is the desorption procedure and this has to be investigated for target analytes, as one of the first steps in SPME method development. Regarding the desorption step, desorption of the extract from the SPME fibre may be done by either heating or back extraction with a small volume of an organic solvent or mixture of solvents. In SPME-GC, the fibre is introduced into the injector port and analytes are thermally desorbed from the coating, whereas in SPME-LC, desorption is performed in an appropriate interface, which enables the mobile phase to elute the analytes retained on the fibre [18,246].

Similarly to SPE, the adjustment of the pH of the sample improves the recovery of the method for target analytes. This is related to the fact that most of SPME coatings extract better the non-ionic species. By careful tailoring of the pH, acidic and basic analytes can be converted to their neutral forms and extracted by the SPME fibre.

The sample volume should be selected based on the estimated distribution coefficient. The distribution coefficient may also be calculated or determined experimentally by equilibrating the sample with the fibre and measuring the amount of analyte extracted by the coating [18].

The other factors investigated are the addition of an inert salt to the sample, extraction time, agitation and temperature. The addition of a salt to a liquid matrix helps to promote the transfer of analytes to the HS. The addition of a salt, usually NaCl or Na_2SO_4, is an especially important parameter in the extraction of polar analytes [18]. For instance, for polar analytes, such as phenolic compounds

[247,248] or pharmaceuticals [249], the sensitivity can be significantly increased. This addition of a salt usually increases the ionic strength of the sample and decreases the solubility of analytes which are more easily retained. However, this effect also depends on the polarity of the analytes, the salt concentration, and the sample matrix and the fibre coating.

Due to the fact that SPME is based on an equilibrium distribution process, the maximum amount of analyte will be extracted when the equilibrium is reached [18]. Numerous studies have shown that analytes with low distribution constants have long equilibration times [18]. Therefore non-equilibrium conditions are usually selected for their extraction with significantly shorter extraction time [248,249]. However, in these cases, the exposure time must be carefully controlled to ensure reproducible results. The typical extraction time in SPME technique ranges from 15 to 60 minutes [250-252]. Moreover, the agitation of sample improves the extraction and reduces the time required to reach the equilibrium. In the case of aqueous samples, agitation is often required in order to facilitate mass transport between the aqueous sample and the fibre. To accelerate the extraction process in manual SPME experiments, magnetic stirring is frequently used. However, attention should be paid to control the rotational speed of the stirring bar and to ensure that the base plate does not change temperature during agitation.

Temperature also has an important influence on the extraction equilibrium. If the temperature is elevated, two contrary effects on the SPME process can be noted. Primarily, an increase in temperature during extraction enhances the diffusion of analytes towards the fibre, so the time needed to reach the equilibrium is lower. Secondly, due to the decrease of the distribution constant, the amount of extracted analytes may be lower. In the HS-SPME-mode, heating of the sample and cooling of the fibre can increase analyte concentration at equilibrium [241]. High temperatures are expected to release more analytes into the HS allowing better retention as the temperature increases due to the improved mass transfer. Moreover, in the HS-SPME sampling mode, the temperature helps transfer analytes to the HS. In the analysis of very volatile components, analyte equilibrium concentration in the HS can be increased by heating the sample and by cooling the fibre [246]. In contrast, in the case of DI-

SPME, the influence of temperature is rather negligible. Most authors, when using commercially available fibres, performed the extraction process at 50-80 ºC [252,253]. For instance, in one study [253], a decrease in extraction efficiency was observed with an increase of temperature from 25 ºC to 65 ºC or above, with a subsequent decrease related to the lower sorption distribution coefficient at higher temperatures (about 70 ºC). A temperature of 65 ºC was finally selected as optimal for the determination of alkylphenols using poly(acrylate) (PA) fibre. In the case of in-house prepared fibres, most of the studies were performed at 20-50 ºC [254-256].

An essential aspect of SPME procedure is the choice of sorptive material, in such a way that it should have a strong affinity for target analytes. The range and choice of fibres available for sorption is ever-increasing. The first commercially available SPME fibres were polydimethylsiloxane (PDMS) and PA. PDMS presents high affinity for the extraction of non-polar compounds due to its apolar character. In contrast, PA is more suitable for extracting polar compounds thanks to its polar character. However, it is well known that both phases, due to their linear structure (low specific surface area), present limited retention when polar compounds are extracted. Other commercially available coatings are DVB, carboxen (CAR), carbowax (CW) and recently commercialised, suitable for the extraction of polar analytes, polyethylene glycol (PEG) fibre. Also, blends of phases such as PDMS/DVB, PDMS/CAR, CW/DVB, DVB/CAR/PDMS and CW/templated resins (CW/TPR) have been used to combine different sorption processes. All these materials with different fibre thicknesses (7-100 μm) and assemblies are commercialised by Supelco. All of them have been widely used for the extraction of a wide range of compounds with different polarities from variety of samples. For instance, PDMS/CAR fibre was successfully applied for the determination of VOCs traces in indoor air [257], while CW/DVB fibre was employed to determine SVOCs in groundwater and treated drinking water [258]. In turn, among commercially available SPME fibres designed for the extraction of polar compounds, PA and PDMS/DVB phases were frequently applied due to their higher affinity for phenolic groups. As an example, Pan *et al.* [253] reported SPME procedure using PA fibre for the determination of alkylphenols in water samples providing good extraction efficiencies, however the analytes were derivatised prior to GC analysis to improve

the quality and sensitivity of separations.

Recently, other triple-phase DVB/CAR/PDMS fibre has been widely used in HS-SPME for the determination of N-nitrosoamines [252] and pesticides [259] from environmental water samples, providing high selectivity and sensitivity. In SPME of N-nitrosoamines, Llop *et al.* [252] compared the HS-SPME performance of DVB/CAR/PDMS fibre to CAR/PDMS and PEG fibres, where it was found that DVB/CAR/PDMS exhibited superior performance than those obtained with CAR/PDMS and PEG.

Although the variety of fibres available enables good SPME performance, an extra step in the analytical procedure is often required for the determination of more polar analytes. The most simple and cost-effective way is derivatisation, which is straightforward and can be readily automated. Derivatisation is usually performed by substitutions on the polar function using acetylation (pre-extraction) or silylation reaction (post-extraction) [260]. Derivatisation can be performed in solution prior to sampling, directly on the fibre or during desorption in the GC injection port, depending on necessity. The details of derivatisation for the SPME technique have also been reviewed [261,262]. Among the several approaches of treatment of polar analytes to make them more easily extracted, the simplest one is a pre-extraction derivatisation based on affinities of the analytes to the extracting phase. For instance, Cháfer-Pericás *et al.* [263] reported two approaches of derivatisation for SPME for the determination of amphetamines in various matrices, namely derivatisation in the sample matrix prior to SPME, and the SPME followed by on-fibre derivatisation. It was found that both approaches gave similar analytical performance in water samples, but the sensitivity achieved using derivatisation prior to SPME was considerably better. The use of SPME with derivatisation not only increases extraction efficiencies, but also can enhance separation, sensitivity or compound identification [261]. SPME-based post-extraction derivatisation was found to be useful for the analysis of highly polar or labile analytes.

Although the application of SPME fibres is increasingly gaining widespread acceptance, they still present important drawbacks such as limited capacity (due to the small amount of extracting phase), fragility and swelling in organic solvents, the damage of the fibre, the stripping of coatings and relatively low recommended

operating temperature (240-280 ºC) to broaden the availability to extract more compounds. Several research groups have focused on designing approaches to improve the SPME materials and enhance their extraction efficiency [264].

An overview of recent applications of different in-house prepared SPME fibres in environmental, biological and pharmaceutical analysis is shown in Table 2. Among the various approaches to developing new extracting phases for SPME fibres, sol-gel technology represents an interesting alternative in this field, since it can effectively create a chemically bonded, porous, and highly crossed coating on the fibre surface, therefore it provides a convenient route to creating new advanced materials for SPME. The idea of sol-gel technique is based on the attachment or immobilisation of different functional group to the fibre. The principle of this technique is the hydrolysis and condensation reaction of organometallic compounds in alcoholic solutions [20]. Typically, fused silica (FS) fibres are used as the bases in sol-gel SPME. The preparation of the sol-gel SPME fibres consists of pretreatment of FS surface, preparation of the sol-gel solution, coating of FS surface and ultimately conditioning of the coated surface [20,265,266]. The silica fibres should be properly pretreated to introduce large number of binding sites on the silica fibre and to facilitate the preparation of well-attached sol-gel coating *via* chemical bonding with the silica fibre surface. Depending on the desired coating, different precursors have been used in the preparation of sol-gel SPME fibres, such as titania [265,267], zirconia and alumina [266] among others. As an example, Li *et al.* [265] investigated the performance of titania-based sol-gel coating for determining polar compounds, such as PAA. Due to the variety of sorptive sites of titania and their acid-base characteristics, polar analytes were effectively extracted from environmental water samples followed by TD and GC analysis, providing recoveries higher than 96% for most of PAA studied and LODs ranged from 0.22 to 0.84 $\mu g\ L^{-1}$.

The developed titania [265,267], zirconia and alumina [266] sol-gel coatings for SPME, when compared to conventional sol-gel silica-based fibres, presented superior performance by exhibiting excellent pH resistance, high thermal stability, good chemical inertness and mechanical strength. Moreover, in comparison with commercially available fibres, such as PDMS, PDMS-DVB and PA, the extraction

Table 2. Examples of in-house SPME coatings and their applications.

SPME phase	Preparation technique	Analytes	Matrix	Analytical technique	Ref.
Al/OH-PDMS Al(sBuO)/OH-PDMS	Sol-gel technology	Volatile alcohols and fatty acids	Alcoholic beverage	HS-SPME-GC-FID	[266]
Ti/OH-TSO TNBO/OH-TS		Polar compounds	Wastewater	HS-SPME-GC-FID	[265]
Ti-chitin on a silver wire		Aliphatic alcohols	Juice	HS-SPME-GC-FID	[256]
Hydroxyfullerene		PCBs, PAHs & PAA	Wastewater	HS-SPME-GC-ECD	[268]
Crown ethers		Phenolic compounds, BTEX	Water	HS-SPME-GC-FID	[255]
Derived calix[4]arene		Aromatic amines, chlorobenzenes	Environ. waters	HS-SPME-GC-FID HS-SPME-GC-ECD	[269]
poly(MAA-co-EGDMA)	Monolith synthesis	Amphetamines	Urine	In-tube SPME-HPLC	[270]
Hydroxylated poly(GMA-co-EDMA)		Phenolic compounds	Environ. waters	In-tube SPME-HPLC	[271]
poly(MAA-co-EGDMA)		Aldehydes	Biological fluids	PMME-SPME-HPLC	[272]
MIP		Triazines	Soil, food	DI-HPLC-UV	[273]
MIP		Drugs of abuse	Water, urine	DI-GC and GC-MS	[274]
Disposable $[C_8MIM][PF_6]$	Immobilisation of IL on the FS fibre support	BTEX	Paints	HS-SPME-GC-FID	[165]
$[ViHDIm][NTf_2]$		Esters and fatty acid methyl esters	Wine	HS-SPME-GC-FID	[275,276]
$[MTPIM][NTf_2]$		Methyl tert-butyl ether	Gasoline	HS-SPME-GC-FID	[277]
$[ViHDIm][NTf_2]$		PAHs and substituded phenols	Water	DI-SPME-GC-MS	[278]
$[EeMIm][NTf_2]$		Amphetamine & methamphetamine	Urine	HS-SPME-GC-MS	[279]
PANI	Electrodeposition	Aromatic amines	Wastewater	HS-SPME-GC-FID	[280]

Table 2. (cont.)

Electrospun nanofibre	Electrospinning	Phenolic compounds, BTEX	Water	HS-SPME-GC-FID	[281]
Polyamide-based nanofibres		Phenolic compounds	Environ. waters	HS-SPME-GC-MS	[282]
NHAP	Biomineralisation pHAF	PAHs	Water, soil	DI-SPME-GC-FID	[283]
PAN/C_{18}, PAN/RP-CONH & PAN/CW/TPR	Covering stainless steel wires & modification of commercial fibre	Pharmaceuticals	Plasma	SPME-LC(ESI)MS	[284]
ADS (restricted access)	Immobilisation of ADS particles on the silica fibre	Benzodiazepines	Biological fluids	SPME-HPLC	[285]
XDS (restricted access)	Covering stainless steel wires	Angiotensains	Blood	SPME-LC	[286]

ADS: Alkyl-diol silica; Al(tBuO): Aluminum sec-butoxide; [C_8MIM][PF_6]: 1-octyl-3-methylimidazolium hexafluorophosphate; CW: carbowax; DI-SPME: direct immersion solid-phase microextraction; DVB: divinylbenzene; [EeMim][NTf_2]: 1-Ethoxyethyl-3-methylimidazolium bis(trifluoromethane) sulphonylimide; FS: fused silica; GMA: Glycidyl methacrylate; [MTPIM][NTf_2]:1-methyl-3-(3-trimethoxysilyl propyl) imidazolium bis(trifluoromethylsulphonyl) imide; NHAP: nanostructured hydroxyapatite; OH-TSO: hydroxyl terminated silicone oil; PAN: Polyacrylonitrile; PANI: polyaniline; PMME: Polymer monolith microextraction; PDMS: polydimethylsiloxane; pHAF: polydopamine-assisted hydroxyapatite formation; poly(MAA-EGDMA): poly(methacrylic acid-ethylene glycol dimethacrylate); TNBO: titanium n-butoxide; TPR: template resin; [ViHIm][NTf_2]: poly(1-vinyl-3-hexylimidazolium) bis[(trifluoromethyl)sulphonyl]imide; XDS: Exchange diol silica.

abilities of sol-gel-coated fibres were comparable or even superior to those of the tested commercial fibres [265,266]. In addition, Al-based coating showed better affinity for polar compounds, such as fatty acids, phenols or alcohols, than silica-based fibres [266].

Although FS fibres have been widely used for the preparation of sol-gel SPME fibres, recently metallic wires have also been used to improve the mechanical stability of the fibre. For instance, Farhadi *et al.* [256] used silver wires for the preparation a sol-gel titania-chitin fibre for HS-SPME to extract aliphatic alcohols from juice samples followed by GC-FID, exhibiting LODs of between 0.3 and 1.5 μg L^{-1}, and recoveries higher than 93%. The enhanced properties of sol-gel coatings, such as mechanical, thermal or chemical stability, are very important for the coupling of SPME with both GC and LC.

Other examples of SPME coatings were prepared *via* sol-gel technique using crown ethers. Several research groups have prepared fibres modified with crown-ether of different chemical compositions and different chain lengths. For instance, Yun *et al.* [255] developed SPME fibres coated with open crown ether stationary phase using sol-gel technique, for the determination of polar compounds, such as phenols, and non-polar, such as BTEX. The results demonstrated that the prepared fibre exhibited higher extraction efficiencies for aromatic amines than those obtained using PDMS or CW/DVB fibres, since the three-dimensional network in the coating structure provided a higher surface area and sample capacity. Sol-gel technology has also been applied to the derivatisation of the fibres with calix[4]arene [287] and diglycidyloxycalix[4]arene [269], which have cavity-shaped structures with polar and apolar properties. These calix[4]arene fibres were applied for the determination of aromatic amines and chlorobenzenes, respectively, from environmental water samples, providing better sensitivity for most of the investigated analytes compared with commercially available fibres (e.g. PDMS, PDMS/DVB, PA and CW/DVB).

Another approach for increasing extraction efficiency is the use of polymer monolithic material, which can be easily synthesised in-situ. As an example of this approach, Fan *et al.* [270] developed a poly(MAA-EGDMA) monolithic capillary column for extraction of amphetamines from urine samples. The

poly(MAA-EGDMA) monolithic capillary was introduced successfully into in-tube SPME coupled to LC, for the direct determination of target analytes in urine samples with recoveries higher than 98%. Moreover, the results indicated that the excellent recoveries of the target analytes were obtained thanks to hydrophobic and ion-exchange interactions.

In another study, Wen *et al.* [271] prepared a hydroxylated poly(GMA-co-EDMA) monolithic capillary for in-tube SPME for the determination of polar organic contaminants in lake water. The prepared in-tube capillary exhibited high capillary stability and high extraction capability towards polar compounds. More recently, in order to simplify SPME setup and organic solvent consumption Xu *et al.* [272] developed a novel extraction disk-monolithic frit format that has been adopted in SPME. The extracting phase based on the poly(MAA-co-EGDMA) monolithic frit was applied for the determination of aldehyde biomarker candidates in biological samples. The obtained recoveries ranged from 71% to 89%, and the maximum volume of sample that could be passed through the monolith frit was only 4 mL. While this format seems to be a promising approach, the extraction efficiency and lifespan of the monolithic frit should be further improved.

In the past few years, some researchers have combined the SPME technique with selectivity of MIPs, by incorporating selective binding sites to the fibres. For instance, Turiel *et al.* [273] reported their work that dealt with the use of MIP coatings on SPME fibres for the determination of triazinic herbicides in environmental and food samples. The MIP-coated SPME-fibres were prepared using a FS capillary that was filled with the polymerisation mixture (propazine/MAA/EGDMA) and then the polymerisation process was induced inside. Once the monolithic SPME fibres were obtained, the silica was etched away. The SPME performance of MIP-coated fibre demonstrated high selectivity, allowing the detection of target analytes at very low concentration levels, without any clean-up step. More recently, the same group prepared MIP monoliths and evaluated their use in SLM-protected MI-SPME [288]. Analogously, Tan *et al.* [289] recently synthesised MIP for SPME fibres for the selective extraction of bisphenol A in complex samples. The MIP fibres were used for the selective extraction of the target analyte in tap water, human urine, and milk samples, providing good recoveries. Djozan *et al.* [254,274] also

reported preparation of selective monolithic SPME fibres based on MIPs for the determination of drugs of abuse, such as diacetylmorphine [254] or methamphetamine [274]. The MIP fibres showed good extraction ability and indicated great potential for further development.

As mentioned previously, ILs were also used in SPME as extracting phases. Recently, the applications of ILs, as innovative sorbent materials for SPME, have been reviewed by Ho *et al.* [290]. As examples, some authors reported introducing ILs onto FS fibre. One of the first application of ILs in SPME was a disposable IL-based coating physically absorbed on the surface of stainless steel wire or FS tubing, introduced by Liu *et al.* [165] for the extraction of BTEX in paints. These developed disposable 1-octyl-3-methylimidazolium hexafluorophosphate $[C_8MIM][PF_6]$ coated fibres that presented reproducibility comparable with comercially available SPME fibres. However, its sensitivity was rather low due to the relatively thin coating. Similarly, Zhao *et al.* [276] reported the development of ILs-based SPME coating, poly(1-vinyl-3-hexadecylimidazolium) bis[(trifluoromethyl)sulphonyl]imide $[ViHDIm][NTf_2]$, and its application for selective extraction of esters and fatty acid methyl esters from complex matrices. ILs-based fibres provided good performance in terms of recovery and repeatability, which was comparable to commercially available PDMS fibre without the need for recoating after every extraction. As indicated in Table 2, these ILs-based coatings were limited to HS-SPME of non-polar analytes, so there was a need for the development of coatings that could extract analytes with different polarities being applied to other extraction modes. Another polymeric IL-based SPME coating that can be used is $[ViHDIm][NTf_2]$, as López-Darias *et al.* [278] described, which is immobilised on FS fibre support. The extraction performance of the poly(ViHDIm-NTf_2) fibre was evaluated on a selected group of water pollutants, including PAHs and substituded phenols, and performed using DI-SPME coupled with GC-MS, achieving high recoveries. The IL-based SPME coating has also been employed in forensic applications. As an example, He *et al.* [279] prepared a novel 1-ethoxyethyl-3-methylimidazloium bis(trifluoromethane) sulphonylimide $[EeMIm][NTf_2]$ extracting phase, by fixing through cross-linkage IL impregnated silicone elastomer on the surface of a FS fibre. The material obtained has been applied for determining amphetamines in human urine, providing promising sample preparation methods.

Another effective strategy for increasing the polarity of the fibres is the electrodeposition of conductive polymers onto the fibre. For instance, Minjia *et al.* [280] prepared stainless steel wires fibres based on polyaniline (PANI). The stainless steel wires were used instead of FS fibre to enhance the mechanical strength of the new fibre. As can be seen in Table 2, the fibre coated with PANI has been successfully applied for extraction of aromatic amines from environmental wastewater samples at trace levels, achieving recoveries higher than 90% for most analytes under study.

More recently, Zewe *et al.* [281] employed electrospun nanofibres for the extraction of BTEX and phenolic compounds following HS-SPME (Table 2), providing good extraction efficiencies. The results obtained were compared to commercially available PDMS, PDMS/DVB and PA fibres, demonstrating enhanced or comparable extraction efficiencies for both the non-polar and polar compounds. Similarly, Bagheri *et al.* [282] prepared polymeric fibres by electrospinning polyamide onto stainless steel wires for the determination of phenols and chlorophenols from environmental samples. The method developed was successfully applied to the extraction of phenolic compounds from real water samples, providing recoveries between 84% and 98%, for most of the analytes studied.

Recently, Feng *et al.* [283] introduced a novel SPME coating on the chemically inert stainless steel wire. The fibre, with nanostructured hydroxyapatite (NHAP) as coating, was prepared by a process of polydopamine-assisted biomineralisation. The extraction performance of the fibre was investigated on group of selected PAHs in environmental water samples, achieving high recoveries ranging from 77% to 113%.

Other types of SPME coatings were developed by Pawliszyn *et al.* [284]. The coatings were prepared by covering flexible stainless steel wires with a mixture of polyacrylonitrile (PAN) and different extracting phases, such as octadecyl silica (PAN/C_{18}), or RP-amide (PAN/RP-CONH), providing good mechanical stability. PAN can be also used for covering commercially available fibres with a biocompatible layer (PAN/CW/TPR). These types of biocompatible SPME coatings can be used for in vivo and in vitro extractions, in direct contact with

biological fluids. The extraction performance of prepared fibres was tested by developing an SPME/LC method for the determination of selected pharmaceuticals in human plasma. The coatings were demonstrated to be biocompatible because they did not adsorb proteins, and were successfully applied for fast drug analysis and assay of drug plasma protein binding.

Another interesting highly-selective approach is the use of RAM, such as alkyl-diol silica (ADS) [285] or ion-exchange diol silica (XDS) [286], as a coating for SPME fibres. For instance, Pawliszyn's group [285] immobilised the ADS particles on the silica fibre with a suitable adhesive. The ADS-SPME fibre obtained was used for the simultaneous fractionation of protein from biological samples, while directly extracting several target analytes, achieving good binding capacity, extraction efficiency, and reproducibility of the fibre over a wide range of benzodiazepine concentrations in urine samples. In a further study [286], the same group developed XDS with cation-exchange properties for the extraction of the peptides angiotensins from blood samples. The XDS particles were immobilised on a stainless steel wire with a binding agent. In this approach, only analytes of low molecular mass were retained and extracted selectively, since they had access to the binding centres at the inner pore surface, covered with hydrophobic alkyl chains and ion-exchange groups.

Over recent years, new approaches of designing and using SPME have advanced which is very clear from the developments of SPME devices and a number of new coatings. The development of new materials has significantly enhanced the range of compounds capable to direct extraction by SPME.

1.3. Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) is an equilibrium technique, similar to SPME, which is based on the partitioning of a solute between the sample matrix and the extracting phase. However, in the SBSE technique, instead of a polymer coated fibre, a magnetic stir bar is usually covered with a glass jacket on which a polymeric coat is placed. The volume of extracting phase is much higher compared to SPME (about 50-250 times higher), which increases the capacity.

Sample extraction is performed by placing the stir bar in the sample and agitating for a certain period of time (see Figure 6). After sampling, the stir bar is removed from the sample, dried with a lint free tissue, followed by thermal or liquid desorption and chromatographic or electrophoretic analysis.

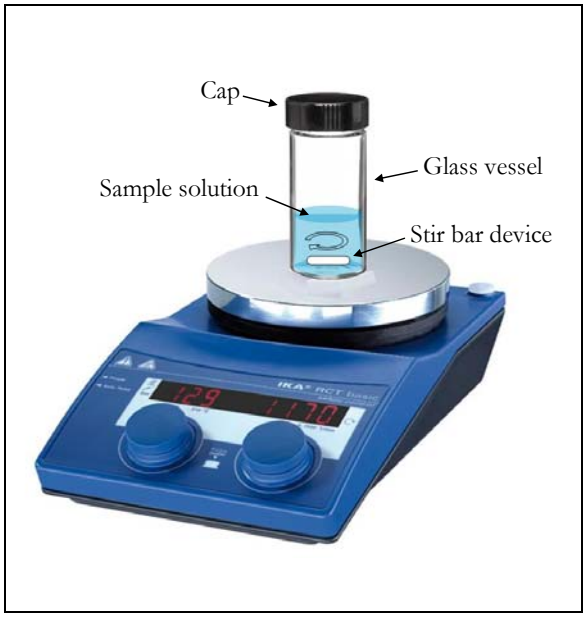

Figure 6. Schematic diagram of SBSE set-up.

Another mode of SBSE is headspace sorptive extraction (HSSE) in which sampling is performed in the headspace above a sample, similar to HS-SPME. SBSE can be performed both dynamically, when sampling is terminated before establishment of equilibrium, and statically, when extraction under equilibrium conditions is carried out.

Analogously to SPME, the parameters that have been considered for SBSE include the extraction and desorption steps. As mentioned previously, the desorption step may be performed thermally or using a small volume of an organic solvent. Thermal desorption (TD) is a commonly-used technique for extracting volatile compounds from various matrices [291-293], followed by GC analysis. TD is an environmentally-friendly approach, since no solvent is used, that offers greatly improved analytical sensitivity, because the sample is not diluted. After extraction, the stir bar is placed in a thermal desorption unit coupled to GC, ensuring the determination of all the analytes from sample retained on the stir bar. However, the common drawbacks of this approach include incompatibility with some solid sorbents and possible degradation of extracting phase that can generate interferences. In contrast to SPME, where TD can be readily performed using an ordinary GC injector, in the SBSE technique, TD requires suitable instrumentation. Liquid desorption (LD) is always used before the LC or CE analysis, and sometimes before GC, when the desorption unit is not available. It can be easily performed using a small amount of suitable solvent. However, in this approach, unlike TD, only a fraction of desorbing solvent is analysed. With respect to the LD, the choice of a suitable solvent is the primary goal. The selected solvent should not react with the sample or the sorbent, and also exhibit high affinity for the extracting phase (to release retained analytes completely). The solvents most commonly used are acetonitrile, methanol, ethyl acetate and dichloromethane. The selection of adequate desorption conditions is as fundamental as the choice of extraction parameters.

As regards the extraction step, the other factors investigated are sample pH, addition of an inert salt, addition of an organic modifier, sample volume, agitation speed, temperature and extraction time. Similarly to other sorptive techniques, sample pH is an especially important factor in the extraction of analytes that contain a pH-dependent functional group. For instance, the extraction efficiency of a PDMS-coated stir bar will enhance at a pH level that generates more undissociated forms of the analytes, since these forms will partition into the stir bar coating [294]. It should also be taken into account that extremely acidic (pH < 2) or very basic (pH > 9) values of sample pH can damage PDMS-phase and reduce stir-bar lifetime [294,295].

With respect to the addition of salt, similarly to SPME, the recoveries can be higher or lower, depending on the amount of additive as well as target analytes. A small amount of salt usually leads to better extraction efficiency due to the salting-out effect, while high salt addition may result in a decrease in efficiency, which can be attributed to electrostatic interactions between analytes and salt ions at molecular level. In some cases, it has been noted that salt addition did not enhance the extraction efficiency of hydrophobic analytes, but even led to a reduction [295,296]. In contrast, in the case of polar analytes, the recovery increases with the addition of inert salts [291,295]. As an interesting example, Ochiai *et al.* [297] developed a method consisting of a dual SBSE performed simultaneously on a sample containing 30% NaCl and the same volume of unmodified sample. One extraction with 30% NaCl was mainly targeting solutes with low $K_{o/w}$ and another extraction with the sample solution without salt was targeting solutes with medium and high $K_{o/w}$.

For the extraction of highly apolar analytes (e.g. pesticides) from water samples, an organic modifier, such as methanol or acetonitrile, is usually added to reduce wall adsorption and matrix effects [297,298]. For instance, higher recoveries were observed during the determination of UV-filters [296] or PAHs [299] when the organic modifier was added. Conversely, lower recoveries were obtained for several polar steroid hormones [298].

Another significant parameter that may lead to higher extraction efficiency is agitation speed (stirring rate). The agitation speed increases the diffusion rate by reducing the static aqueous layer surrounding the stir bar. However, excessive agitation speed might damage the stir bar coating due to the direct contact of the stir bar device [300], especially in the case of in-house prepared extracting phases. Typical agitation speed values using commercially available stir bars range between 900-1100 rpm [292,293,296] or even 1500 rpm [291,301]. However, in cases of new extracting phases authors recommend values in the range of 300-750 rpm in order to avoid mechanical damage [302,303].

Similarly to SPME, the temperature of the SBSE process should be optimised experimentally. Using a commercially available stir bar at increased temperature, the extraction equilibrium is attained quickly [294,304], but the partition

coefficient of the analytes and the extraction efficiency decrease [300]. The majority of authors observed an increase of extraction efficiency until 40-60 ºC [294,304] and a subsequent decrease linked to a decrease of the sorption distribution coefficient at higher temperatures (about 70 ºC) [294,304]. In another study [305], when determining organophosphorus pesticides (OPPs) using a sol-gel stir bar coating based on PDMS-poly(vinylalcohol) (PDMS-PVA), a decrease in extraction efficiency was observed with the increase of temperature from 15 ºC to 50 ºC. Thus, in the case of in-house prepared coatings, most of the studies were performed at room temperature [306,307].

The extraction time in SBSE, typically 60-240 minutes [298,308,309], is much longer than in SPME due to the greater amount of coating. However, in several cases, to trade off the sensitivity and shorten the analytical procedure, the authors decided to work under non-equilibrium conditions and selected an extraction time shorter than 1 hour [294,304,310] to determine the target analytes using different SBSE coatings. The kinetic of the extraction in SBSE is controlled as in SPME. The agitation speed, stir bar dimension and partition coefficient are the factors which determine the optimal extraction time. Similarly to SPME, optimisation is performed by plotting the extraction efficiency versus the extraction time to fix the time where no additional recovery is observed when increasing of the extraction time further.

Another parameter that undoubtedly plays a key role in extraction performance is the stir bar coating. Until very recently, the only commercially available stir bar was coated with polydimethylsiloxane (PDMS). These SBSE devices - Twister (Gerstel, Mulheim a/d Ruhr, Germany) are available in lengths of 10 and 20 mm which have 0.5 and 1 mm phase thicknesses, respectively. Normally, the 10 mm stir bars are used for 1-50 mL of sample, whereas the 20 mm stir bars are used for 100-250 mL of sample volume. Applications of SBSE techniques using PDMS-coated stir bar have been intensively investigated [31,308,310-312]. SBSE has been successfully applied in determining environmental pollutants such as pesticides, PAHs, PCBs, personal care products and others [308,312]. A PDMS-coated stir bar was employed in clinical and forensic analysis for the extraction of serotonin reuptake inhibitors [311] from different biological matrices (plasma, urine, brain tissue). It has been also successfully applied in the determination of a

wide range of non-polar analytes including pesticides [310] and volatile compounds [292], in oenological and food analysis.

Although SBSE has been widely applied to extract non-polar and semipolar compounds, it does not work well when extracting polar compounds unless they have been derivatised [313-315]. As is well-known, compounds that contain polar functional groups cannot be easily retained by PDMS. Thus, improved affinity for polar analytes to the extracting phase can be provided by the derivatisation step (e.g. acetylation of the phenolic groups), which can be performed in the sample prior to the SBSE step [315,316].

In comparison to SPME, that offers several types of polymeric phases with a wide range of polarity available, one of the main limitations of SBSE is the apolar nature of PDMS. In spite of the superior performance of SBSE compared to SPME in most instances, the fact that until very recently PDMS was the only commercially available phase for SBSE inevitably limits extractability of highly polar compounds. This year a new commercial stir bar, EG/Silicone Twister (from Gerstel) with more polar extracting phase, was introduced. EG/Silicone Twister (length: 10 mm, phase volume: 32 μl) is based on ethylene glycol (EG) and PDMS. At present, some pilot tests of a new polar extracting phase - PA Twister (Gerstel) are performed with a view to commercialisation. PA Twister (length: 10 mm, phase volume: 25 μl), which is based on polyacrylate (PA) combined with polyethylene glycol (PEG), was applied for the first time to extract benzothiazole from untreated wastewater [301]. These results indicate a great potential in SBSE of polar analytes, since it provided good analytical performance without the need for previous filtration or a cleaning step. However, as this new extracting phase is not yet commercially available, further studies should be carried out to explore extractability and affinity for a range of polar analytes.

Before the commercialisation of these new polar stir bars, in response to demand for more suitable phases for SBSE, several in-house procedures for stir bar coating have been developed. These new phases and their applications will be discussed below and summarised in Table 3.

The first attempt to overcome the limitation of PDMS towards the extraction of polar and highly volatile compounds was the stir bar dual-phase, which was introduced by Bicchi *et al.* [317]. The dual-phase stir bar combines the capacity of two or more extracting phases containing an outer PDMS coating combined with different adsorbents inside. In this approach, the commercially available stir bar was modified with activated carbons. The extractability of dual-phase stir bars was evaluated by using them for the HSSE and SBSE from different matrices. High recovery of very volatile compounds emitted from plant material was demonstrated as well as higher recoveries for more polar analytes in water samples. In another study, Barletta *et al.* [318] reported a similar approach preparing a new extracting phase based on PDMS and activated carbon (PDMS-ACB) for SBSE. It was prepared by the attachment of the extracting phase using a Teflon/glass capillary mold setup. The developed PDMS-ACB-coated stir bar demonstrated good stability and resistance to organic solvents for numerous extractions (more than 150). The extractability of PDMS-ACB stir bar was evaluated to determine pesticides in sugarcane juice samples.

Several problems have been identified with the synthesis of SBSE coatings with various polarities directly onto the glass tube as well as problems with obtaining a mechanically stable thick layer of extraction phase. To overcome these problems, several different approaches for preparation of new coatings have been described.

As explained in the section on SPME, one approach was based on sol-gel technology, which is a suitable procedure for preparing a thick film with high thermal stability, low bleeding and long lifetime, resulting from strong adhesion between the coating and glass surface. The results obtained show this material's applicability to extract both polar and non-polar compounds.

Stir bars with hydroxy-terminated PDMS as a coating layer prepared by the sol-gel technique have been employed for determination of pesticides [300]. The factors influencing the extraction were investigated. It was found that the stirring can affect the mechanical stability of the film during extraction process. Several new stir bars were also prepared using sol-gel technology by the introduction of various groups, such as β-cyclodextrin [305,319], DVB [319] or poly(vinylalcohol) [320] into the PDMS network. Although good performance and extractability were demonstrated,

some problems were noted with mechanical stability, such as cracking of the polymer layer, which led to a progressive loss of extracting phase over time. Several applications using various extracting phases prepared by the sol-gel technique, for SBSE in different matrices are included in Table 3. It also includes other relevant examples of extracting phases prepared in-house for SBSE application that uses other approaches.

Recently, Lan *et al.* [303] reported the preparation of an organic-inorganic hybrid titania-hydroxy-terminated silicone oil (Ti-OH-TSO) stir bar coating by the sol-gel technology. The extraction performance of Ti-OH-TSO-coated stir bar was evaluated and compared with PDMS, PDMS-DVB, PDMS-β-cyclodextrin (PDMS-β-CD) and C_{18} coated stir bars. A Ti-OH-TSO coating was applied for the determination of polar drugs of abuse in urine samples [303].

The Ti-OH-TSO-coated stir bar showed good chemical stability, preparation reproducibility, highly pH-resistant ability, high extraction efficiency and superior selectivity compared to other phases. To avoid cracking problems, more recently, Ibrahim *et al.* [321] prepared and characterised another extracting phase for SBSE based on tetraethoxysilane-polydimethylsiloxane (TEOS-PDMS) using sol-gel technology. The synthesis of TEOS-PDMS coating was optimised and its applicability was successfully demonstrated in the extraction of selected OPPs from water solutions [321].

New materials such as PDMS/polypyrrole (PPy) [294] were also proposed as extracting phases for SBSE coating. The stir bar coating was prepared by the polymerisation of PDMS and pyrrole directly onto the Teflon mold. Its porous structure provides a high specific surface area that can improve the extraction capacity. A PDMS/PPy stir bar was evaluated for determining antidepressants in plasma and presented high extraction efficiency (sensitivity and selectivity).

Another possible option is the use of monolithic material. Monolithic materials are obtained by polymerisation of a monomer mixture with a porogen, forming a porous polymer containing a network of pores. In consequence, monolithic materials present good permeability, which accelerates the mass transfer between extracting phase and the analytes in the sample.

Table 3. Applications of different in-house prepared coatings for SBSE.

Coating	Preparation technique	Analytes	Matrix	Analytical technique	Ref.
PDMS/ACB	Attachment of polymeric phase on Teflon support	Pesticides	Juice	GC-MS	[318]
PDMS-β -CD	Sol-gel technology	Oestrogens, BPA	Drinking water	LC-UV	[305]
Ti-OH/TSO		Drugs of abuse	Urine	LC-UV	[303]
TEOS/PDMS		OPPs	Water	LC-UV	[321]
PDMS/β -CD		Brominated flame retardants	Soil dust	LC-UV	[322]
PDMS/β -CD /DVB		PAHs, PASHs	Water	LC-UV	[319]
PDMS/PPY		Antidepressants	Plasma	LC-UV	[294]
PDMS/PVA		OPPs	Honey	GC-FID	[320]
Sol-Gel PDMS		n-alkanes, PAHs	Water	GC-FID	[323]
OcMA/EDMA	Monolith synthesis	Anabolic steroids, PAHs	Seawater and urine	LC-UV	[324]
MASE/EDMA		Steroid Sex Hormones	Urine	LC-UV	[325]
MASPE/DVB		Quinolones and nitroimidazoles	Wastewater Honey	LC-UV	[302,309]
MAOMA/DVB		Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}	Water	LC-UV	[326]
VI/DVB		Aromatic amines	Water	LC-UV	[327]
VP/EDMA		Phenols	Water (lake and sea)	LC-UV	[328]
VP/EDMA		Steroid hormones	Water	LC-UV	[307]
VPD/DVB		PAHs, phenols, anilines, heavy metal ions and hormones	Water	LC-UV	[329]
MIP		Triazine herbicides	Food and soil	LC-UV	[330,331]
MIP		Ractopamine	Animal tissue	LC-FL	[332]
MIP		Sulpha drugs	Animal tissue	LC-UV	[333]
MIP		Monocrotophos	Soil	GC-NPD	[334]

Table 3. (cont.)

ADS/RAM	Immobilisation of extracting phase using binding agent	Caffeine and metabolites	Rat Plasma	LC-UV	[32]
PU	Covering of stir bar with extracting phase	Acidic pharmaceuticals	Water (river, sea and wastewater)	LC-UV	[306]
PU		Testosterone and methenolone	Urine	LC-UV	[335]

ACB: activated carbon; ADS/RAM: alkyl-diol silica restricted access material; β-CD: β-cyclodextrine; BPA: bisphenol A; DVB: divinylbenzene; EDMA:. ethylene dimethacrylate; FID: flame ionisation detector; FL: fluorescence detector; MASPE: methacrylic acid-3-sulphopropyl ester potassium salt; MASE: methacrylic acid stearyl ester; MIP: molecularly imprinted polymer; MAOMA: 2(methacryloyloxy) ethyltrimethylammonium chloride; NPD: nitrogen-phosphorus detector; OcMA: octyl methacrylate; OOPs: organophosphorus pesticides; PAHs: polycyclic aromatic hydrocarbons; PASHs: polycyclic aromatic sulphur heterocycles; PDMS: polydimethylsiloxane; PPY: poly(pyrrole); PU: polyurethane; PVA: poly(vinylalcohol); Ti-OH-TSO: titania-hydroxy-terminated silicone oil; VI: vinylimidazole; VP: vinylpyridine; VPD: vinylpyrrolididone.

The outstanding features of these materials are simple synthesis, high permeability, excellent mass transfer characteristics and low cost. Thus, the monolithic materials, thanks to the suitable combination of monomers used for their preparation, allow the effective extraction of both non-polar and polar analytes.

Huang *et al.* synthesised series of different monolithic polymeric phases for SBSE (Table 3). For instance, octyl methacrylate and ethylene dimethacrylate (OcMA-EDMA) were used to synthesise a sorptive material for the extraction of PAHs in seawater and anabolic steroids in urine [324]. Then, to obtain more polar material, vinylpyridine-EDMA (VP-EDMA) was combined to prepare a polymeric phase [307], which was able to extract phenols from environmental water samples [328]. To further increase the polarity of the synthesised polymers, other monomers mixtures were evaluated, e.g. methacrylic acid-3-sulphopropyl ester potassium salt and divinylbenzene (MASPE-DVB) [302,309], methacrylic acid stearyl ester (MASE-EDMA) [325], 2-(methacryloyloxy)ethyl-trimethyl ammonium chloride-DVB (MAOMA-DVB) [326], vinylimidazole-DVB (VI-DVB) [327] or vinylpyrrolididone DVB (VPD-DVB) [329,336]. These monolithic coatings were used in SBSE of different compounds from biological and environmental matrices (for more details, see Table 3). The SBSE performance of monolithic coatings was also compared to other phases. For instance, the SBSE performance of VI-DVB-coated stir bar in extraction of polar amines were compared to PDMS-coated stir bar and other monolithic coatings. The results clearly showed the superior performance of VI-DVB in the extraction of polar aromatic amines compared to other extracting phases (VP-EDMA and VPD-DVB) [327]. In a recent study [302], the extractability of, for example, nitroimidazoles on the MASPE-DVB was also compared with a commercially available PDMS-coated stir bar and an in-house prepared stir bar based on VP-EDMA monolith. Therefore, it was demonstrated that MASPE-DVB phase can extract nitroimidazoles much better than commercial SBSE and VP-EDMA.

As can be observed in Table 3, other selective coatings have also been prepared using MIP technology. Hu *et al.* [330,331] prepared an MIP-based extracting phase using an iron bar placed inside a glass tube with the outer surface of the glass sililated. Then, the pretreated bar was inserted into a pre-prepared mixture of PDMS, with β-cyclodextrin as the template molecule and further chemically

bonded to the glass bar in order to enhance the stability of the coating. These novel SBSE coatings were applied for the extraction of herbicides and fungicides from complex samples, providing good analytical performance. More recently, Xu *et al.* [332,333] reported the synthesis of MIP-coated stir bars, which were applied for trace analysis of β-agonists [332] and sulphonamides in complex samples, such as animal tissues [333], exhibiting satisfactory recoveries. Although the MIP-coated stir bars have the advantages of easy preparation, resistance and promising extraction capability, they still present some limitations. The issue of selectivity and effective clean-up step should be further investigated.

In another study, Zhu *et al.* [334] prepared stir bars coated with a polymeric film of Nylon-6 polymer imprinted with monocrotophos. In this case, the authors prepared a mixture of Nylon-6, formic acid and L-glutamine as the template molecule and a previously prepared magnetic rod was inserted to this colloid solution. The MIP extracting phase prepared was successfully applied to the selective extraction of monocrotophos and their analogues from environmental soil samples. In comparison to the PDMS stir bar, the MIP-coated film presented higher selectivity and faster equilibrium adsorption.

Moreover, more selective stir bars have been developed using alkyl-diol silica (ADS) restricted access material (RAM). Lambert *et al.* [32] prepared and evaluated SBSE based on RAM for the direct extraction of caffeine and metabolites from biological fluids, achieving recoveries close to 100%. The ADS-based extracting phase fractionated the protein components from the sample, providing clean extract.

Other materials used as SBSE coatings were polyurethane (PU) foams [306]. It was found that PU foams show considerable thermal stability and mechanical resistance to organic solvents. In SBSE followed by LD, PU foams were demonstrated to have superior performance for polar analytes than the commercially available PDMS stir bar. PU foams were applied in SBSE to determine testosterone and methenolone in urine matrices [335], achieving negligible matrix effects and good analytical performance. These new polymeric extracting phases seem to be promising materials for SBSE. Although the extraction efficiencies for polar and semi-polar compounds were satisfactory,

they were still low for highly polar analytes. In addition, when using these materials, derivatisation is not required to extract analytes directly from an aqueous matrix [328].

In spite of the good performance and better extractability of polar analytes, these in-house materials can be applied only to LD due to limited thermal stability.

Despite all these efforts, there is still demand for extracting phases which can cope with the current requirements and provide better extractability for wide range of polar organic compounds, expanding the range of SBSE applicability further.

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CHAPTER 2. OBJECTIVES

The main aim of this Doctoral Thesis is to develop new polymeric materials for sorptive extraction techniques, such as solid-phase extraction and stir bar sorptive extraction, to improve the extraction of polar compounds from water samples.

This general objective can be detailed in:

- Development of new polymeric materials that improve capacity and selectivity for solid-phase extraction.

- Preparation of new stir bars with polar monolithic coatings.

CHAPTER 3. EXPERIMENTAL PART AND RESULTS

As discussed in the introduction to this Thesis, sorptive materials and their applicability in a variety of matrices represent a very diverse and continuously evolving field of research. Although there have been significant developments in the area of new materials for sorptive extraction techniques, the extraction of polar analytes is still considered the bottleneck of the extraction process. With this in mind, efforts have been undertaken to improve capacity and selectivity during extraction and clean-up of samples. For this reason, one of the main objectives of this Thesis was to develop new materials to extract polar organic contaminants from water samples.

This chapter includes the experimental part, results and discussion of different studies that have been developed in this Doctoral Thesis. These results have already been published, or are currently due to be published, in several international scientific journals. They are presented here in each section in journal paper format. These studies have been classified into four sections. For each section, a brief introduction is included to establish the aspect of the research, and the most notable results are also discussed. The list of articles published as a result of this Doctoral Thesis is included in Annex II.

The first section reports the synthesis of new hypercrosslinked polymers with hydrophilic character in the form of microspheres and their evaluation as novel SPE sorbents for the extraction of polar contaminants from different water samples, followed by LC analysis.

In the second section, two new mixed-mode ion-exchange hypercrosslinked sorbents have been synthesised and used for the selective SPE of polar pharmaceuticals. Both precursor particles for mixed-mode hypercrosslinked sorbents were obtained by precipitation polymerisation (PP). Depending on the moieties of the polymer, they acted as a weak cation-exchange or a strong anion-exchange sorbent. The sorbents were used for the extraction of a group of target analytes (basic and acidic pharmaceuticals, respectively) from complex environmental waters that included a clean-up step, in order to eliminate interfering compounds. The SPE performances of these new sorbents were compared to commercially available mixed-mode sorbents.

The synthetic parts of the above studies were carried out in collaboration with Prof. Peter A.G. Cormack of the Polymer Research Group of the Department of Pure and Applied Chemistry of the University of Strathclyde (Glasgow, Scotland, UK).

The third section focuses on the synthesis of imidazolium-based supported ionic liquids phases (SILPs) containing different anions, and their evaluation as SPE sorbents for selectively extracting acidic pharmaceuticals from aqueous samples. This is one of the first studies in which polymeric SILPs act as strong anion-exchange sorbents in SPE. This study was carried out in collaboration with Prof. Andrzej W. Trochimczuk of the Polymer and Carbon Materials Research Group of the Department of Chemistry of Wrocław University of Technology (Wrocław, Poland).

The fourth section presents the development of two polar monolithic extracting coatings for the SBSE of polar contaminants. The monolithic stir bar coatings were prepared and applied in SBSE with liquid desorption followed by LC-MS/MS. Furthermore, the SBSE performances of the prepared extracting phases were compared to commercially available PDMS-coated stir bars. The coatings were prepared during my stay at the University of Strathclyde under the supervision of Prof. Peter A.G. Cormack.

3.1. New materials with hydrophilic character for solid-phase extraction

Over the past several years, in order to enhance the extractability of polar analytes from liquid samples, efforts have been focused on the development of new SPE materials. In this field, our research group has a broad experience in the synthesis and application of chemically modified polar sorbents [1], sorbents with a hydrophilic monomer [2], molecularly imprinted polymers [3,4], and the latest developments in SPE materials centring on hypercrosslinked polymeric sorbents [5].

As mentioned in the Introduction, hypercrosslinked sorbents were first introduced by Davankov [6]. Professor Sherrington's research group also adapted a hypercrosslinking procedure using VBC-DVB particles as the starting material [7]. In one of the latest studies [8], they found that, depending on the VBC isomer, a certain degree of hydrophilicity can be conferred. Since then, they have been widely applied in different sorption processes [8-11], including SPE [8,9] with good results, mainly for less polar compounds. The prepared hypercrosslinked sorbents presented different degrees of polarity and specific surface areas [8], depending on the precursors used in the synthesis, which were obtained by suspension polymerisation. When using the VBCmix (mixture of VBC isomers ~70% m-; ~30% p-) as a precursor, the hypercrosslinked sorbent (HXLGmix) showed a higher specific surface area (~1900 $m^2 g^{-1}$) with a hydroxyl group content of ~1.5 mmol g^{-1}, whereas when the precursor was VBCp, the hypercrosslinking reaction generated an HXLGp sorbent that combined a larger number of hydroxyl moieties (~2.4 mmol g^{-1}) with a high specific surface area (~900 m^2 g^{-1}) [8]. The differences in performance between these hypercrosslinked sorbents were observed in their SPE performance in the on-line extraction of a group of polar compounds, in which the best results were provided by HXLGp. Therefore, to enhance the capacity of the sorbent, the hydrophilicity must be balanced. Although the prepared resins provided a certain degree of polarity, this hydrophilic character of hypercrosslinked sorbents was not controlled. Therefore, to gain more control over the hydrophilicity of the prepared sorbents, another approach that included the introduction of a polar monomer during synthesis has been developed in this Thesis.

This assumption is also corroborated in other studies focused on batch equilibration experiments, where various hydrophilic hypercrosslinked resins were

evaluated as sorbents for the removal and subsequent recovery of aromatic pollutants from water [10,11]. These hypercrosslinked resins were functionalised with carbonyl groups [10] and multiple phenolic hydroxyl groups [11], and applied to the removal of p-nitrophenol and p-nitroaniline from aqueous samples. In comparison to commercially available macroporous Amberlite XAD-4, these hypercrosslinked resins provided larger sorption capacity and higher sorption affinity due to their hydrophilic character. Thus, once again it was demonstrated that larger sorption capacity results from the high content of polar functional groups in the polymer structure, rather than high specific surface area.

With respect to the development of hypercrosslinked resins as SPE materials, other sorbents were prepared from the precursor particles obtained by precipitation polymerisation (PP), and their sorption properties were enhanced by hypercrosslinking reactions [9]. These novel hypercrosslinked sorbents presented improved properties as SPE materials compared to conventional polymeric SPE sorbents, since they possessed hypercrosslinked structures and small micrometer monodisperse particles (~4 μm). The essential features of hypercrosslinked sorbents, such as high specific surface area, relatively small pore size, excellent sorption capacity for a wide range of medium and low polarity analytes, as well as an easy regeneration process, make them useful as sorptive materials in extraction techniques.

As stated in the Introduction, the hydrophilicity of the sorbent is a determining parameter that can be tuned by introducing a polar monomer into the polymer skeleton. The monomers, such as 2-hydroxyethyl methacrylate (HEMA), are polar due to the presence of hydroxyl and acrylic groups, and can be considered good candidates for the preparation of hydrophilic sorbents. Polymers derived from the precursors that contain HEMA and DVB obtained by PP are attractive polymeric materials and can be employed for the extraction of a whole range of polar compounds. Their size, functionality of the base polymer, morphology of the polymer beads, and the degree of crosslinking are the main factors involved in controlling the properties and their sizes are all in the low micrometer range (varying from 1 to 7 μm) depending on the content of the methacrylic comonomer [12]. This characteristic makes these monomers suitable for the preparation of hydrophilic materials for SPE applications. Therefore, the goal of

the research was to obtain hydrophilic hypercrosslinked materials that contain HEMA, which can be applied in SPE for the extraction of polar analytes, and to compare them to commercially available sorbents.

The results obtained in these studies are published in the *Journal of Chromatography A* 1217 (2010) 3238-3243.

3.1.1. Hydrophilic hypercrosslinked polymeric sorbents for the solid-phase extraction of polar contaminants from water

HYDROPHILIC HYPERCROSSLINKED POLYMERIC SORBENTS FOR THE SOLID-PHASE EXTRACTION OF POLAR CONTAMINANTS FROM WATER

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Abstract

Three new hypercrosslinked polymers with hydrophilic character arising from hydroxyl moieties in their skeletons have been prepared in microsphere format and applied to the off-line solid-phase extraction (SPE) of polar compounds from water samples. For sample volumes of 1000 mL, the recoveries of various polar pesticides, such as oxamyl, methomyl, selected phenolic compounds, as well as some pharmaceuticals, were close to 90%. The HXLPP-polar polymer with the best performance characteristics was applied to real samples. Its performance was also compared to commercially available sorbents, such as LiChrolut EN (hydrophobic, hypercrosslinked), Oasis HLB (hydrophilic, macroporous) and Isolute ENV+ (hydrophilic, hypercrosslinked); the new sorbent outperformed the commercially available sorbents. The polymer was applied successfully in off-line SPE of river water samples followed by liquid chromatography and ultraviolet detection, providing a good linear range and detection limits of 0.2 μg L^{-1} for the majority of the compounds, with the exception of oxamyl, methomyl, guaiacol and salicylic acid where the detection limit was 0.5 μg L^{-1}.

Keywords: *Hydrophilic hypercrosslinked sorbents; polymer microspheres; solid-phase extraction; polar contaminants*

1. INTRODUCTION

In recent years, solid-phase extraction (SPE) has become the preferred extraction technique to enrich pollutants from aqueous samples. An important consideration in SPE is the rational selection of the sorbent depending on the characteristics of the analytes to be extracted and also on the complexity of the sample matrix. For this reason, over the last few years several sorbents with a broad range of properties have been developed and commercialised.

Traditionally, sorbents have been divided into silica-based, carbon-based and polymer-based sorbents, with polymeric sorbents being particularly attractive in light of their chemical stability and easily tailored physico-chemical characteristics [1-3].

A very important feature of polymeric sorbents is their porous character, which provides the surface area necessary for sorption of the compounds, therefore porous character is directly related to the efficacy of the sorbent in the SPE process [4].

Conventional, commercially available polymeric sorbents are normally based on styrene-divinylbenzene copolymers with macroporous structure and hydrophobic characteristics.

Therefore, they are more effective in the retention of non-polar compounds than the retention of polar compounds [5,6].

In order to improve the retention of polar compounds on SPE sorbents, several sorbents that increase both the specific surface area (expressed in $m^2 g^{-1}$) and the hydrophilicity have been developed [3].

Regarding the enhancement of the specific surface area, hypercrosslinked polymers belong to a new generation of permanently porous polymeric resins. They have high specific surface areas (up to ~2000 $m^2 g^{-1}$), and their high micropore content makes them particularly well suited for sorption processes [7].

The hydrophilicity of a sorbent can be increased by introducing a polar co-monomer into the polymerisation [8-11], or by post-polymerisation chemical modification to introduce polar functional groups into the polymer structure [12-14].

Commercially available hydrophilic sorbents include, for instance, Oasis HLB (from Waters), which is a macroporous poly(N-vinylpyrrolidone-co-divinylbenzene) copolymer with a specific surface area of ~800 $m^2 g^{-1}$ or Strata-X (from Phenomenex), which is poly (styrene-co-divinylbenzene) modified chemically with pyrrolidone groups, with a specific surface area of ~800 $m^2 g^{-1}$.

Previous studies [8,14-19], which include the SPE of various families of analytes from several aqueous matrices, have demonstrated that the retention properties of sorbents for polar analytes are enhanced as the specific surface area and the degree of hydrophilicity of the sorbent is increased.

This relationship was exemplified in a previous study [16] in which a hypercrosslinked sorbent with a styrenic

skeleton and pendent hydroxyl groups performed better than the hydrophobic analogues. Moreover, in a recent study with hydrophobic sorbents [15] it was demonstrated clearly that the lower the particle size of the sorbent the better the SPE performance.

Considering these facts, an ideal hypercrosslinked sorbent for the efficient capture of polar analytes ought to combine the features of hydrophilicity and low particle size.

The aim of this study was therefore to synthesise new hypercrosslinked polymers in the form of small particles with hydroxyl moieties in place in the skeleton to confer hydrophilic behaviour, and then to evaluate the polymers as novel sorbents for the retention of polar pollutants using SPE.

2. EXPERIMENTAL

2.1. Reagents and standards

The polar pollutants selected to evaluate the performance of the sorbents included: pesticides such as oxamyl, methomyl and (4-chloro-2-methylphenoxy) acetic acid (MCPA) obtained from Riedel-de-Haen (Seelze, Germany); phenolic compounds such as phenol, 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), guaiacol; pharmaceuticals such as antipyrine, salicylic acid and ibuprofen obtained from Sigma-Aldrich (Steinheim, Germany). Their chemical structures are shown in Fig. 1S.

Standard solutions for each compound were prepared at a concentration of 2000 mg L^{-1} in methanol. A mixture of all the compounds was prepared by diluting the standard solutions with Milli-Q water (Millipore, Bedford, MA, USA).

HPLC grade acetonitrile and methanol were from SDS (Peypin, France). Hydrochloric acid, used to adjust the pH of the mobile phases and the samples prior to SPE, was from Probus (Barcelona, Spain).

The reagents for the polymer syntheses were divinylbenzene (DVB) (80% grade) and 2-hydroxyethyl methacrylate (HEMA) (98% grade) supplied by Aldrich, *para*-vinylbenzylchloride (VBC) (95% grade) supplied by Fluka (Steinheim, Germany). The monomers were purified by passing them through short neutral alumina columns. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and was purified by recrystallisation from acetone. Ferric chloride ($FeCl_3$) and anhydrous 1,2-dichloroethane (DCE), from Aldrich, were used in the hypercrosslinking reactions.

2.2. Resin preparation

The complete synthetic procedure is outlined in Fig. 1. In more detail, micrometer-sized, spherical precursor particles (PP-polar) were obtained by an optimised precipitation polymerisation (PP) method [20]. The crosslinking monomer (DVB) and AIBN (2 mol% relative to the total number of

polymerisable double bonds present in all three comonomers) were added to acetonitrile (200 mL) in a polypropylene bottle (250 mL). The monomer solution was de-oxygenated with N_2 at 0 °C and the bottle then placed on a low-profile roller (Stovall, Essex, UK) in a temperature-controllable incubator (Stuart Scientific, Surrey, UK). The temperature was ramped from ambient to 60 °C over a period of 2 h and the polymerisation was allowed to proceed at 60 °C for 6 h, at which point the reaction mixture started to get milky. The reaction mixture was cooled to room temperature and the remaining monomers (HEMA and VBC) then added to the milky suspension (N.B. the total monomer concentration was 2%, w/v relative to solvent). This mixture was de-oxygenated with N_2 at 0 °C and the bottle placed back into the low-profile roller in the temperature-controllable incubator at 60 °C for a further 48 h. The resulting particles were filtered on a 0.2 µm nylon membrane filter and washed successively with MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 °C.

The mole ratio of comonomer in the feed was varied systematically, with the hydrophilic comonomer (HEMA) being fed at three different levels (25, 20 and 10 mol%). Table 1 details the monomer feed ratios for each polymerisation, as well as information on the characterisation of the products.

The hypercrosslinking of the PP-polar particles was carried out using a procedure described previously [20] (see detailed information in Supporting information).

The hypercrosslinked resins (HXLPP-polar) were characterized by measuring their specific surface areas using a BET treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The chlorine and nitrogen contents for the resins were obtained with elemental microanalysis using a Carlo-Erba EA 1106 instrument. The ATR mode FTIR spectra were recorded using Perkin-Elmer Spectrum One Spectrometer. Microsphere diameters and homogeneity in size were determined using ImageJ software analysis of 100 individual particles in scanning electron microscopy (SEM) images, which were acquired using a JEOL 6400 Instrument [20]. Fig. 1 also shows the SEM image for HXLPP-polar microspheres. The characterisation data obtained for all the resins produced is detailed in Table 1.

2.3. Chromatographic equipment and conditions

The chromatographic experiments were performed with an HP 1090 Liquid Chromatograph and UV-Detector (Hewlett-Packard, Waldbronn, Germany) equipped with an injection valve with a 20 µl loop. The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain).

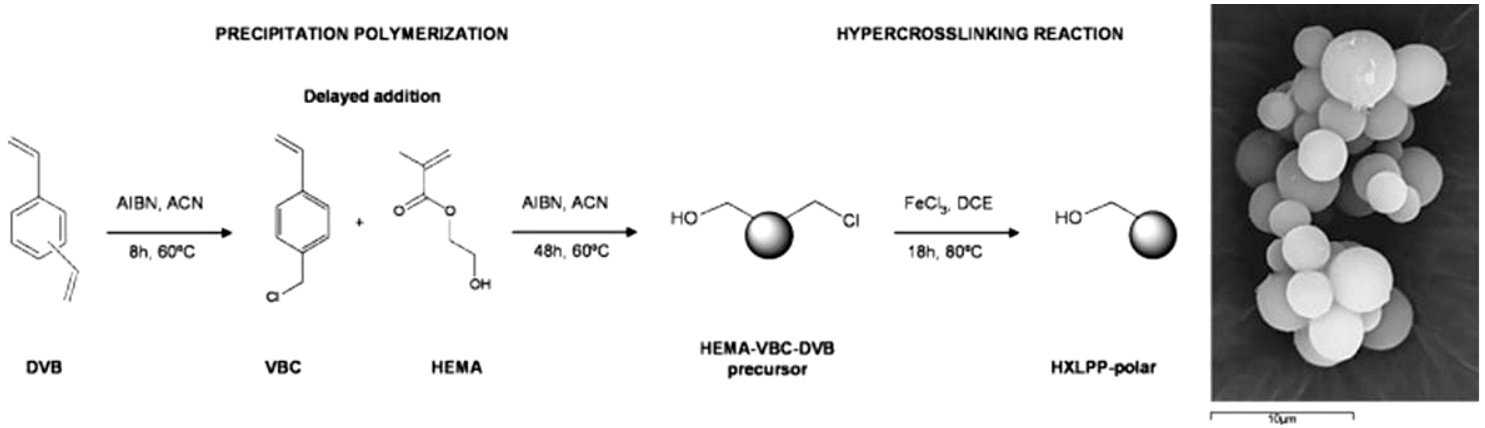

Fig. 1. Schematic representation of the synthetic procedure used in the production of HXLPP-polar resins and SEM images of HXLPP-polarB resin (the applied acceleration voltage of the incident electron beam was 20 kV).

Table 1. Monomer feed and analytical data for PPpolar precursor particles and their hypercrosslinked derivatives (HXLPP-polar).

Resin	HEMA/VBC/DVB (mole ratio)	D[a] (μm)	Coef. Var.(%)	Yield (%)	%C[b]	%H[b]	%Cl[b]	%O[c]	O[c] (mmol g^{-1})	S.A.[d] ($m^2 g^{-1}$)
PP-polarA	25/25/50	n.d.	n.d.	54	77.6	7.6	5.3	9.5	1.98	~5
PP-polarB	20/40/40	n.d.	n.d.	44	76.6	7.3	8.6	7.5	1.56	~5
PP-polarC	10/50/40	n.d.	n.d.	48	78.5	7.1	10.1	4.3	0.90	~5
HXLPP-polarA	25/25/50	5.7±2.4	42.4	90[e]	80.2	7.5	3.8	9.5	1.89	670
HXLPP-polarB	20/40/40	5.5±1.8	33.4	90[e]	81.2	6.6	4.9	7.5	1.56	850
HXLPP-polarC	10/50/40	3.9±1.3	33.8	88[e]	84.1	6.4	3.9	4.3	0.90	925

n.d. no data; [a] Average particle diameter ± standard deviation (S.D.) calculated from the image analysis of 100 individual particles in SEM micrographs (using ImageJ software); [b] Obtained experimentally with elemental microanalysis [c] Obtained by substraction with the rest of elements based on monomer feed [d] Specific surface area computed from N_2 sorption isotherms and BET analysis. [e] Relative to the mass of the corresponding (non-hypercrosslinked) precursor particles.

The mobile phase was: Milli-Q water adjusted to pH 2.8 with HCl and acetonitrile (ACN). The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 65 °C. The gradient profile was 20% of ACN initially, held for 8 min, then to 25% ACN in 7 min, to 80% ACN in 5 min, to 100% ACN in 2 min, held for 4 min, after which time the mobile phase was returned to the initial conditions (20% ACN) in 2 min. The total run time was 28 min.

The wavelengths used to detect the compounds were 240 nm (oxamyl and methomyl), 210 nm (antipyrine, phenol, guaiacol, 4-NP, salicylic acid and 2,4-DNP), and 220 nm (MCPA and ibuprofen).

2.4. Solid-phase extraction

200 mg of the synthesised sorbents (HXLPP-polarA, HXLPP-polarB and HXLPP-polarC) in the form of 4-6 μm particles were packed in 6 mL poly-propylene syringes with the sorbents being retained by two frits (a metal frit of 2 μm pore size on the bottom and a polyethylene frit with 20 μm pore size on the top). The retention capabilities of the sorbents were compared to the commercial cartridges LiChrolut EN (200 mg, 6 mL) from Merck (Darmstadt, Germany), Oasis HLB (200 mg, 6 mL) from Waters (Milford, MA, USA) and Isolute ENV+ (200 mg, 6 mL) from International Sorbent Technology (Cambridge, UK). A vacuum manifold (Teknokroma, Barcelona, Spain) was used to manipulate the cartridges in the off-line SPE procedure.

Prior to the extractions, all samples were adjusted to pH 2.5 with HCl. The SPE procedure for all cartridges was the same: the cartridge was activated with 10 mL of MeOH followed by 5 mL of Milli-Q water adjusted to pH 2.5 with HCl, then the sample was loaded at a flow rate of 10 mL min^{-1} using the vacuum manifold connected to the cartridge. Finally, the compounds were eluted from the cartridge using 7 mL of MeOH.

Before injection onto the LC system, the eluate was evaporated to 1 mL and 1 mL of Milli-Q water was added to obtain a MeOH:H_2O mixture (1:1) suitable for injection onto the HPLC.

Real samples from the Ebre river and tap water were filtered through 0.22 μm nylon membranes (Supelco, Bellefont, PA, USA) prior to the pre-concentration step to eliminate the particulate matter present in real samples.

3. RESULTS AND DISCUSSION

3.1. Synthesis of the hypercrosslinked resins

Three hydrophilic, hypercrosslinked polymer resins (HXLPP-polarA, HXL-PP-polarB and HXLPP-polarC) were synthesised. The resin characterisation data is detailed in Table 1. The polymer precursors used in the hypercrosslinking reactions were three swellable, gel-type

resins (PP-polarA, PP-polarB and PP-polarC) prepared by precipitation polymerisation (PP).

PP is a simple and straightforward polymer synthesis method used to obtain, in a single step, micrometer-sized, spherical particles which, as has been demonstrated previously [15], show advantages in SPE. PP has been studied widely for polyDVB production (for examples, see [21,22]), but also for some copolymerisations which include poly(VBC-co-DVB) [23] and poly (HEMA-co-DVB) [24]. However, there are few studies reporting terpolymer production by PP, possibly because of the difficulty in establishing suitable polymerisation conditions. The aim of the present paper was to synthesise the terpolymer poly(HEMA-co-VBC-co-DVB) and thereby a resin which combines hydrophilicity (through HEMA) in a hypercrosslinked polymer (hypercrosslinking reaction through VBC) with control of shape and form of the particles (through DVB). Various attempts to establish suitable conditions for the terpolymerisation failed to deliver the desired products when all three comonomers were present at the outset of the polymerisations (data not shown). As detailed in the Experimental section, an alternative synthesis strategy was attempted which turned out to be successful. This strategy involved the delayed addition of the HEMA and VBC comonomers to a DVB polymerisation; HEMA and VBC were added once the nucleation phase of the reaction was complete. Fig. 1 outlines the synthetic procedure. As shown in Table 1, using this strategy we obtained a set of PP-polar precursor resins with variable chlorine and oxygen contents, which indicates that all three monomers were incorporated into the final resins. The incorporation of the HEMA and VBC monomers was confirmed by the FTIR spectra of PP-polar particles: absorption bands for HEMA: at ~3400 cm^{-1}, indicative of the OH group, ~1730 cm^{-1}, indicative of the C=O group and at ~1200 cm^{-1}, indicative of the C-O group; and, for VBC: by the sharp band at ~1265 cm^{-1}, which is characteristic of the CH_2Cl group.

The pendent chloromethyl groups from VBC in the PP-polar resins were subsequently consumed in hypercross-linking reactions to generate the hypercrosslinked resins, such that we produced a series of HXLPP-polar resins with variable specific surface areas and degrees of hydrophilicity, properties that were dependent upon the initial monomer contents in the polymerisation feeds. The FTIR spectra, for the HXLPP derivatives, also confirm the disappear-ance of the CH_2Cl band; meanwhile, the bands characteristic for HEMA remain relatively unchanged. It should be highlighted that, to the best of our knowledge, this is the first time that a hydrophilic hypercrosslinked polymer has been synthesised. In a previous study [16] a hypercrosslinked resin with residual -OH groups was synthesised starting from a hydrophilic monomer.

However, the -OH content was difficult to control since the -OH groups arose from hydrolysis of VBC during polymer synthesis.

Regarding particle size, all three resins had average diameters in the range 3.9-5.7 μm (see Table 1), thus are well suited for SPE purposes. However, none of the particles was monodisperse (coefficient of variation was >33%, in all cases) which can be attributed to the presence of three different co-monomers in the PP and the conditions under which the polymerisations were performed.

As an example, Fig. 1 also depicts the SEM image for HXLPP-polarB, to show the spherical shape of the particles produced, as well as their relatively narrow particle size distribution.

3.2. Evaluation of the sorbents

Before exploiting the new sorbents in SPE, the LC separation was optimised. The linear range of the method was determined by directly injecting 20 μl of the standard solutions (0.1-10 mg L^{-1} of analytes) onto the LC column. The regression coefficients (r^2) were good and ranged from 0.9989 for ibuprofen to 0.9998 for phenol and 2,4-DNP.

In the SPE process, we firstly optimised the nature and volume of the elution solvent; in this regard, several volumes of acetonitrile and methanol were tested. The recovery values obtained for 100 mL of standard solution with 10 mL acetonitrile in the elution step were not satisfactory, especially for 2,4-DNP and MCPA (35% and 28%, respectively). Thus, we decided to use methanol as the elution solvent. The recovery values obtained with 10 mL of methanol were noticeably higher in the case of 2,4-DNP and MCPA than those obtained with acetonitrile as the elution solvent. Then, we reduced the volume of methanol to below 10 mL and noticed that 5 mL of methanol was insufficient to complete elute all the studied analytes. Finally, 7 mL was found to be a sufficient volume of methanol to obtain excellent recoveries (greater than 90% for all compounds). Therefore, we chose 7 mL of methanol since it provided the quantitative elution of all compounds.

To test the performance of the resins we needed to select the SPE conditions in order to increase the retention of the compounds. We evaluated the behaviour of the resins under acidic (pH 2.5) and neutral pH (pH 7) conditions; better results were obtained under acidic conditions where ionisation of the analytes is prevented; therefore, the pH was set at 2.5 in subsequent experiments. The next step was to identify the highest sample volume which could be loaded onto the SPE cartridges without significant breakthrough. To do this, volumes from 100 to 1000 mL of Milli-Q water at pH 2.5 (with HCl) were spiked with the analytes in concentrations from 5 to 50 μg L^{-1}, depending on the volume, and then extracted.

Table 2 compares the recoveries of analytes obtained with the three hypercrosslinked materials for the higher

sample volumes percolated (*i.e.* 500 and 1000 mL). From the results shown, it can be seen that the recoveries of all analytes, even the most polar ones such as oxamyl, methomyl and phenol, have values close to 90% or higher, even for a sample volume of 1000 mL. Only for one of the sorbents (HXLPP-polarA) did phenol show a lower recovery (73%), and this was when a 1000 mL sample was extracted. This result can be explained by the lower specific surface area of HXLPP-polarA compared with the other two resins, and is in keeping with expectations [3].

In view of these preliminary evaluation results we selected the HXLPP-polarB sorbent to evaluate further the application of this type of novel sorbent to real samples. The selection of HXLPP-polarB was based mainly on the recoveries for phenol, but also because this resin offers a balance of specific surface area (850 $m^2 g^{-1}$) and hydrophilicity (7.5% O) over all the resins tested.

To broaden the scope of application of these sorbents, three additional pharmaceutical compounds which are problematic in SPE processes with conventional sorbents were tested with the HXLPP-polarB sorbent.

These compounds, which were selected in order to check the recovery of analytes with a wide range of polarity, were the two polar pharmaceuticals antipyrine and salicylic acid, and the less polar ibuprofen; antipyrine and salicylic acid are known to be particularly difficult to handle in SPE [25-27].

The recoveries (Table 3) for the SPE of 1000 mL of Milli-Q water spiked with 50 $\mu g\ l^{-1}$ of each compound were 91%, 89% and 93% (with %RSD (n = 3) < 6) for antipyrine, salicylic acid and ibuprofen, respectively.

Table 2. Recoveries (%) of the analytes obtained with different sorbents in off-line SPE for different sample volumes spiked with the analyte mixture at 50 $\mu g\ L^{-1}$ in Milli-Q water.

	Recovery (%)					
Analyte	HXLPP-polarA (670 $m^2 g^{-1}$)		HXLPP-polarB (850 $m^2 g^{-1}$)		HXLPP-polarC (925 $m^2 g^{-1}$)	
Sample volume	500 mL	1000 mL	500 mL	1000 mL	500 mL	1000 mL
Oxamyl	98	95	95	90	96	96
Methomyl	98	93	99	97	99	99
Phenol	83	73	86	85	84	83
Guaiacol	87	84	88	89	84	84
4-NP	98	95	95	90	92	93
2,4-DNP	91	88	94	91	91	92
MCPA	84	85	89	90	90	87

% Relative standard deviations (RSD) (n=3) were lower than 7.

In view of the highly satisfactory results obtained for the HXLPP-polar resins (Table 2), we tested and compared one of these resins to the commercially available sorbents LiChrolut EN, Oasis HLB and Isolute ENV+ (Table 3).

LiChrolut EN (1200 $m^2 g^{-1}$) is a hydrophobic hypercrosslinked ethylvinylbenzene-divinylbenzene copolymer, Oasis HLB (~800 $m^2 g^{-1}$) is a macroporous copolymer derived from two monomers, the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone (these monomers create a hydrophilic-lipophilic balance), and Isolute ENV+ (~1000 $m^2 g^{-1}$) which is a hypercrosslinked hydroxylated poly(styrene-co-divinylbenzene) co-polymer. From the recovery values listed in Table 3, one can see that the hypercrosslinked sorbent HXLPP-polarB gave better recovery values for the most polar compounds than commercially available LiChrolut EN and Oasis HLB, and similar recovery values to Isolute ENV+.

Table 3. Recoveries (%) of analytes on the HXLPP-polarB sorbent, LiChrolut EN, Oasis HLB and Isolute ENV+ in off-line SPE for 1000 mL of standard solution spiked with 50 μg L^{-1} of each compound in Milli-Q water

Analyte	Recovery (%)			
	HXLPP-polarB 850 $m^2 g^{-1}$	LiChrolut EN 1200 $m^2 g^{-1}$	Oasis HLB 800 $m^2 g^{-1}$	Isolute ENV+ 1000 $m^2 g^{-1}$
Oxamyl	91	84	74	95
Methomyl	91	86	66	95
Antipyrine	91	83	84	68
Phenol	86	79	78	92
Guaiacol	91	85	82	97
4-NP	91	85	86	95
Salicylic Acid	89	88	85	94
2,4-DNP	88	84	86	80
MCPA	90	84	84	93
Ibuprofen	93	86	86	99

% Relative standard deviations (RSD) (n=3) were lower than 6.

For instance, when 1000 mL of sample was percolated the recovery of methomyl was 91%, 86%, 66% and 95% for HXLPP-polarB, LiChrolut EN, Oasis HLB and Isolute ENV+, respectively. The recovery of oxamyl on all HXLPP-polar resins (see Table 2) at the same sample volume was around 95%.

The observed variation in the recovery values with the HXLPP-polarB, LiChrolut EN, Oasis HLB and Isolute ENV+ sorbents is more significant with antipyrine, since for the same sample volume (1000 mL) the antipyrine recoveries were 91%, 83%, 84% and 68%, respectively; again; the best results were

for the new HXLPP-polarB material.

Thus, in view of the detailed SPE comparison which has been carried out for all these sorbents, and bearing in mind their wide range of chemical and physical characteristics which includes variations in the type of network (macroporous or hypercrosslinked), specific surface area and hydrophilicity, the clearly beneficial features of the HXLPP-polar resins would appear to be derived from the combination of both high specific surface area, hydrophilicity, and their homogeneity in shape, relatively small particle size and relatively low particle size distribution. In particular, the impressive extraction performance of the HXLPP-polar sorbents may be ascribed to the efficiency and ease with which they can be packed reproducibly into the columns and the high chromatographic efficiency when applied in column format [15].

3.3. Application to real samples

The performance of the HXLPP-polarB sorbent when applied to real samples was investigated for three environmental water samples: mineral water, tap water and Ebre river water.

In this set of experiments, after the SPE process the eluate was evaporated to improve the overall sensitivity of the method and thereby to allow quantification of the analytes at those concentration levels typically encountered when handling real water samples. However, the eluate was not evaporated to dryness; instead, the volume was nearly reduced to 1 mL, since the recovery of volatile phenols (e.g., phenol and guaiacol) was observed to fall when the eluate was evaporated under a nitrogen stream to dryness [28,29].

Table 4 shows the SPE results for the different water samples. As can be seen, the data for mineral and river water is excellent, with recovery values close to 90% and very similar to the values obtained for extractions from Milli-Q water, demonstrating yet again the impressive ability of these sorbents to retain the most polar compounds even when in the presence of matrix interferences. Moreover, the real water SPE results for HXLPP-polarB are superior to those reported [30] for Oasis HLB and Isolute ENV+; in this study, the results obtained with commercial sorbents after percolating 350 mL of river water were 60% and 70% (Oasis HLB) and 29% and 52% (Isolute ENV+) for oxamyl and methomyl, respectively. By comparison, in the present study the recovery results with HXLPP-polarB for 1000 mL of river water sample were 93% and 91% for the same analytes, respectively. With respect to tap water, the results obtained with the HXLPP-polarB sorbent were excellent for the majority of the compounds, except for the first three compounds eluted. For oxamyl, the recovery was 29%, whereas methomyl and antipyrine were not recovered.

Generally speaking, for the rest of compounds the recoveries were rather good, especially when taking into account the fact that 1000 mL of sample was being extracted. The analogous results from the same previous study [30], support these low recoveries; for example the recoveries obtained for oxamyl were 59% (Oasis HLB) and 50% (Isolute ENV+). This effect may be due to the presence of chlorine in the sample; we did try to reduce the chlorine content prior to SPE, but were not successful in increasing the recoveries for these problematic analytes.

Since the SPE results for river water were highly satisfactory, we proceeded to validation of the method using the same type of samples. First, we aimed to reduce the initial band which appears at the beginning of the chromatogram.

Fig. 2 shows the chromatograms of 1000 mL of Ebre river sample (b) and the same sample spiked with a standard solution at 2 μg L^{-1} for each compound (a). The initial band is due to the presence of humic and fulvic acids in the sample; previous studies reported [31] that this band decreases when Na_2SO_3 is added to the sample. We investigated a similar approach by adding 10 and 2 mL of 10% (w/v) Na_2SO_3 aqueous solution per 1000 mL of sample. Unfortunately, although the band decreased slightly the salt precipitated during the SPE loading step and partially blocked the cartridge.

Table 4. % Recoveries of the analytes on the HXLPP-polarB sorbent in off-line SPE for 1000 mL of standard solution spiked at 50 μg L^{-1} with each compound in mineral, tap and Ebre river water.

	Recovery (%)		
Compound	**Mineral water**	**Tap water**	**River water**
Oxamyl	93	29	93
Methomyl	89	-	91
Antipyrine	93	-	93
Phenol	78	76	84
Guaiacol	90	81	92
4-NP	97	93	97
Salicylic Acid	97	90	100
2,4-DNP	96	92	98
MCPA	97	137	97
Ibuprofen	98	96	96

% RSD (n=3) were lower than 8.

Therefore, in spite of the good results reported in a previous paper where this strategy was applied successfully, we discarded this as a viable option. Several samples of river water were analysed. Subsequent analyses of water samples taken from different locations on the Ebre river revealed that in one of the

samples three peaks appeared at the retention time corresponding to methomyl, phenol and 2,4-DNP (see the Ebre river blank chromatogram Fig. 2b), but this observation needs to be confirmed in an independent study using a more powerful detector, such as a mass spectrometer.

In the validation studies using 1000 mL of river water, all the analytes exhibited good linearity from 0.5 to 20 μg L^{-1}, with the exception of oxamyl and methomyl (1-20 μg L^{-1}), with regression coefficients (r^2) higher than 0.9995.

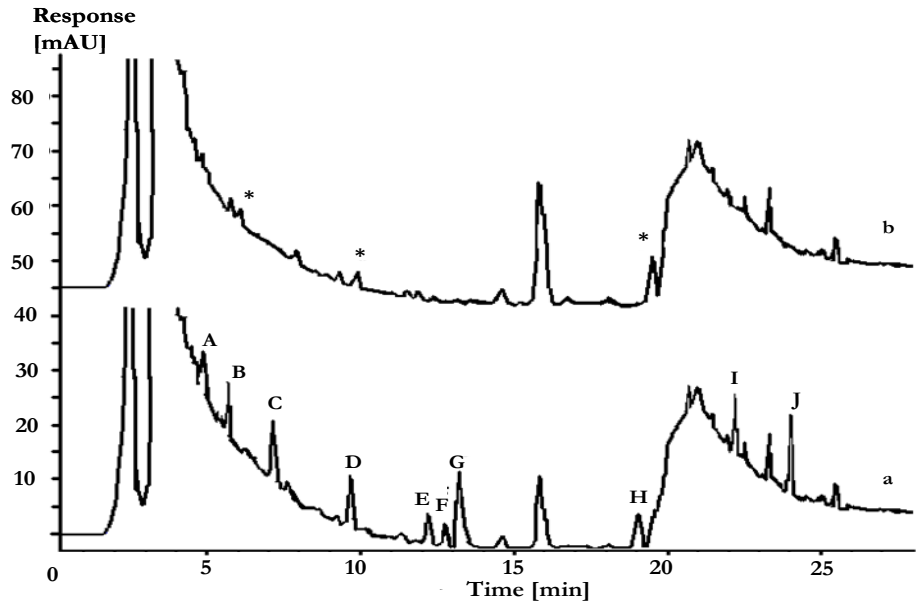

Fig. 2. Chromatograms obtained by off-line trace enrichment of 1000 mL of Ebre river water samples with (a) and without (b) the addition of 2 μg l^{-1} level of analytes. Peak designation: (A) oxamyl, (B) methomyl, (C) antipyrine, (D) phenol, (E) guaiacol, (F) 4-NP, (G) salicylic acid, (H) 2,4-DNP, (I) MCPA, (J) ibuprofen. *Peaks at the same retention time as the studied analytes.

The detection limits (LODs), calculated using a signal to noise ratio of ≥3, were 0.2 μg L^{-1} for most of compounds, with the exception of oxamyl, methomyl, guaiacol and salicylic acid (0.5 μg L^{-1}). The repeatability and reproducibility of the method, expressed as the relative standard deviation (RSD) of three analyses of 1000 mL of Ebre river water spiked at 1 μg L^{-1} were lower than 15% for all compounds. Although the limits of detection are not as low as those reported elsewhere for these kind of samples, they could be lowered significantly by use of a more sensitive detection method such as mass spectrometer, or even tandem mass spectrometry. In any case, the excellent performance of the HXLPP-polarB sorbent has been demonstrated unequivocally.

4. CONCLUSIONS

Three hydrophilic, hypercrosslinked sorbents in the form of micrometer-sized polymer microspheres were synthesised successfully from poly (HEMA-co-VBC-co-DVB) precursors of varying chemical composition.

The hypercrosslinked sorbents varied in terms of their hydrophilicity and had tuneable specific surface areas. The three sorbents were evaluated in off-line SPE studies for the extraction of polar analytes from water samples, and were found to perform well. The sorbent with optimal performance characteristics (HXLPP-polarB) was selected for further evaluation.

In comparative SPE studies with the commercial sorbents LiChrolut EN, Oasis HLB and Isolute ENV+, the HXLPP-polarB sorbent significantly out-performed the commercial materials.

When real samples were extracted, recoveries of analytes from 1000 mL of mineral or river water were high, even for the most polar compounds, such as oxamyl, methomyl, phenol and salicylic acid.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.chroma.2009.08.091.

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Supplementary information

Hypercrosslinking reaction

For the hypercrosslinking reactions, the PP-polar particles (2 g) and 1,2-DCE (40 mL) were placed in a round-bottomed flask (100 mL), and the mixture left under nitrogen for 1 hour to swell the beads. Then, $FeCl_3$ (in a 1:1 molar ratio of CH_2Cl:$FeCl_3$) suspended in DCE (40 mL) was added. The mixture was then heated rapidly to 80 ˚C and kept at this temperature for 18 hours.

The hypercrosslinked particles were filtered on a nylon membrane filter and washed with MeOH and then washed several times with aqueous HNO_3 (pH 1). They were then extracted overnight with acetone in a Soxhlet extractor and washed again with MeOH and diethyl ether before drying overnight *in vacuo* at 40 ˚C.

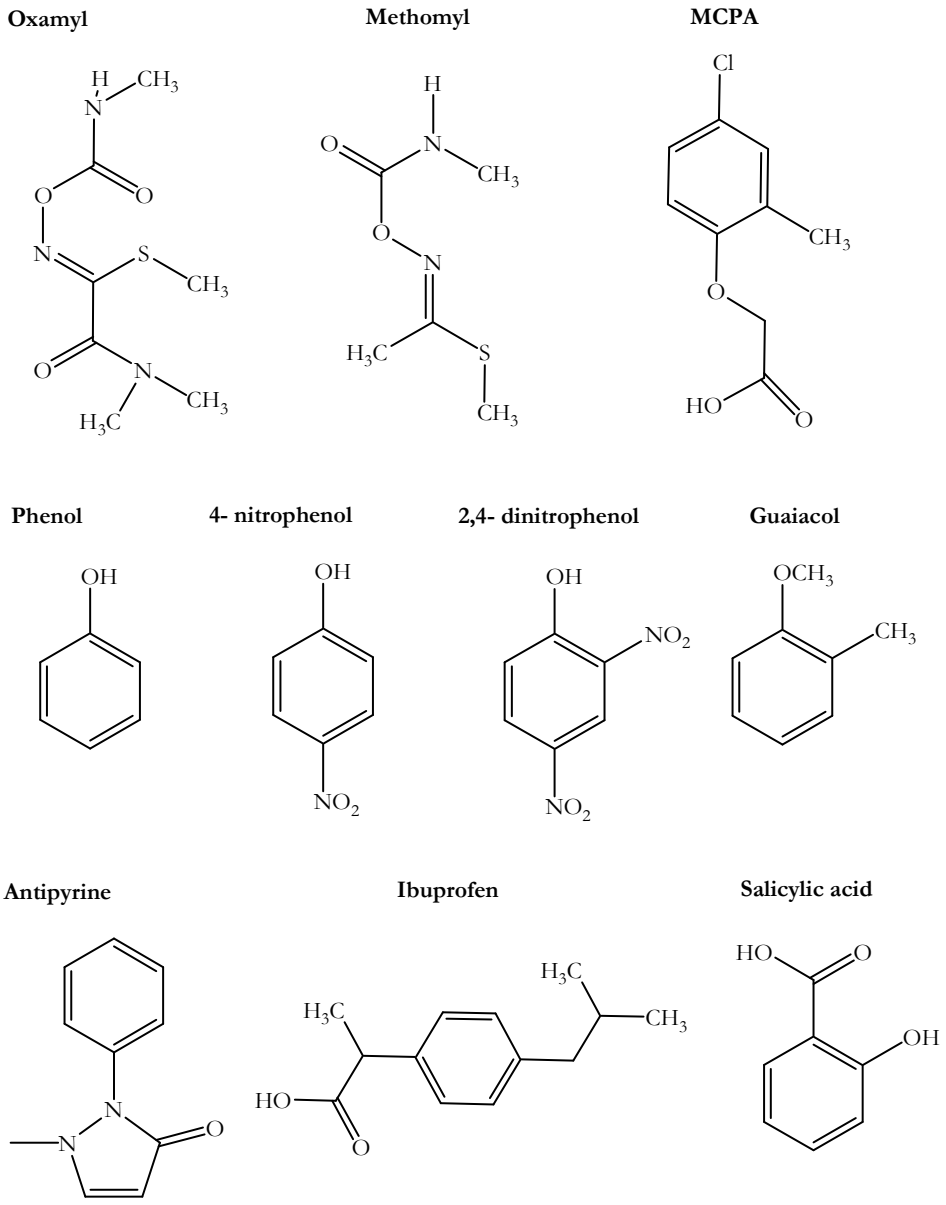

Fig. 1S. Chemical structures of the compounds studied.

3.1.2. Discussion of results

In the presented paper, three different hypercrosslinked sorbents have been successfully synthesised from copolymeric precursors prepared by PP, introducing polarity into the hypercrosslinked polymer structure.

The results demonstrate that PP is a powerful tool for preparing suitable precursors in form of monodisperse polymer microspheres for the subsequent hypercrosslinking process. The great advantage of this procedure is that a hypercrosslinked sorbent suitable for SPE applications can be obtained without using any additional stabiliser during polymerisation. Consequently, the presence of undesirable residuals in the final system is minimal.

As previously mentioned, the final sorbents consist of smooth, spherical particles. Although none of the synthesised particles was totally monodisperse, they exhibited relatively narrow size distribution (e.g. 3.9±1.3 or 5.7±2.4 μm) within the suitable range for SPE applications.

As can be seen from the results, the application of a sorbent with a balance between the content of hydroxyl group and specific surface area provided highly satisfactory results. In comparison to commercially available polymers designed to retain a broad range of molecules, including polar ones, the superior performance of the HXLPP-polarB sorbent in the retention of the studied analytes suggests that the lower particle size of the HXLPP-polarB sorbent may provide better contact with the extracted analytes, and therefore increase extraction efficiency.

Taking into consideration the excellent SPE performance and desired properties of the sorbent, the HXLPP-polarB sorbent can be considered a good candidate as a sorbent in on-line SPE applications. On-line SPE has been already performed with the HXLPPa (1320 $m^2 g^{-1}$) sorbent, which is a hydrophobic hypercrosslinked sorbent, whose precursors were also obtained by PP [9]. In the extraction of polar analytes from similar samples, HXLPPa had already demonstrated that hypercrosslinked sorbents with micron-sized particles can be successfully applied to on-line SPE, providing quantitative results for a group of polar analytes.

Comparing the results of off-line SPE using HXLPP-polarB and those from on-line SPE performance using HXLGp and HXLGmix sorbents [8,13] which also

contain hydroxyl moieties due to the hydrolysis process, it can be concluded that their presence in the polymer skeleton enhances the retention of polar compounds. The excellent performance of these various sorbents indicates that hydrophilic hypercrosslinked polymeric sorbents with a suitable combination of polar character and specific surface area are very efficient for the extraction of polar analytes. In addition, the combination of hydrophobic and hydrophilic interactions is desirable for their great retention capabilities.

The promising results obtained from the enhanced hydrophilic hypercrosslinked materials encourage further development in the synthesis and application of new polymers with hydrophilic characteristics. The synthesis of sorbents with a polar character, large specific surface area and microporous structure is of great potential in SPE to enhance the extraction of polar analytes. Hypercrosslinked sorbents provided a significant increase in capacity. However, as can be observed in some applications, the improvement of their selectivity is still a challenge, since they are able to retain indistinctly all compounds from the samples. As we can see in this study, we did not manage to remove all the interferences (mainly humic and fulvic acids) with the addition of Na_2SO_3 to the sample before extraction. Therefore, it would be desirable to prepare sorbents that, on the one hand, maintain their capacity, but on the other hand, possess a certain degree of selectivity in order to prevent the presence of certain interferences. Therefore, future steps in the development of new sorptive materials for SPE will focus on improving selectivity in the extraction of polar compounds.

3.1.3. References

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3.2. Synthesis and application of new mixed-mode sorbents for solid-phase extraction

As stated in the Introduction, over the past few years, the research into polymeric sorbents has been directed towards enhancing selectivity and capacity. Continuing our research and using synthetic approaches, we aimed to design and tailor the sorptive materials to interact with the analyte or analytes of interest selectively, withdrawing all other compounds, including interferences. Typical examples of selective sorbents are immunosorbents (IS) or molecularly imprinted polymers (MIPs). However, in most cases, one of the drawbacks of these materials is their limited capacity. Although the selectivity and the capacity can be magnified separately, recent developments are focused on the dual-phase or mixed-mode sorbents, which combine these properties in a single material [1].

Generally speaking, SPE procedure using mixed-mode sorbents may provide cleaner extracts compared to reversed-phase (RP) sorbents. It is also worth mentioning that the selectivity of mixed-mode sorbents arises from the additional clean-up step. As is well-known, qualitative and quantitative analysis of complex matrix samples requires clean extracts from the SPE procedure that eliminates interferences and retains target analytes, and thus gains in both sensitivity and selectivity. Furthermore, highly clean extracts have become more important in efforts to avoid ion suppression/enhancement effects when SPE extracts are analysed using LC-(ESI)MS/MS [2].

Therefore, in further analysis, clean extracts allows sensitive detection of analytes and improves the overall performance of the method. For instance, Galera *et al.* [3] used commercially available Oasis MCX for the determination of β-blockers in groundwater. In order to decrease matrix effects, the authors included a clean-up step in the SPE procedure prior to the elution of the target compounds that resulted in cleaner extract with lower amounts of co-extractives and negligible matrix effects, providing quantitative recoveries.

Although there are several commercially available mixed-mode sorbents commonly used in SPE, such as the mixed-mode series of Oasis or Strata-X, all of them are macroporous and their capacity can be improved. Therefore, ongoing research aims to find ways to enhance their properties including capacity and selectivity. One approach to improve the capacity of the sorbents is by synthesising hypercrosslinked sorbents, whose high content of micropores

provides large specific surface areas, and thus more interaction sites with the analytes, as demonstrated in the previous section.

Previously, our group disclosed two hypercrosslinked mixed-mode weak anion-exchange resins, which were further modified with either ethylene diamine or piperazine (HXLPP-WAX) to prepare two materials combining ion-exchange interactions and high micropore content [4,5]. These very promising results encouraged us to extend the hypercrosslinked mixed-mode sorbent series.

In this chapter, we report on the preparation of hypercrosslinked sorbents with weak cation-exchange (WCX) and strong anion-exchange (SAX) characters and their applicability for the selective extraction of basic or acidic analytes, respectively, from different water samples. Based on our research group's previous experience in developing new mixed-mode materials [4,5], we also selected PP to generate the precursor polymers. However, the synthesis protocols were slightly different, considering the functional group of the sorbents.

The WCX sorbent is modified by a weak anion group and the chargeability of the sorbent and the analytes can be tuned due to the weak acidity of the ionic group which modifies the sorbent. Meanwhile, in the case of SAX sorbents, a strong cationic moiety modifies the polymeric skeleton and the chargeability of the analytes or interferents, but not the chargeability of the sorbent due to its strong alkalinity. These features of the WCX and the SAX sorbents make them suitable for selective extraction of basic and acidic compounds, respectively. Bearing in mind that the selectivity of the extraction process depends not only on the selection of a suitable sorbent, but also on the suitable SPE protocol, we optimised the SPE procedures for each type of sorbent to enhance its specific properties. Another aspect that we were interested in was the comparison of the SPE performance of these new mixed-mode hypercrosslinked sorbents to commercially available materials.

The goal of the presented work was to show the great potential of hypercrosslinked mixed-mode sorbents to enhance the selective extraction of ionisable analytes. All the results derived from the studies related to the HXLPP-WCX sorbent were published in the *Journal of Chromatography A* 1217

(2010) 1575-1582. The manuscript including the results derived from the application of HXLPP-SAX has been submitted for its publication in the same journal.

3.2.1. Synthesis and application of hypercrosslinked polymers with weak cation-exchange character for the selective extraction of basic pharmaceuticals from complex environmental water samples

SYNTHESIS AND APPLICATION OF HYPERCROSSLINKED POLYMERS WITH WEAK CATION-EXCHANGE CHARACTER FOR THE SELECTIVE EXTRACTION OF BASIC PHARMACEUTICALS FROM COMPLEX ENVIRONMENTAL WATER SAMPLES

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Abstract

The synthesis of high specific surface area sorbents (HXLPP-WCX) in the form of hypercrosslinked polymer microspheres with narrow particle size distributions, average particle diameters around 6 μm, and weak cation-exchange (WCX) character, is described. The WCX character arises from carboxylic acid moieties in the polymers, derived from the comonomer methacrylic acid. A novel HXLPP-WCX sorbent with an attractive set of chemical and physical properties was then used in an off-line solid-phase extraction (SPE) protocol for the selective extraction of a group of basic compounds from complex environmental samples, a priority being the clean separation of the basic compounds of interest from acidic compounds and interferences. The separation power of the new sorbent for basic pharmaceuticals was compared to two commercially available, mixed-mode sorbents, namely Oasis WCX and Strata-X-CW. Under identical experimental conditions, HXLPP-WCX was found to deliver both higher capacity and better selectivity in SPE than either of the two commercially available materials. In an optimised SPE protocol, the HXLPP-WCX sorbent gave rise to quantitative and selective extractions of low μg L^{-1} levels of basic pharmaceuticals present in 500 mL of river water and 250 mL of effluent waste water.

Keywords: *Hypercrosslinked sorbents; Polymer microspheres; Mixed-mode weak cation-exchanger; Solid-phase extraction; Basic compounds*

1. INTRODUCTION

Solid-phase extraction (SPE) is a powerful analytical tool used widely for preconcentration, fractionation and purification of analytes of interest from complex environmental [1-4] and biological samples (urine, blood and plasma) [5,6]. In recent years, a group of analytes of increasing interest is pharmaceuticals, since they are dispersed continuously into the environment as a result of human use [5,7], which can give rise to problems including health concerns for humans, therefore there is a demand for analytical methods which enable the accurate determination of pharmaceuticals in the environment, even when the pharmaceuticals are present at low levels. To satisfy this demand, and meet the appropriate detection limits, there is a requirement for suitable preconcentration techniques which can both concentrate and clean-up the analytes present in the complex environmental matrices. SPE is excellent choice in this regard since it can provide high enrichment factors of the target compounds and eliminate interferences from the sample to be analysed. Polymeric materials are the most important group of sorbent used in SPE, since they offer attractive advantages such as good retention of analytes and sorbent stability under a much broader range of analysis conditions than for sorbents of other types (i.e., silica- and carbon-based sorbents). Several polymeric sorbents have been developed and applied to the extraction of pharmaceuticals [8-10].

In recent years, SPE technology has expanded to offer the use of mixed-mode, polymeric ion-exchange media, which combines the attributes of reversed-phase chemistry and ion-exchange interactions into one single material [11]. Mixed-mode ion-exchange sorbents are designed to interact with ionic species, but they can also retain non-charged species effectively through hydrophobic or hydrophilic interactions [11,12].

Mixed-mode sorbents are classified as either strong or weak ion-exchange, depending on the ionic groups tethered to the sorbent. An important advantage of weak ion-exchange sorbents is that the ionisation state of the resin may be tuned easily by pH, thus adding more versatility and power to SPE applications [12]. Amongst the most popular, comercially available mixed-mode sorbents are Oasis MCX, Oasis MAX, Oasis WCX, and Oasis WAX (all from Waters), which are classified as strong (MCX, MAX) or weak (WCX, WAX) cation/anion-exchange resins, respectively. All four of these interesting sorbents are derived from an Oasis HLB polymeric skeleton [poly(vinylpyrrolidone-co-divinylbenzene), ~800 $m^2 \cdot g^{-1}$] which has been modified chemically with sulfonic acid and quaternary ammonium groups in the case of the strong ion-exchangers, and carboxylic acid and piperazine groups in the case of the weak ion-exchangers.

In an analogous fashion, Strata-X (a Phenomenex sorbent), which is based on a poly(styrene-co-divinylbenzene) skeleton bearing polar vinylpyrrolidone residues, can be modified chemically to give related sorbents bearing sulfonic acid groups (Strata-X-C), carboxylic acid groups (Strata-X-CW) or ethylene diamine groups (Strata-X-AW).

All of these commercial sorbents have macroreticular structures which give rise to weaker reversed-phase interactions with analytes than do hypercrosslinked polymer resins.

Hypercrosslinked polymers are a new generation of permanently porous, polymeric resins with enhanced analyte retention characteristics arising from their high micropore contents and correspondingly high specific surface areas (>1000 m^2 g^{-1}) [13]. Recently, we disclosed the synthesis of mixed-mode hypercrosslinked sorbents with weak anion-exchange (WAX) character, and the application of these novel sorbents to the SPE of acidic pharmaceuticals from aqueous samples [14].

The present study describes the synthesis of hypercrosslinked polymer resins with weak cation-exchange (WCX) character, where the WCX properties are derived from the presence of carboxylic acid moieties, and the application of these sorbents to the SPE of basic pharmaceuticals from complex environmental samples. The new materials have been bench-marked against Strata-X-CW and Oasis WCX. Although previously porous polymer containing methacrylic acid (MAA) in monolith format has been applied to in-tube-solid-phase microextraction-liquid chromatography (SPME-LC) for the extraction of drugs from complex sample matrices [15]; as far as we are aware, this is the first time that a hypercrosslinked sorbent has been used as a weak cation-exchanger for the selective extraction of basic pharmaceuticals from complex environmental samples, which also contain acidic and neutral compounds.

2. EXPERIMENTAL

2.1. Reagents and standards

The reagents used for the polymer syntheses were divinylbenzene (DVB) (80% grade) supplied by Aldrich (Steinheim, Germany), methacrylic acid (MAA) (98% grade) and para-vinylbenzyl chloride (VBC) (95% grade) supplied by Fluka (Buchs, Switzerland). DVB and VBC were purified by passing through short columns packed with neutral alumina. MAA was purified by vacuum distillation. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck, Darmstadt, Germany). Ferric chloride ($FeCl_3$) and anhydrous 1,2-dichloroethane (DCE), from Aldrich, were used in the hypercrosslinked reactions.

The pharmaceutical analytes selected to evaluate the performance of the

sorbents in SPE were: acetaminophen, caffeine, antipyrine, propranolol, carbamazepine, naproxen and diclofenac (all obtained from Sigma-Aldrich). The chemical structures and pK_a values of the analytes are presented in Table 1.

Standard solutions at 1000 mg L^{-1} in methanol were prepared for each analyte. The mixture of all the analytes was prepared by diluting the standard solutions with MeOH:H_2O (1:1, v/v).

LC-grade acetonitrile and methanol (SDS, Peypin, France) and Milli-Q water (Millipore, Bedford, MA, USA) were used to prepare the mobile phases. Hydrochloric acid (Probus, Barcelona, Spain) was used to adjust the pH of the mobile phase and the sample before SPE. Other reagents used in SPE procedures were: ammonium hydroxide (NH_4OH) (Merck), formic acid (HCOOH) (Probus) and trifluoroacetic acid (TFA) (Fluka).

2.2. Resin preparation and characterisation

The micron-sized spherical particles (PP-WCX) used as swellable precursors in the production of the hyper-crosslinked resins (HXLPP-WCX), were synthesised using an optimised precipitation polymerisation (PP) protocol [16]. The monomers (10% MAA, 50% VBC and 40% DVB [w/w%]) and AIBN (2 mol% relative to polymerisable double bonds) were dissolved in acetonitrile (200 mL) in a polypropylene bottle (250 mL) at a total monomer concentration of 2 w/v%. The monomer solution was de-oxygenated with N_2 at 0 ºC and the bottle then placed on a low-profile roller (Stovall, Essex, UK) in a temperature-controllable incubator (Stuart Scientific, Surrey, UK).

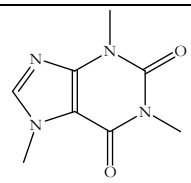
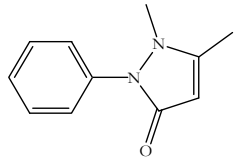
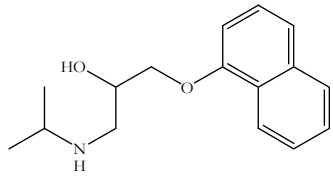
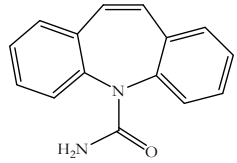
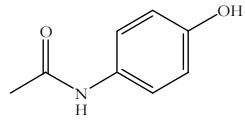
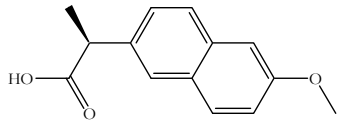
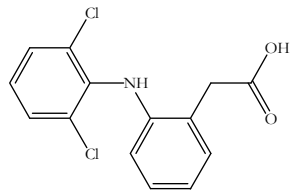
The temperature was ramped from ambient to 60 ºC over a period of 2 h and the polymerisation allowed to proceed at 60 ºC for a further 46 h. The resulting particles were filtered on a 0.2 μm nylon membrane filter and washed successively with MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 ºC.

The hypercrosslinked reactions of the MAA-VBC-DVB precursors were carried out as described in previous study [16], using a well-established reaction for the VBC-DVB precursor.

The hypercrosslinked resin (HXLPP-WCX) was characterized by measuring specific surface area using a BET treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The carbon (83.1 w/w%), hydrogen (7.2 w/w%), chlorine (2.5 w/w%) and oxygen (6.3 w/w%, calculated by difference) contents for the resin were obtained by elemental microanalysis using a Carlo-Erba EA 1106 Instrument. The cation-exchange capacity was calculated from the microanalytical data using the theoretical values.

The average microsphere diameter and homogeneity in size (particle size distribution) were calculated using ImageJ software from the image analysis

Table 1. Chemical structures and pK_a values of the selected analytes.

Compound	Type of compound	Structural Formula	pK_a [a]
Caffeine	CNS[b] stimulant		13.4
Antipyrine	Analgesic		13.3
Propranolol	β-blocker		9.5
Carbamazepine	Anti-epileptic		13.7
Acetaminophen	Analgesic		9.7
Naproxen	NSAID[c]		4.8
Diclofenac	NSAID[c]		4.2

[a] pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs)
[b] Central nervous system
[c] Non-steroidal anti-inflammatory drug.

of 100 individual particles in scanning electron microscopy (SEM) images, which were acquired using a JOEL 6400 Instrument. SEM image for the HXLPP-WCX resin is included in Figure 1S of the Supported Information Section. A schematic representation of the structure of HXLPP-WCX is depicted in Fig. 1.

The characterisation data for all the sorbents studied is detailed in Table 2.

2.3. Chromatographic equipment and conditions

The chromatographic experiments were performed with an HP 1090 Liquid Chromatograph equipped with an injection valve with a 20 µl loop and an Agilent 1200 UV spectrophotometric detector (Agilent, Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm I.D. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain). The mobile phases were Milli-Q water adjusted to pH 3 with HCl (solvent A) and acetonitrile (solvent B). The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 65 ºC. The gradient profile was from 10 to 15% ACN in 5 min, then to 100% ACN in 25 min (held for 2 min), then the mobile phase was returned to the initial conditions (10% ACN) in 3 min.

The detection wavelength for all the compounds was 210 nm.

2.4. Solid-phase extraction

SPE cartridges (6 mL, polypropylene) were packed with 200 mg of the HXLPP-WCX sorbent. The sorbent was retained by two frits: a 2 µm pore size metal frit at the bottom, and a 20 µm pore size polyethylene frit at the top. The retention capabilities of the novel sorbent was compared to commercial SPE cartridges from Phenomenex (Strata-X-CW; 200 mg/6 mL) and Waters (Oasis WCX; 200 mg/6 mL) (which was packed manually).

A vacuum manifold (Teknokroma) was used to manipulate the cartridges in the off-line SPE process. One single sorbent cartridge of each type was used for the whole study. The three sorbent structures are presented in Fig. 1.

Prior to the SPE extractions, the pH of the sample was adjusted to 7 with HCl or NaOH. The procedure used for all cartridges was identical: the cartridge was activated with 5 mL of MeOH followed by 2 mL of Milli-Q water, and the sample then loaded at a flow rate 10 mL min^{-1}. After equilibration, the cartridge was washed with 2 mL 5% NH_4OH in MeOH. Finally, the compounds were eluted from the cartridge using 5 mL of 2% TFA in MeOH.

Prior to LC analyses, the SPE eluates were evaporated to dryness and then reconstituted in 1 mL of MeOH:H_2O (1:1, v/v).

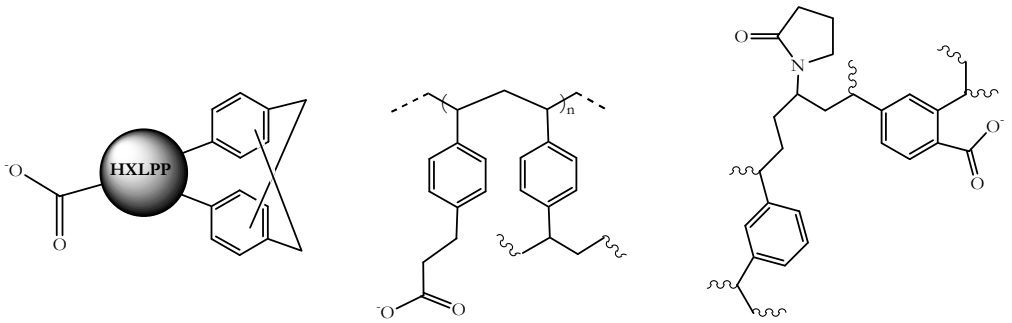


Fig. 1. Chemical structures of the sorbents tested: HXLPP-WCX, Strata-X-CW and Oasis WCX.

To keep the samples under proper conditions, real water (Ebre river water and effluent waste water from a treatment plant) were adjusted to ~pH 3 with HCl and kept at 4 °C before analysis. They were filtered through 0.22 μm nylon membranes (Supelco, Bellefonte, PA, USA) prior to the preconcentration step to eliminate the particulate matter which is normally present in real samples.

3. RESULTS AND DISCUSSION

3.1. Preparation of the HXLPP-WCX sorbent

The novel WCX hypercrosslinked sorbent (HXLPP-WCX) was derived from a swellable copolymer precursor (PP-WCX) prepared by precipitation polymerisation (PP). PP is a simple, straightforward and reproducible method for obtaining, in one single preparative step, spherical polymer particles with average diameters in the low-micron size regime which, as has been demonstrated previously [14,17, 18], perform well as novel sorbents in SPE applications. The aim of the present work was to synthesise a hypercrosslinked derivative of the ter-polymer poly(MAA-co-VBC-co-DVB), and thereby access a resin which combined both weak cation-exchange character (through the MAA residues present) and high specific surface area derived from its high micropore content (from hypercrosslinking reactions which consume the pendent chloromethyl groups). During the production of the poly(MAA-co-VBC-co-DVB) precursor polymer, various comonomer ratios were evaluated (data not shown); the

comonomer ratio reported in the present manuscript (i.e., 10% MAA, 50% VBC, 40% DVB [w/w]) was found to offer the optimal balance of properties, i.e., suitable ion-exchange capacity and particle size, and high specific surface area.

The resin characterisation data for HXLPP-WCX is detailed in Table 2. The specific surface area was 1125 $m^2 g^{-1}$ and the cation-exchange capacity 0.72 mequiv. g^{-1}. Following on from the development of a convenient synthetic route into an HXLPP-WCX resin, our aim was to evaluate the potential benefits in SPE of introducing carboxylic acid moieties into hypercrosslinked polymer microspheres, and to compare the performance of this new resin to the commercially available sorbents (more specifically, Oasis WCX and Strata-X-CW).

The characterisation data for all three sorbents is detailed in Table 2. The ion-exchange capacity is similar for all three sorbents (~0.75 mequiv. g^{-1}), however, the particle size of the HXLPP-WCX sorbent (~6 μm) is markedly lower than either of the other two materials. The lower particle size of the sorbent might provide better contact with the analytes to be extracted, and, thus, benefit in the SPE process.

3.2. SPE optimization

Pharmaceuticals bear a variety of functional groups and they can be cationic, anionic or zwitterionic depending on the sample pH. Some pharmaceuticals contain nitrogen-containing functional groups which are basic and will therefore be readily protonated to give a cation under certain conditions [19,20]. To evaluate the cation-exchange properties of the HXLPP-WCX sorbent, and establish the scope of its sorption characteristics, we selected a group of acidic and basic pharmaceuticals with variable pK_a values (Table 1).

Table 2. Characterisation data for the sorbents tested in SPE.

	HXLPP- WCX[a]	**Oasis WCX[b]**	**Strata X-CW[b]**
	Laboratory synthesized	Waters	Phenomenex
Yield (%)[c]	85	n.d.	n.d.
I.E.C.[d] (mequiv. g^{-1})	0.72	0.75	0.74
Specific surface area ($m^2 g^{-1}$)	1125	800	800
Average particle size (μm)	6.1±1.6	30	33

n.d. no data
[a] Data measured experimentally
[b] Data provided by the supplier
[c] Relative to the mass of the corresponding (non-hypercrosslinked) precursor particles.
[d] Ion-exchange capacity.

Besides the selection of analytes with a wide range of acidic/basic properties, to test the performance of the WCX sorbents in an accurate and reliable manner it was necessary to optimise the SPE conditions in such a way as to maximise the retention of the analytes on the sorbents. Optimal retention conditions are those for which ionic interactions between the MAA residues in the sorbent and the cationic forms of the analyte are maximised.

3.2.1. Sample loading

Since HXLPP-WCX is a cation-exchange material, the analyte retention mechanism is based on ionic interactions between carboxylic acid groups in the polymer and the pharmaceuticals. Thus, the pH of the sample during analyte extraction by the sorbent is an important parameter to be optimised.

To investigate the retention properties of the HXLPP-WCX sorbent, 100 mL volumes of two separate samples (at pH 3 and pH 7, respectively) were percolated through SPE cartridges packed with the sorbent. At pH 3 the carboxylic acid groups of the acidic compounds and the sorbent are primarily in their non-ionised form, whereas the basic compounds are fully ionised.

In contrast, at pH 7 the carboxylic acid-containing acidic compounds are deprotonated and are eluted during the SPE washing step, while the carboxylic acid residues in the polymer are ionised and retain the basic pharmaceuticals (protonated) by ionic interactions.

Thus, cation-exchange phenomena are expected to be more effective at pH 7 than at pH 3. When preliminary SPE experiments were performed to confirm these expectations, the recoveries of the basic compounds were found to be lower at pH 3 and very high at pH 7. For this reason, samples were adjusted to pH 7 in all the subsequent SPE experiments.

3.2.2. Washing step

The aim of the washing step was to eliminate interferences (including acidic and neutral compounds) bound to the sorbent through reserved-phase mechanisms, while retaining on the sorbent the basic compounds bound through cation-exchange interactions. 1 mL volumes of various neat organic solvents (such as methanol and acetonitrile) were applied in the washing step, but in such cases all the analytes were eluted. Thus, we decided to use a solution of NH_4OH in organic solvent as the washing solution, to maintain the desired ionisation state of the analytes and the sorbent. In this regard, the following solutions were evaluated: 5% NH_4OH in MeOH; 5% NH_4OH in ACN; 5% NH_4OH in MeOH/ACN (1/4). Of these three options, 5% NH_4OH in MeOH gave higher recoveries for all analytes than the other two washing solution and was thus selected as the washing solvent of choice. Thereafter, the next step was to evaluate the optimum volume of the washing solvent to be used in the SPE

protocol. For these experiments, where the sample matrix was Milli-Q water, 1 mL of 5% NH_4OH in MeOH was used initially. Although this volume of washing solvent was found to be not enough to elute all the acidic compounds quantitatively, 2 mL of 5% NH_4OH in MeOH was found to be effective for this purpose so was established as the optimal volume of washing solvent required to elute acidic compounds and interferences, whilst still allowing total retention of the analytes of interest (i.e., basic compounds).

3.2.3. Elution of basic compounds

For the elution step, in which the aim was to elute the basic compounds bound to WCX sorbents through ionic interactions, various acidic solutions were tested (acidification protonates the carboxylic acid residues on the sorbents, breaks the cation-exchange interactions and leads to release of the basic analytes from the sorbents thanks to the elution strength of the organic solvent also present in the solution). For this purpose, 5 mL aliquots of 2% HCOOH in MeOH, 2% TFA in MeOH and 2% TFA in MeOH/ACN (1/4) were investigated. Since 2% TFA in MeOH delivered the best results (higher recoveries than for 2% HCOOH in MeOH), and did not give any significant disturbance in the LC separation of the analytes, it was selected for use in the elution step. 2% TFA in MeOH/ACN (1/4) delivered good results also, but required longer evaporation times. 5 mL of 2% TFA in MeOH was found to be sufficient to elute completely all of the basic compounds, so was set as the optimal volume of elution solvent.

3.2.4. Volume of sample

Once the SPE protocol had been established, the effect of varying the volume of sample in the loading step (from 100 to 1000 mL) was investigated as a manner to predict the extraction capacity of the sorbent. The HXLPP-WCX sorbent gave rise to good recoveries of analytes even when the sample volume was 1000 mL (Table 3). Typically, the recoveries of the basic analytes were close to 100% for the HXLPP-WCX sorbent. Only for antipyrine did the HXLPP-WCX resin gave rise to a small degree of fractionation; for example, when 1000 mL of sample spiked at 20 $\mu g\ L^{-1}$ with the analyte mixture were extracted, the recovery of antipyrine in the elution step was 79%, with the remainder (15%) being eluted in washing step. In view of the pK_a (13.3) and chemical structure of antipyrine, this behaviour may be attributable to the stronger retention of antipyrine through hydrophobic interactions.

Therefore, we have demonstrated that the HXLPP-WCX sorbent is highly effective in extracting basic analytes in a quantitative manner from high volume (1000 mL) aqueous samples, after a washing step with 2 mL of 5% NH_4OH in MeOH, a feature which helps greatly in the removal of interferences from the sample matrix.

3.3. Comparison to commercial sorbents

The SPE performance of the HXLPP-WCX sorbent was compared to Strata-X-CW and Oasis WCX. The former had a specific surface area of 1125 $m^2 \cdot g^{-1}$ (arising from the high micropore content) whereas the commercially available sorbents, which are not hypercrosslinked, have lower specific surface areas (800 m^2 g^{-1}). A second notable difference between the HXLPP-WCX sorbent and the commercially available sorbents is the particle size; the HXLPP-WCX sorbent is in the form of microspheres with average particle diameter around 6 μm, whereas the average particle size of Strata-X-CW and Oasis WCX are both significantly larger at around 30 μm. The SPE results arising from use of the three different resins are presented in Table 3. It can be seen that the analyte recoveries were higher for all compounds with HXLPP-WCX than either Strata-X-CW or Oasis WCX. When varying sample volumes were percolated through the Strata-X-CW and Oasis WCX cartridges, most of the compounds were either eluted in the washing step or fractionated between the washing and the elution steps; the retention of certain analytes was also low compared to HXLPP-WCX.

When 1000 mL of sample was percolated through Strata-X-CW, the recoveries of the acidic analytes in the washing step were 9% for acetaminophen, and close to 50% for naproxen and diclofenac. It was also observed that all of the basic analytes were fractionated (see Table 3).

Table 3. Recovery values (%) when the HXLPP-WCX, Strata-X and Oasis WCX sorbents were applied in SPE for the preconcentration of 1000 mL of a Milli-Q sample spiked at 20 μg L^{-1} with the analyte mixture.

Analytes	Type	HXLPP-WCX		Strata X-CW		Oasis WCX	
		Wash	Elution	Wash	Elution	Wash	Elution
Caffeine	Basic	5	93	47	17	60	5
Antipyrine		15	79	55	11	91	0
Propranolol		0	93	48	40	73	13
Carbamazepine		4	107	31	46	90	15
Acetaminophen	Acidic	87	0	9	0	17	0
Naproxen		99	6	47	17	74	3
Diclofenac		94	13	45	22	77	11

For the experimental conditions, see text.
%RSD (n=3) were lower than 12% for %R >10%.

The Oasis WCX sorbent, which has properties similar to Strata-X-CW, was found to be even less useful than Strata-X-CW for the capture of basic pharmaceuticals; for Oasis WCX all the compounds retained very poorly and were eluted primarily during the washing step.

Another interesting feature relates to the retention behaviour of naproxen and diclofenac, which have pK_a values of 4.8 and 4.2, respectively. These compounds were eluted nearly quantitatively (%R ~100%) during the washing step when percolated through the HXLPP-WCX sorbent, but they were not recovered completely by the commercial sorbents, which can be attributed to losses of these analytes during the loading step. This behaviour for this pair of analytes may be due to the weaker reversed-phase retention mechanisms operating for the commercially available sorbents. In any case, it is evident that, the HXLPP-WCX sorbent gives higher recoveries for all of the target analytes than the two commercial WCX sorbents, which, due to its not suitable results were not further tested.

3.4. Application to real samples

Given the highly promising SPE data obtained with HXLPP-WCX when the SPE protocol was applied to Milli-Q water, an analogous protocol was applied to the analysis of Ebre river water and effluent waste water. As is common practice, for the analysis of real water samples the sample volume loaded onto the SPE cartridges is normally lower than the sample volume applied when the analytes are in Milli-Q water due to the presence of interferences in real samples which compete with the analytes for binding to the sorbent and thereby reduce the analyte capture efficiency. To establish the utility of the HXLPP-WCX sorbent for the analysis of real water samples, the initial SPE experiments involved the percolation of 500 mL sample of Ebre river water spiked at 1 μg L^{-1} through cartridges packed with the sorbent (thereafter, the remainder of the SPE protocol was as detailed in Section 2).

Table 4 summarises the recovery values obtained for the various analytes on the HXLPP-WCX sorbent. From these results it can be observed that when 500 mL of a river water sample was loaded onto the SPE cartridge the recovery values for the analytes were high and similar to those obtained for Milli-Q water, with the exception of antipyrine which showed a higher level of fractionation than for the Milli-Q water case.

Table 4. Recovery values (%) obtained when the HXLPP-WCX sorbent was applied in SPE for the preconcentration for different real samples spiked with the analyte mixture.

Analytes	Type	Ebre River (1 μg L^{-1}) 500 mL		Effluent WWTP (5 μg L^{-1}) 250 mL	
		Wash	Elution	Wash	Elution
Caffeine	Basic	20	90	26	82
Antipyrine		50	54	76	0
Propranolol		0	90	0	92
Carbamazepine		11	90	30	70
Acetaminophen	Acidic	113	0	100	0
Naproxen		94	0	91	0
Diclofenac		98	0	93	0

For the experimental conditions, see text.
%RSD (n=3) were lower than 14% for %R >10%.

Fig. 2 shows the chromatograms obtained following preconcentration on HXLPP-WCX of 500 mL of non-spiked (Fig. 2b and d) and spiked (at 1 μg·l^{-1} for each analyte; Fig. 2a and c) Ebre river water. For the river water samples, a signal was detected at the retention time corresponding to caffeine (see the non-spiked Ebre river water chromatogram, Fig. 2d), but further analysis by a confirmatory technique such as mass spectrometry (MS) may be appropriate here.

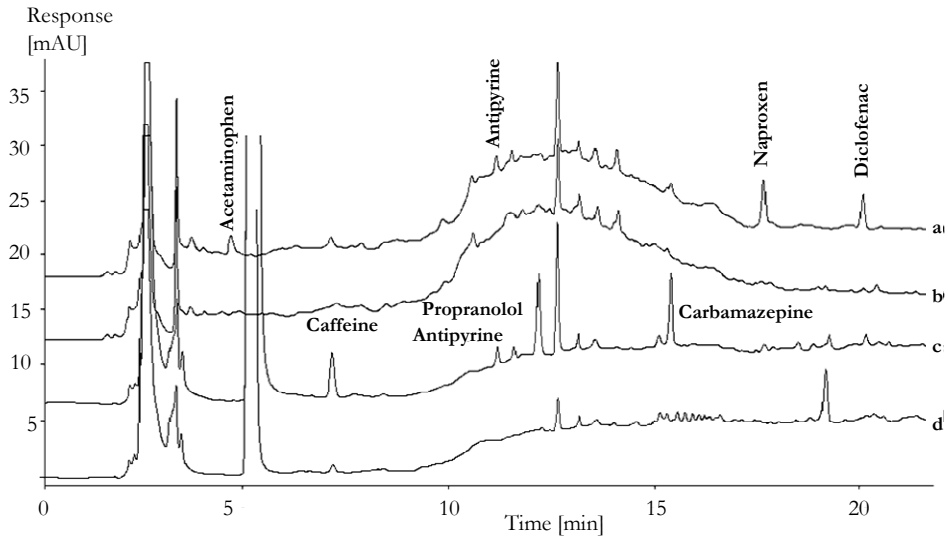


Fig. 2. Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 500mL of Ebre river water sample with (a and c) and without (b and d) addition of a 1 μg L^{-1} level of analyte mixture: washing step (a and b) and elution step (c and d).

Typical chromatograms for the washing step, where all the interferences and acidic analytes retained on the cartridges through reversed-phase mechanism are eluted from the sorbents, are shown in Fig. 2a (spiked) and Fig. 2b (non-spiked). Typical chromatograms for the elution step, where the target analytes retained through weak cation-exchange interactions (i.e., mainly the basic analytes) are eluted from the sorbents, are shown in Fig. 2c (spiked) and Fig. 2d (non-spiked). It is important to note the cleanliness of the chromatograms, an observation which is particularly striking when one considers the fact that a non-selective detector (UV) was used in these analyses. Both the selectivity and sensitivity of the analyses could be improved further by using more powerful detector such as mass spectrometer.

To demonstrate the selectivity of the HXLPP-WCX sorbent, a further set of SPE experiments was performed using dirtier sample matrices, including effluent water from a wastewater treatment plant (WWTP). The recovery values obtained when 250 mL of effluent WWTP samples, spiked at 5 μg L^{-1}, was percolated through the HXLPP-WCX sorbent, are shown in Table 4. In general, the HXLPP-WCX sorbent gave good recoveries for most of the analytes studied, with the exception of antipyrine and carbamazepine. The recovery of carbamazepine in the elution step was 70%, the remaining 30% being eluted in washing step.

As regards antipyrine, its elution profile, when loaded onto the HXLPP-WCX sorbent, was the reverse of that expected, i.e., it was eluted in the washing step.

In fact, antipyrine had already presented retention problems when present in other aqueous matrices, and these problems may be magnified when antipyrine is present in more complex samples since natural organic matter and other compounds present in wastewater matrices give rise to increased competetion for binding to the sorbent. Fig. 3 shows the elution chromatograms obtained after percolation of 250 mL of an effluent WWTP sample through the HXLPP-WCX sorbent without (Fig. 3b) and with (Fig. 3a) the addition of the mixture of analytes at the 5 μg L^{-1} level. To emphasise the importance and effectiveness of the washing step for this complex sample matrix, we performed this particular analysis without a washing step; after the loading of 250 mL of effluent wastewater spiked at the 5 μg L^{-1} level with the mixture of analytes, all the analytes were eluted directly with 5 mL of 2% TFA in MeOH without any prior washing step being used. The effect of re-introducing the methanol-based washing step was then examined in a effort to remove interferences. Fig. 4 shows the washing (Fig. 4a and b) and elution (Fig. 4c and d) chromatograms obtained after the percolation of a 250 mL effluent WWTP sample through the HXLPP-WCX sorbent without (Fig. 4b and d)

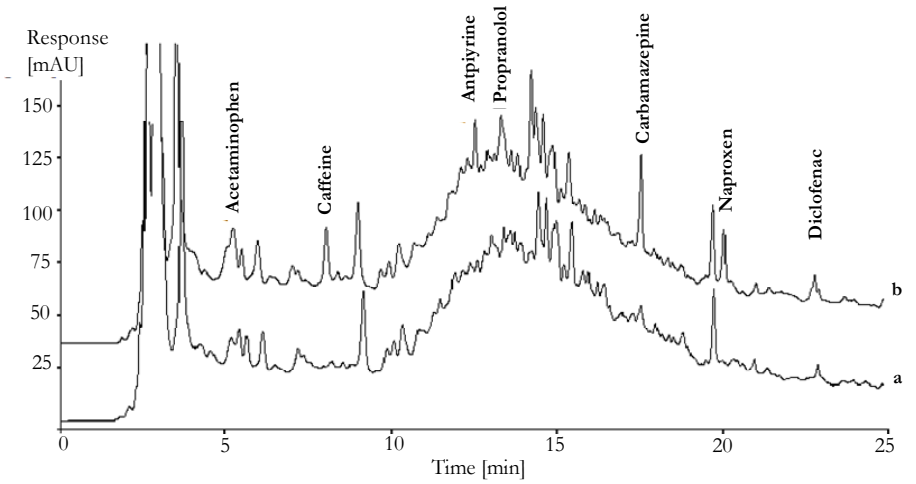


Fig. 3. Chromatograms obtained after off-line trace enrichment with the HXLPP-WCX sorbent of 250 mL of effluent WWTP sample with (a) and without (b) the addition of a 5 μg L^{-1} level of an analyte mixture (without a washing step).

and with (Fig. 4a and c) the addition of the mixture of analytes at the 5 μg L^{-1} level. For the effluent WWTP sample, peaks were observed at the retention times corresponding to antipyrine, naproxen and diclofenac (Fig. 4b) and caffeine (Fig. 4d), but these results should be confirmed by a more powerful detector.

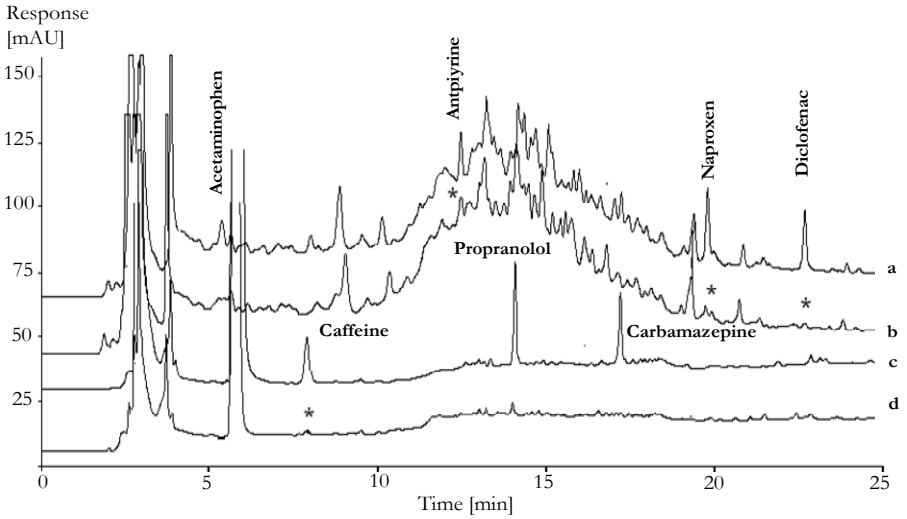


Fig. 4. Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 250 mL of effluent WWTP sample with (a and c) and without (b and d) the addition of a 5 μg L^{-1} level of analyte mixture: washing step (a and b) and elution step (c and d). *Peaks at the same time of studied analytes.

In addition to the marked improvements in the quality of the chromatograms, the new sorbent allows a more accurate quantification of analytes at lower concentration levels in complex matrices without the analytes being masked by interferences. The main point of all is the fact that the recovery of all the basic analytes of interest is complete in these complex environmental samples, on account of the WCX interactions which lead to high analyte recoveries (and clean chromatograms). In addition, the cleanliness of the extracts obtained after SPE with the HXLPP-WCX sorbent is an added advantage in respect of the potential to reduce or avoid ion-suppression effects in the case of determination by LC-MS with electrospray ionisation.

In validation studies using 500 mL of river water and 250 mL of effluent WWTP, all the basic analytes exhibited good linearity. In river water all the analytes exhibited a linear range from 0.5 to 50 μg L^{-1}, with determination coefficients (r^2) greater than 0.992. The limits of detection (LODs), calculated on the basis of a signal to noise ratio ≥3, were 0.1 μg L^{-1} for all the basic analytes. The repeatability and reproducibility of the method, expressed as the relative standard deviation (% RSD) of three analyses of 500 mL of Ebre river water spiked at 1 μg L^{-1}, were less than 14% for all the basic analytes. For effluent waste water, all the analytes exhibited a good linear range (1-50 μg L^{-1}) with r^2 greater than 0.984. The LODs were 0.5 μg L^{-1} for most of compounds, with the exception of caffeine where the LOD was 1 μg·L^{-1}. Although the LODs are not as low as those reported for some environmental water samples [21,22], they could be decreased markedly by the introduction of a more sensitive detection system, such as tandem mass spectrometry.

Moreover, the cleanliness of the chromatograms will tend to reduce or prevent ion-enhancement/suppression effects when LC–MS is used.

4. CONCLUSIONS

In this study the synthesis of hypercrosslinked polymer resin with weak cation-exchange is described, and a detailed investigation carried out with respect to the application of this sorbent to the SPE of basic pharmaceuticals from complex environmental samples. The resin was produced *via* the hypercrosslinking of swellable polymers precursors which were synthesised *via* precipitation polymerisation. The WCX properties are derived from the presence of carboxylic acid moieties in the polymer.

This is the first time that a hypercrosslinked polymer resin has been exploited as weak cation-exchanger for the SPE of basic pharmaceuticals. Following optimization of the SPE protocol, it was found that the novel HXLPP-WCX sorbent enabled essentially quantitative recovery, and adequate selectivity, of most of the

analytes tested, and performed well as a weak cation-exchanger. In contrast, the commercially available sorbents Strata-X-CW and Oasis WCX were unable to completely retain basic analytes *via* an ion-exchange mechanism and remove acidic analytes during the washing step. The highest extraction efficiency was achieved with the HXLPP-WCX sorbent. Overall, the HXLPP-WCX sorbent proved to be highly effective for the preconcentration of basic analytes present in complex environmental water samples.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.03.

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Appendix A. Supplementary data

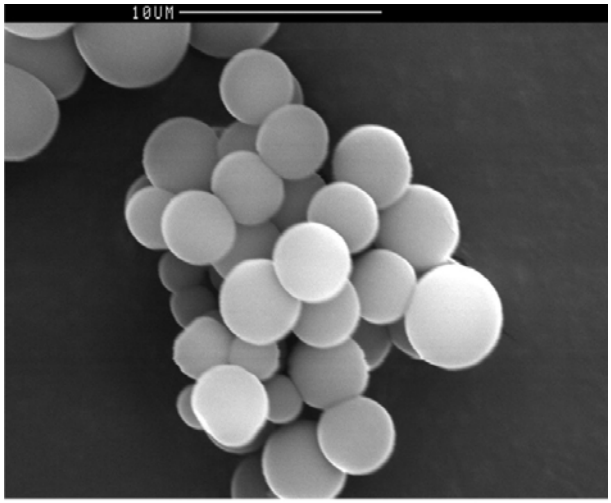

Fig. 1S. SEM image of the HXLPP-WCX resin. The applied acceleration voltage of the incident electron beam was 25 kV.

3.2.2. Hypercrosslinked polymers with strong anion-exchange character for the selective extraction of acidic pharmaceuticals from environmental water samples

HYPERCROSSLINKED POLYMERS WITH STRONG ANION-EXCHANGE CHARACTER FOR THE SELECTIVE EXTRACTION OF ACIDIC PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

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Abstract

This paper presents the synthesis and detailed solid-phase extraction (SPE) evaluation of two novel high-specific surface area polymeric sorbents (HXLPP-SAXa and HXLPP-SAXb). The novel sorbents under study are both members of a large family of hypercrosslinked polymer microspheres, some of which are designed specifically to offer ion-exchange properties; the specific polymers of interest in the current work have been chemically modified in such a way as to impart a tuneable level of strong anion-exchange (SAX) character onto the sorbents. The novel sorbents were applied as SAX sorbents in SPE studies, with the goal being to selectively extract a group of acidic compounds from complex environmental samples in an efficient manner.
Of two HXLPP-SAX resins evaluated in this study, it was found that the sorbent with the lower ion-exchange capacity (HXLPP-SAXa) gave rise to the best overall performance characteristics and, indeed, was found to compare favourably to the SPE performance of commercial SAX sorbents. When the HXLPP-SAXa sorbent was applied to the SPE of real water samples, the result was quantitative and selective extraction of low levels of acidic pharmaceuticals from 500 mL of river water and 100 mL of effluent wastewater.

Keywords: *Hypercrosslinked sorbents; Polymer microspheres; Mixed-mode strong anion-exchanger; Solid-phase extraction; Acidic pharmaceuticals*

1. INTRODUCTION

Due to an increasing recognition of the serious problems which can arise from the appearance of pharmaceuticals in the environment (e.g., contaminated water courses), and especially problems relating to (human) health, researchers are motivated to develop sensitive analytical methods which allow the determination of pharmaceuticals at low levels [1-4]. To realise this goal, there is a need for suitable preconcentration techniques, which can concentrate the analytes and clean-up the complex matrix samples [5] prior to analysis.

Solid-phase extraction (SPE) is a widely used sample preparation technique for enrichment, isolation and purification of components of interest from different complex liquid matrices, such as environmental, biological and food samples. SPE is a well-established and widely-used technique which is simple to perform and provides excellent recovery and reproducibility [6-9].

In recent years, many developments in SPE technology have been introduced, with one of the recent trends being the implementation of approaches that improve the selectivity of SPE (e.g., mixed-mode sorbents, molecularly imprintted polymers and immunosorbents [9-13].

Much attention has been focused recently in the field of mixed-mode sorbents [10,14]. Mixed-mode sorbents are a new generation of sorbents which, through a single material, enhance the selectivity and the capacity of the extraction process. They combine reversed-phase retention with ionic interactions (cationic or anionic) into one single material, which makes it possible to extract analytes with acidic or basic characteristics in a selective manner [10,15].

Depending on the functional group(s) attached to the mixed-mode sorbent, the sorbent can be classified either as anionic or cationic, but also as either a strong or weak ion-exchanger. Anion-exchange sorbents typically contain quaternary ammonium groups, or weakly basic functional groups such as primary or secondary amines. Cation-exchange sorbents contain strongly acidic groups, such as aromatic or aliphatic sulfonic acid groups, or else weakly acidic functional groups such as carboxylic acids. These functional groups improve the selectivity of the SPE process when the sorbents are used under appropriate experimental conditions, and hence enhance the sensitivity of the determination of acidic or basic components from complex samples [10].

There are various examples of comercially available mixed-mode sorbents with differing ion-exchange properties. As far as SAX sorbents are concerned, the most widely used commercially available sorbent is Oasis MAX (Waters). This sorbent is based on a copolymer of *N*-vinylpyrrolidone and divinylbenzene (DVB) which has been modified chemically to give polymer-bound, quaternary ammonium groups which transform the sorbent into a

mixed-mode SAX material. Similarly, Strata-X-A (Phenomenex) is a DVB-based polymer which has had pyrrolidone groups introduced; the polymer is then modified further to put in place quaternary ammonium groups. Another example of a commercially available mixed-mode SAX sorbent is SampliQ SAX (Agilent Technologies); this is yet another DVB-based polymer which has quaternary ammonium groups as an integral part of the structure.

In relation to in-house produced mixed-mode sorbents, our research group has developed a range of mixed-mode sorbents based upon hypercrosslinked structures.

These hypercrosslinked mixed-mode polymeric sorbents are somewhat different to the commercially available materials, in that the non-commercial materials have a hypercrosslinked, microporous structure which seems to enhance the hydrophobic interactions and, therefore, promote the retention of analytes during the SPE loading.

Recently, mixed-mode hypercrosslinked sorbents bearing carboxylic acid groups (HXLPP-WCX) [16], and ethylene-diamine or piperazine groups (HXLPP-WAX) [17,18] were synthesised and exploited successfully as WCX and WAX sorbents for the selective extraction of basic [16] and acidic [17,18] pharmaceuticals, respectively, from aqueous samples.

The current paper describes the synthesis and SPE evaluation of new hypercrosslinked polymeric sorbents which are both microporous and contain tuneable amounts of a SAX moiety (quaternary ammonium groups).

2. EXPERIMENTAL

2.1. Reagents and standards

The analytes selected to evaluate the sorbents were pharmaceuticals with basic and acidic groups: paracetamol, caffeine, antipyrine, propranolol, carba-mazepine, salicylic acid, fenoprofen, ibuprofen and diclofenac, all of which were obtained from Sigma-Aldrich. The chemical structures and pK_a values of the analytes are shown in Fig. 1.

A standard solution (1000 mg L^{-1} for each compound) was prepared in methanol (SDS, Peypin, France). The mixture of all the compounds was prepared by diluting the standard solution in Milli-Q water (Millipore, Bedford, MA, USA). Acetonitrile (ACN; from SDS) and Milli-Q water were used to prepare the mobile phase. Sodium hydroxide (NaOH) from Panreac (Barcelona, Spain), and hydrochloric acid (HCl) and formic acid (HCOOH) from Probus (Barcelona, Spain), were used to adjust the pH of the mobile phase and the sample prior to SPE. The reagents used for the polymer syntheses were divinylbenzene (DVB) (80% grade) and *para*-vinylbenzylchloride (VBC) (95% grade), both supplied by Aldrich (Dorset, UK). DVB and VBC were purified by passing them through a short column of neutral alumina.

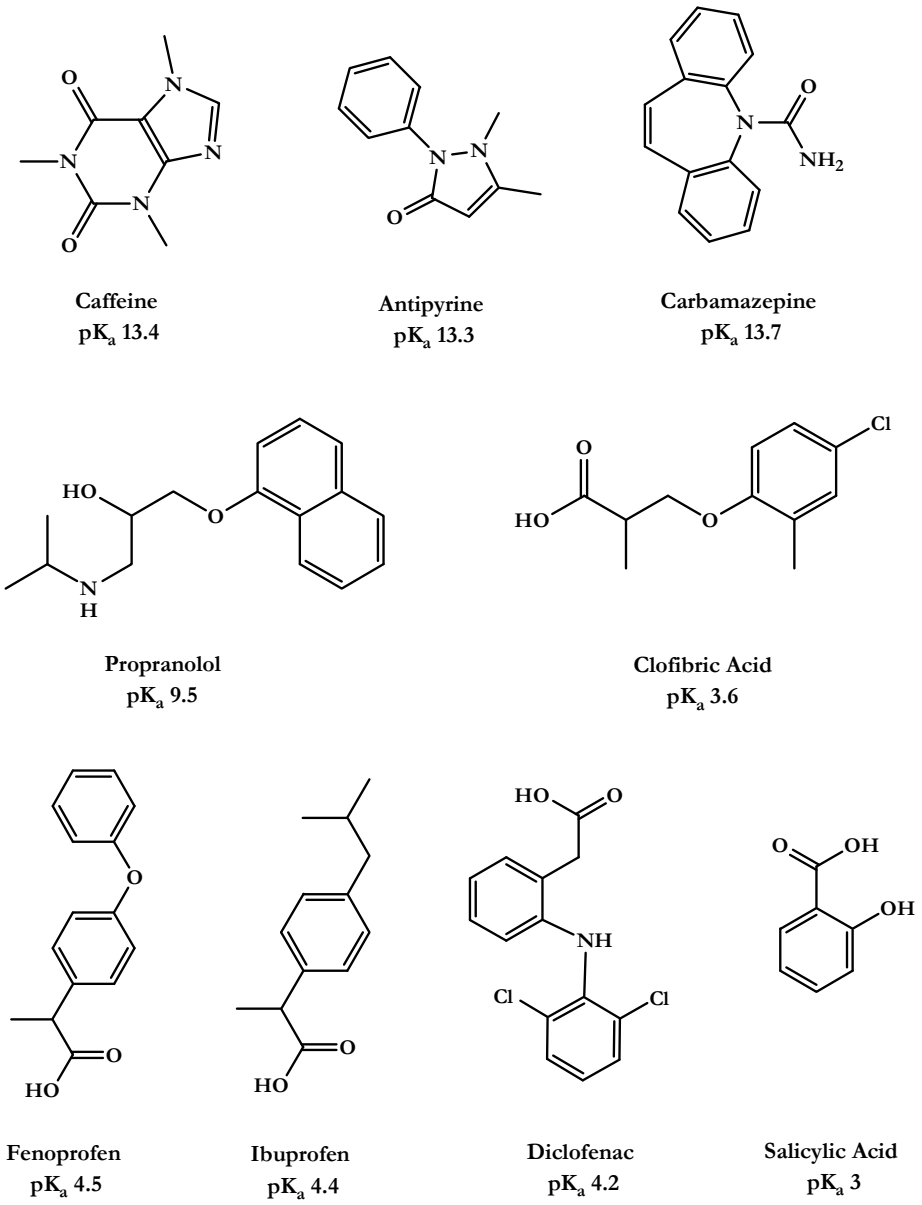

Fig. 1. Chemical structures of the acidic and basic pharmaceuticals used to evaluate the sorbents.

The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Aldrich) at low temperature). Dimethylbutylamine (DBMA), from Aldrich, was used in the quaternisation reactions. Ferric chloride ($FeCl_3$) and anhydrous 1,2-dichloroethane (DCE), from Aldrich, were used in the hypercrosslinking reactions.

2.2. Polymer synthesis and characterisation

The HXLPP-SAX resins were synthesised by adapting methods which have been developed previously in our laboratories [17]. Swellable precursor particles in the form of microspheres were obtained by precipitation polymerisation (PP). In a typical PP, the monomers (DVB and VBC, in a 25/75 [w/w] ratio and at a total monomer concentration of 2% (w/v) relative to the solvent) and intitiator (AIBN; 2 mol% relative to the total number of polymerisable double bonds) were dissolved in acetonitrile (500 mL) in a polypropylene bottle fitted with a screw-cap. The monomer solution was de-oxygenated by sparging with N_2 at 0 °C, the bottle then sealed under nitrogen and placed on a low-profile roller (Stovall, Essex, UK) in a temperature-controllable incubator (Stuart Scientific, Surrey, UK).

The temperature was ramped from ambient to 60 °C over a period of around 2 hours, and the polymerisation allowed to proceed at 60 °C for a further 46 hours. The polymer particles which had formed were filtered on a 0.2 μm nylon membrane filter and then washed in sequence with MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 °C.

For the quaternisation reactions which were used to produce PP-SAXa and PP-SAXb, the precursor particles (1.5 g and 2 g, respectively) and DMBA (74 mg and 48 mg, respectively) were added to DCE (30 mL) in a round-bottomed flask (100 mL) and the mixture heated at 80 ºC under N_2 for 24 hours. After cooling, the product particles were washed with deionized water until the washings were neutral.

The products was then washed with methanol (100 mL) and oven-dried under vacuum at 40 ºC for 24 hours.

The hypercrosslinking of the quaternised polymeric precursors was carried out as described in previous study [20]. For these hypercrosslinking reactions, PP-SAXa and PP-SAXb were added to DCE (40 mL) in round-bottomed flasks (100 mL) and left to swell fully under N_2 at room temperature for 1 hour. Then, $FeCl_3$ (1:1 mole ratio with respect to the chloromethyl content of the particles) in DCE (30 mL) was added and the mixture heated at 80 ºC for 2 hours. The resultant particles were recovered as described above and washed with MeOH, and several times with aqueous HNO_3 (pH 1). The particles were then extracted overnight with acetone in a Soxhlet extractor and were washed again with MeOH and diethyl ether before drying *in vacuo* overnight at 40 ºC. Figure 2 shows a schematic representation of the HXLPP-SAX structure, as well as the chemical structures of the two commercial sorbents tested.

The hypercrosslinked resins (HXLPP-SAXa and HXLPP-SAXb) were characterized by measuring their specific surface areas using N_2 sorption isotherm

data generated on a Micromeritics ASAP 2000 porosimeter. The chlorine (w%) and nitrogen (w%) contents of the resins were obtained using a titration method and elemental microanalysis using a Perkin Elmer 2400 Series II Analyser, respectively. The anion-exchange capacity was calculated from the elemental microanalytical data. Microsphere diameters and particle size distributions were calculated using ImageJ software from the image analysis of 100 individual particles in scanning electron microscopy (SEM) images, which were acquired using a Cambridge Instruments Stereoscan 90 instrument. The characterisation data obtained from the resin evaluation is detailed in Table 1.

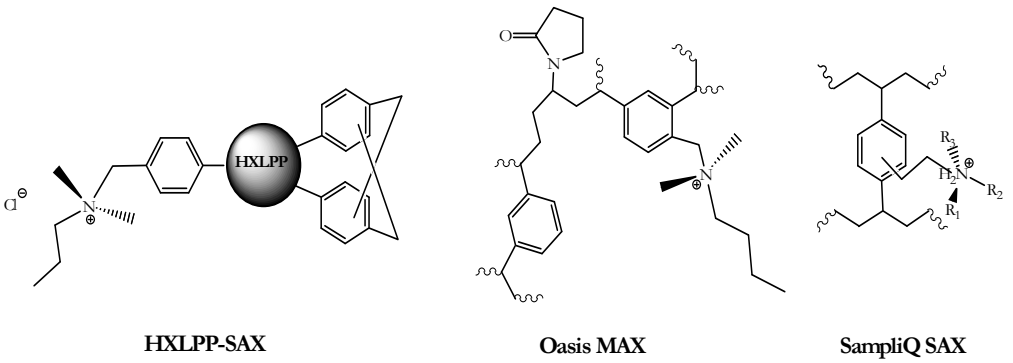

Fig. 2. Schematic representations of the chemical structures of the sorbents tested: HXLPP-SAX, Oasis MAX and SampliQ SAX.

2.3. Chromatographic equipment and conditions

The chromatographic experiments were performed with an Agilent 1100 liquid chromatograph equipped with an injection valve with a 20 μL loop and a UV spectrophotometric detector (Agilent, Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain).

The mobile phase was Milli-Q water adjusted to pH 3 with HCl and acetonitrile (ACN). The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 30 ˚C. The UV detection was set at 210 nm.

The gradient profile was initially from 20% to 25% ACN in 15 min, next to 60% ACN in 15 min then to 100% ACN in 10 min (held 2 min), after which time the mobile phase was returned to the initial conditions (20% ACN) in 3 min.

Table 1. Characterisation data for the sorbents tested in SPE.

	Sorbent	IEC (meq g^{-1})	Specific surface area ($m^2 g^{-1}$)	Average particle diameter (μm)
Laboratory synthesised	HXLPP-SAXa[a]	0.2	1470	4.5±0.8[c]
	HXLPP-SAXb[a]	0.4	1290	4.3±0.9[c]
Commercially available	Oasis MAX[b] Waters	0.3	~800	25-35
	SampliQ SAX[b] Agilent	0.09	~600	25-35

IEC - Ion-exchange capacity
[a] Data measured experimentally
[b] Data provided by supplier
[c] Average particle diameter ± standard deviation (SD) calculated from the image analysis of 100 individual particles in SEM micrographs (using ImageJ software).

2.4. Solid-phase extraction

SPE cartridges were packed manually by weighting 200 mg of each HXLPP-SAX sorbent and placing them into 6 mL polypropylene syringes. In each case the sorbent was retained by two frits (a metal frit of 2 μm pore size at the bottom of the sorbent bed and a polyethylene frit of 10 μm pore size at the top of the sorbent bed). The retention capabilities of the sorbents were compared to the commercial cartridges (SampliQ SAX 200 mg/6 mL from Agilent, and Oasis MAX 200 mg/6 mL from Waters), which were manually packed. The cartridges were placed in an SPE manifold (Teknokroma), which was connected in turn to a vacuum pump.

Prior to the extraction, the pH of the sample was adjusted to 7 with HCl or NaOH as appropriate.

The procedure with all cartridges was as follows: cartridges were activated with 5 mL of MeOH and 5 mL of Milli-Q water, and then the sample was loaded at a flow rate 10 mL min^{-1}. After equilibration, the cartridge was washed with 10 mL of MeOH. Finally, the compounds were eluted from the cartridge using 10 mL of 10% HCOOH in MeOH.

Before injection, the eluate was evaporated to dryness and then reconstituted with 500 μL of 1:1 MeOH:H_2O (v/v), prior to LC analysis.

Real water samples from the Ebre River and effluent water from a waste water treatment plant (WWTP) were filtered through 0.45 μm nylon membranes (Supelco, Bellefont, PA, USA) prior to the SPE. To ensure proper conditions for keeping the samples, the real water samples were adjusted to pH~3 with HCl and kept at 4 ºC prior to analysis.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterisation of the HXLPP-SAX sorbents

Two HXLPP-SAX sorbents (HXLPP-SAXa and HXLPP-SAXb) were prepared in the form of microporous polymer microspheres with relatively

narrow particle size distributions (mean particle diameters: 4.5±0.8 μm and 4.3±0.9 μm, respectively) by: copolymerisation of DVB and VBC under typical precipitation conditions; conversion of 5 % (in the synthesis of HXLPP-SAXa) or 10 % (in the synthesis of HXLPP-SAXb) of the VBC-derived chloromethyl groups in the swellable precursor particles to quarternary ammonium groups by treatment with DMBA; finally, hypercrosslinking of the quaternised swellable precursors to deliver the final products in good yields.

It was discovered that it was more efficient to introduce the quaternary ammonium groups into the polymers prior to the hypercrosslinking reactions rather than afterwards. The resin characterisation data is detailed in Table 1. The specific surface areas of the two polymers were 1470 $m^2\ g^{-1}$ (HXLPP-SAXa) and 1290 $m^2\ g^{-1}$ (HXLPP-SAXb), with the high specific surface areas arising from the high micropore content. The ion-exchange capacities (IEC) of the two polymers were calculated to be 0.2 meq g^{-1} and 0.4 meq g^{-1}, respectively.

Compared to commercially available mixed-mode SAX sorbents, such as Oasis MAX and SampliQ SAX, the HXLPP-SAX sorbents have significantly higher specific surface areas. As noted above, high specific surface areas were obtained because HXLPP-SAXa and HXLPP-SAXb are microporous. In contrast, Oasis MAX and SampliQ SAX are not microporous, and thus have somewhat lower specific surface areas. Since it can be expected that binding capacity will rise as the specific surface area is increased, it was anticipated that the microporous sorbents would have relatively high binding capacities. In addition, since HXLPP-SAXa and HXLPP-SAXb had significantly lower average particle diameters than either of the two commercial sorbents, it was anticipated that additional benefits would accrue from improved packing of the sorbents into the SPE cartridges and higher column/cartridge efficiency.

3.2. SPE optimisation

To evaluate the anion-exchange properties of the HXLPP-SAX sorbents, and assess their sorption capacity, we selected a group of acidic and basic pharmaceuticals with different pK_a values (Fig. 1).

When using a SAX sorbent in SPE, the procedure involves loading of the analytes in their anionic form onto the cationic sorbent. This step allows for the selective ionic retention of the analytes by the sorbent, while the interferences (basic and neutral analytes) can be eliminated by the washing step since the interferences are retained by reversed-phase interactions alone.

Therefore, the parameters evaluated to establish the optimal experimental conditions for the SPE procedure included the study of the pH of the sample, as well as the loading volume, composition and volume of the eluting

and washing solvents. In experiments designed to optimise the performance of the SAX sorbents, the HXLPP-SAXa sorbent was selected for study, since HXLPP-SAXa and HXLPP-SAXb are based on the same common polymer backbone and were expected to show similar performance.

All of the experiments carried out over the course of the SPE optimisation were performed by loading 100 mL of Milli-Q water spiked at 100 μg L^{-1} with the analyte mixture.

3.2.1. Loading step

First of all, the pH of the sample applied in the loading step was optimised. In this regard, the behaviour of the sorbents at different pH values was evaluated: acidic (pH 5) and neutral (pH 7). After loading of the samples onto the cartridges, the analytes were eluted using 10 mL of 2% HCOOH in MeOH.

The results obtained under neutral conditions were somewhat better than those obtained at pH 5, therefore the pH of the sample was set at 7 in subsequent experiments. At pH 7 the acidic analytes are expected to be predominately in their anionic (deprotonated) forms and free to bind to the SAX sorbents through an ion-exchange mechanism. The non-acidic analytes (*i.e.*, neutral and basic interferences) will be in either a neutral or basic form at pH 7; thus any retention must therefore be due to weak, reversed-phase (RP) interactions, and the non-acidic analytes can be eliminated easily thereafter by washing with an organic solvent.

3.2.2. Elution step

In order to select an appropriate eluting solvent for the retained acidic analytes, we tested different percentages of HCOOH in MeOH. Lowering the pH protonates the bound acidic compounds; this breaks the ionic interactions between the analytes and the sorbent and allows the analytes to be released.

Accordingly, 10 mL aliquots of varying percentages (2%, 5% and 10%) of HCOOH in MeOH were investigated as elution solvents.

The highest recoveries were achieved when using 10% of HCOOH solution; 2% and 5% solutions of HCOOH were unable to elute salicylic acid completely. In order to optimise the volume of the elution solvent, 5 mL aliquots were tested in turn; it was found that 5 mL of the elution solvent was insufficient (%R ~70%) to elute completely all the acidic analytes; however, the next 5 mL of elution solvent (*i.e.*, 10 mL in total) eluted the remaining acidic pharmaceuticals.

As expected, no traces of analytes were found in the third 5 mL elution fraction. Thus, 10 mL of 10% HCOOH in MeOH was selected as the optimal volume and concentration of elution solvent to elute the acidic analytes of interest.

3.2.3. Washing step

An ideal washing solvent is one which leaves the target analytes bound to the sorbent, but which removes from the sorbent the basic and neutral analytes or interferences retained to the SAX sorbent through RP interactions.

We optimised the nature and volume of the washing solvent required to eliminate interferences bound to the sorbents through RP mechanisms (including basic pharmaceuticals with pK_a>6), while retaining acidic analytes on the sorbent through ion-exchange interactions. In this respect, several different volumes of ACN and MeOH were tested. The elimination of basic analytes with 10 mL of MeOH was noticeably more complete (~80-100%) than when ACN was used as the washing solvent (~70-90%). Thus, MeOH was selected as the preferred washing solvent. Then, the optimum volume of MeOH was established. Volumes of MeOH ranging from 5 to 15 mL were tested. A 5 mL volume of MeOH was insufficient to eliminate all the basic analytes, thus we increased the volume of MeOH to 10 mL. This volume was sufficient to successfully remove all basic analytes, and therefore all possible basic and neutral interferences in the sample, without further losses of acidic analytes. When higher volumes of MeOH were tested (*i.e.*, 15 mL) the recovery values for the acidic pharmaceuticals started to fall. In conclusion, 10 mL of MeOH as washing solvent was found to be a sufficient to remove all basic analytes to a substantial extent (recoveries greater than 90% for all basic analytes).

3.2.4. Sample volume

At this stage of the study, both of the novel sorbents (HXLPP-SAXa and HXLPP-SAXb) were examined to identify the best material for further applications. When the HXLPP-SAXb sorbent was tested under the optimised SPE conditions which had been established, the results obtained were similar to those obtained for HXLPP-SAXa. Now that optimised SPE experimental conditions had been established, the next goal was to determine the highest volume of sample which could be loaded onto the SPE cartridges without significant breakthrough. To this end, different sample volumes were percolated through the two different HXLPP-SAX sorbents. Sample volumes ranging from 100 to 500 mL of Milli-Q water at pH 7.0 were spiked with the analytes in concentrations ranging from 20 to 100 μg L^{-1}, depending on the volume, and applied to the cartridges.

The results obtained for 100 mL and 500 mL sample volumes for the two HXLPP-SAX sorbents are listed in Table 2. As can be seen, the basic compounds are washed out very effectively with methanol.

For the acidic compounds, in the case of HXLPP-SAXa the recoveries of the acidic analytes were close to 100% for all the volumes tested.

Table 2. Recoveries of the analytes on the different HXLPP-SAX sorbents in SPE for different sample volumes spiked with the analyte mixture at 20 μg L^{-1} in Milli-Q water.

Analytes	Type	Recovery [%]							
		HXLPP-SAXa				HXLPP-SAXb			
		100 mL		500 mL		100 mL		500 mL	
		Wash	Elution	Wash	Elution	Wash	Elution	Wash	Elution
Caffeine	Basic	102	0	92	0	102	0	103	0
Antipyrine		101	0	97	0	103	0	104	0
Propranolol		100	0	88	0	101	0	83	0
Carbamazepine		100	0	97	0	100	0	104	0
Salicylic Acid	Acidic	0	100	0	92	0	88	0	89
Clofibric Acid		0	96	0	91	0	100	0	90
Fenoprofen		0	99	0	98	0	98	0	103
Diclofenac		0	102	0	93	0	87	0	81
Ibuprofen		0	95	0	94	0	102	0	102

% Relative standard deviations (RSD) (n = 3) were lower than 7.

Meanwhile, the recovery values of the same analytes on HXLPP-SAXb were marginally lower (80%-100%).

3.3. Comparison to commercially available sorbents

To further illustrate the attractive ion-exchange characteristics of the HXLPP-SAX sorbents, the optimised SPE procedures were applied to the commercially available SAX sorbents, Oasis MAX and SampliQ SAX (see Table 1 for characterisation data). One of the notable differentiating features between the various sorbents is their polarity; the Oasis MAX sorbent is the most polar, due to the presence of vinylpyrrolidone residues in its structure, whereas the other sorbents have are based on hydrophobic monomers. Fig. 2 shows schematic illustrations of the structures of all the sorbents tested.

Regarding the ion-exchange capacity, the values for HXLPP-SAXa and HXLPP-SAXb were found to be 0.2 and 0.4 meq g^{-1}, respectively, whereas the values for Oasis MAX and SampliQ SAX are reported as 0.3 and 0.09 meq g^{-1}, respectively.

A further differentiating feature between the sorbents is their average particle size; the average particle sizes of HXLPP-SAX sorbents (~4.5 μm and 4.3 μm, for HXLPP-SAXa and HXLPP-SAXb, respectively) are significantly lower than either of the other commercially available sorbents. The hydrophobic structure, larger particle size and lower specific surface area of the SampliQ SAX sorbent may explain the low retention of propranolol (68%) on this particular sorbent (see Table 3).

Optimal SPE conditions were adapted to suit the specific SPE requirements of the commercially available sorbents. Table 3 presents the results obtained after the percolation of 500 mL samples through these sorbents; it can be observed that all the acidic analytes were eluted completely during the SPE procedure. Generally speaking, it was demonstrated that all of the sorbents enabled selective extraction of acidic pharmaceuticals. In view of the SPE comparison which has been performed for these four sorbents, it can be stated that the novel HXLPP-SAX materials allow the complete, selective extraction of acidic analytes from aqueous samples, with an application performance which is at least as good as the best commercially available sorbents.

3.4. Application to environmental samples

The extraction of real samples was performed using HXLPP-SAXa, and the goal was to demonstrate the applicability of the sorbent to the extraction of acidic pharmaceuticals from complex environmental matrices, such as river water and effluent water from a WWTP. To further decrease the limits of detection realisable for real samples, an evaporation step was included in the analytical protocol. This evaporation step did not

Table 3. Recoveries of analytes on the Oasis MAX and SampliQ SAX in off-line SPE for 500 mL of Milli-Q water spiked with the analyte mixture at 20 μg L^{-1}.

Analytes	Type	Recovery [%]			
		Oasis MAX		SampliQ SAX	
		Wash	Elution	Wash	Elution
Caffeine	Basic	74	0	87	0
Antipyrine		95	0	92	0
Propranolol		90	0	68	0
Carbamazepine		94	2	94	0
Salicylic Acid	Acidic	0	90	0	100
Clofibric Acid		0	93	0	95
Fenoprofen		0	99	0	98
Diclofenac		0	99	0	99
Ibuprofen		0	94	0	98

%RSD (n = 3) were lower than 11.

adversely affect the recoveries of the analytes.

Recovery values obtained for the target analytes when a 500 mL volume of Ebre river water spiked at 0.5 μg L^{-1} was percolated through the sorbent are listed in Table 4.

It can be seen that the recovery values recorded for the target analytes are high, and similar to those obtained with Milli-Q water, except for ibuprofen which fractionated between the washing (28%) and elution (66%) steps.

The recovery value for diclofenac also decreased slightly (to 74%). This low %R can be explained by the presence of acidic matrix components in real samples which can decrease significantly the recoveries of acidic pharmaceuticals on SPE sorbents due to competition between acidic analytes and acidic interferences for binding to the sorbent.

Fig. 3 shows the chromatograms obtained following preconcentration on HXLPP-SAXa of 500 mL of Ebre river water samples: unspiked (Fig. 3a and c) and spiked at 0.5 μg L^{-1} with the analyte mixture (Fig. 3b and d). Fig. 3c (unspiked) and 3d (spiked) present typical chromatograms for the washing step, where all the interferences, including basic analytes retained on the cartridges through RP mechanisms, were eluted from the sorbent. Fig. 3a (unspiked) and 3b (spiked) show typical chromatograms for the elution step, where the acidic analytes retained through SAX interactions were eluted from the sorbent. No target analytes were detected in unspiked river water.

In order to validate the method for quantification of acidic analytes in river water, linearity, repeatability, reproducibility and limits of detection (LODs) were determined using 500 mL of Ebre river water.

Table 4. Recoveries of the analytes on the HXLPP-SAXa sorbent in off-line SPE for 500 mL of Ebre river water spiked with the analyte mixture at 0.5 μg L^{-1}.

Analytes	Type	Recovery [%]	
		HXLPP-SAXa	
		Wash	Elution
Caffeine	Basic	96	0
Antipyrine		101	0
Propranolol		80	0
Carbamazepine		103	0
Salicylic Acid	Acidic	0	80
Clofibric Acid		0	90
Fenoprofen		0	90
Diclofenac		0	74
Ibuprofen		28	66

%RSD (n = 3) were lower than 12.

All the acidic analytes presented good linearity with a linear range from 0.2 to 5 μg L^{-1}, and determination coefficients (r^2) higher than 0.996. The LODs, calculated at signal-to-noise (S/N) ≥ 3 were 0.05 μg L^{-1} for all acidic analytes, with the exception of clofibric acid and ibuprofen (0.1 μg L^{-1}). These values could readily be improved further, if necessary, by using a more selective detection technique, such as mass spectrometry or mass spectrometry in tandem.

The repeatability and reproducibility, defined as the relative standard deviation (%RSD) of three analyses of 500 mL of river water spiked at 0.5 μg L^{-1}, were less than 12% and 19%, respectively, for all acidic analytes.

It is noteworthy to mention that this selective extraction procedure for sample clean-up minimises the matrix effect that is normal in the analysis of complex samples by LC-MS with electrospray ionisation [21,22].

In order to demonstrate the selectivity of the HXLPP-SAXa sorbent, effluent WWTP samples were also analysed. Due to the high complexity of real samples of this type, we decreased the sample volume percolated from 500 mL (used when analysing Milli-Q water) to 100 mL of effluent wastewater. The recovery values obtained when 100 mL of effluent WWTP samples spiked at the 5 μg L^{-1} level were percolated through the HXLPP-SAXa sorbent are shown in Table 5. The HXLPP-SAXa sorbent gave impressive results for all of the analytes studied, similar to the obser-vations for the SPE of the Milli-Q and river water samples, except for ibuprofen (60% in elution step, 11% in washing step). The recoveries of most of the analytes were similar across both river water and WWTP samples.

Only small differences can be observed for fenoprofen, which was partially eluted between the washing and elution step (*i.e.*, fractionated) with the HXLPP-SAXa sorbent. As was the case for the Milli-Q water samples, we performed, in parallel, the same experiments using the commercially available Oasis MAX and SampliQ SAX.

Table 5 shows the data obtained using these sorbents. All the acidic analytes were eluted completely during elution step (as expected). These recovery results are similar to those generated for the HXLPP-SAXa sorbent, with the exception of diclofenac which was retained better on HXLPP-SAXa.

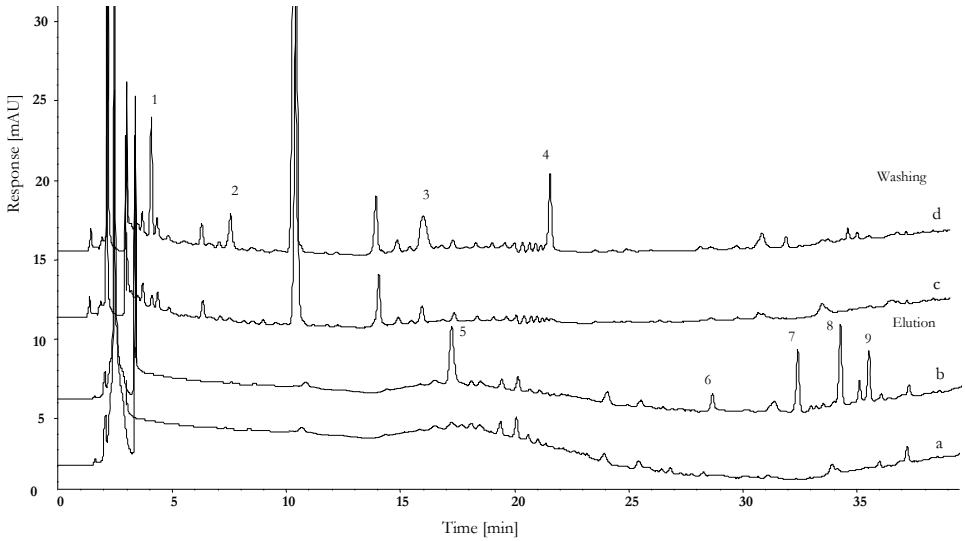

Fig. 3. Chromatograms obtained after off-line trace enrichment with the HXLPP-SAXa sorbent of 500 mL of river water sample without (a and c) and with (b and d) the addition of a 0.5 μg L^{-1} level of analyte mixture: washing step (c and d) and elution step (a and b). Peak assignment: 1: caffeine; 2: antipyrine; 3: propranolol; 4: carbamazepine; 5: salicylic acid; 6: clofibric acid; 7: fenoprofen; 8: diclofenac; 9: ibuprofen.

Fig. 4 shows representative chromatograms when 100 mL volumes of effluent WWTP water were percolated through the HXLPP-SAXa sorbent: unspiked (Fig. 4a and c) and spiked at 5 μg L^{-1} with analyte mixture (Fig. 4b and d). The results obtained from HPLC-UV analysis of SPE extracts of the effluent WWTP samples indicated the possible presence of salicylic acid; a peak in the chromatogram was observed at the same retention time as salicylic acid (Fig. 4a).

To confirm the presence of the salicylic acid in an unequivocal manner, a more powerful detector such as mass spectrometer would need to be used.

The presence of salicylic acid at low μg L^{-1} levels has been reported previously by Pedrouzo *et al.* in samples taken from the same river and WWTP [23].

Table 5. Recoveries of analytes on the HXLPP-SAXa, Oasis MAX and SampliQ SAX sorbents in off-line SPE for 100 mL of effluent WWTP spiked with the analyte mixture at 5 μg L^{-1}.

Analytes	Type	Recovery [%]					
		HXLPP-SAXa		Oasis MAX		SampliQ SAX	
		Wash	Elution	Wash	Elution	Wash	Elution
Caffeine	Basic	82	0	86	0	91	0
Antipyrine		110	0	81	0	85	0
Propranolol		66	0	73	0	80	0
Carbamazepine		99	0	64	0	70	0
Salicylic Acid	Acidic	0	85	0	75	0	88
Clofibric Acid		0	74	0	76	0	79
Fenoprofen		4	90	0	75	0	82
Diclofenac		0	85	0	66	0	67
Ibuprofen		11	60	0	58	0	66

%RSD (n = 3) were lower than 15.

One obvious benefit of using selective sorbents is the cleanliness of chromatograms which are obtained.

As can be seen in Fig. 4, the chromatogram profiles in the washing step (Fig. 4c and d) include many interferences, interferences which were removed from the elution and thereby allowed the elution of a cleaner extract (see Fig. 4a and b), even with UV detection.

Although UV detection may be viewed as less powerful than mass spectrometry in terms of analyte identification it does, of course, allow for accurate quantification of the analytes.

Overall, these results indicate that SPE using these novel HXLPP-SAX sorbents constitutes an attractive means to determine acidic pharmaceuticals in environmental water samples. The ion-exchange character and physical characteristics of the sorbents leads to efficient SPE and clean chromatograms. Low LODs are realisable for even the dirtiest of samples.

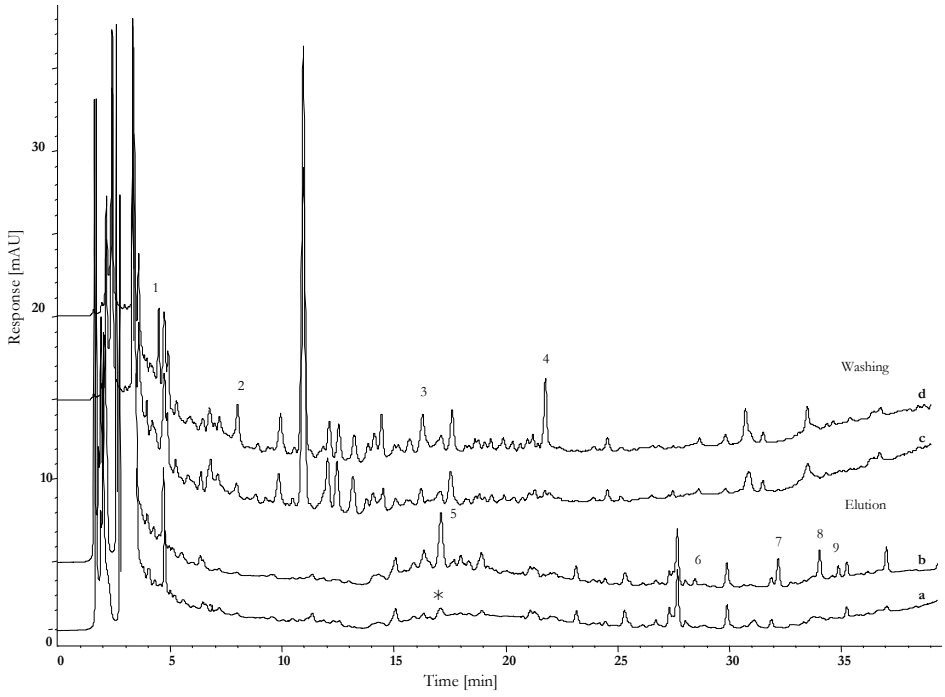

Fig. 4. Chromatograms obtained after off-line trace enrichment with HXLPP-SAXa sorbent of 100 mL of effluent waste water sample without (a and c) and with (b and d) the addition of a 5 μg L^{-1} level of analyte mixture: washing step (c and d) and elution step (a and b). Peak assignment as in Fig. 2; "*" is tentatively assigned to salicylic acid.

4. CONCLUSIONS

In this study, hypercrosslinked polymers with quaternary ammonium groups as an integral part of their structure (HXLPP-SAX) have been synthesised in a well-defined microsphere format, and then applied as SAX sorbents in SPE to enable the selective extraction of acidic pharmaceuticals from environmental water samples. The HXLPP-SAX sorbents were evaluated in off-line SPE studies for the extraction of acidic analytes from water samples, and they compared favourably to the best commercially available sorbents.

The sorbent with the optimal performance characteristics (HXLPP-SAXa) was selected for further evaluation. This sorbent enabled the selective extraction and quantification of a group of acidic compounds from complex environmental water samples. The recoveries of the analytes were very good, the LODs were low, and the effectiveness of the ion-exchange interactions meant that a washing step efficiently eliminated the interferences which were bound to the sorbent by RP interactions. The effectiveness of the cleaning step meant that clean chromatograms were obtained, even when complex river water or WWTP effluent samples were being analysed.

Acknowledgements

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3.2.3. Discussion of Results

As can be seen from the papers presented, two different mixed-mode polymeric sorbents were successfully prepared and evaluated.

With respect to the preparation of these mixed-mode sorbents, both were prepared from precursors obtained by PP, but the protocols of synthesis were different. In the first case, the HXLPP-WCX sorbent was obtained by direct polymerisation of monomers including DVB, VBC and MAA, in such a way that the MAA that contains the carboxyl moieties was directly incorporated into the precursor polymer, and followed by the hypercrosslinking reaction. In contrast, HXLPP-SAX was obtained using post-polymerisation modification reactions on the precursor particles that were functionalised with different amounts of quaternary ammonium residues and, afterwards hypercrosslinked. The HXLPP-WCX sorbent had an IEC of 0.75 meq g^{-1}, while the IEC of HXLPP-SAX sorbents was between 0.2 meq g^{-1} and 0.4 meq g^{-1}. These IEC values reflected the number of available ionic groups on the sorbent capable of ionic interaction. As can be observed, the synthetic approach consisting of the incorporation of ionic moieties into a polymer skeleton exhibits a significantly higher IEC than the polymer in which the moieties were incorporated by post-polymerisation modification on the HXLPP particles. Comparing the results obtained from these studies, it seems that direct synthesis of polymers from monomers that already contain the ionic group have more promising potential than the modification of the HXLPP resin with a functional ionic group.

With regard to the shapes and sizes of the sorbent particles, the polymers obtained by PP have discrete spherical particles with a narrow size distribution. Typically, the particle size ranges between 4.3±0.9 μm (HXLPP-SAX) to 6.1±1.6 μm (HXLPP-WCX). In previous studies [5,6], the use of small particles has been already demonstrated. It is also worth mentioning that the particle size of HXLPP sorbents is a rather small for SPE materials compared to commercially available macroporous sorbents, for which the sizes range between 30 to 60 μm.

It may also be worth noting that the specific surface area provided by the HXLPP-WCX sorbent (1125 m^2 g^{-1}) is higher than that of commercially available sorbents (~800 m^2 g^{-1}), and the specific surface areas exhibited by HXLPP-SAX sorbents

are definitely much higher than those presented above (1290-1470 $m^2 g^{-1}$). This can be attributed to the higher content of VBC (75%) in HXLPP-SAX material, and thus the higher number of interactions points that can be generated during the hypercrosslinking reaction.

With respect to the applications in SPE, the new HXLPP-WCX showed excellent ability at cleaning the extract and selectively retaining the basic pharmaceuticals thanks to it mixed-mode retention mechanism with the presence of both cation-exchange and hydrophobic interaction sites. The SPE using the HXLPP-WCX sorbent, following suitable protocol, achieved significant clean-up during the washing step, where acidic analytes and interferences bound by RP can be removed as well as other neutral interferences. The washing step dramatically reduced the levels of residual matrix components from complex water samples, leading to a significant reduction of matrix effects, which can be easily observed on the presented chromatograms, whereas the same SPE procedure without the washing step contained significant levels of interferences.

Similarly, the HXLPP-SAX sorbent was evaluated for the extraction of acidic analytes from different water samples. The SPE performance using suitable protocol allowed the elimination of the basic analytes and interferences bound to the sorbent through RP interactions during the washing step, and then the elution of the acidic analytes retained on the sorbent through ionic interactions. The SPE procedure using the HXLPP-SAX sorbent was successfully applied to the selective extraction of acidic pharmaceuticals providing clean extract, and thus, allowed their quantitative recovery.

Comparing the SPE performance to previously introduced hypercrosslinked HXLPP-polar sorbents, it can be observed that the added carboxylic groups and quaternary amine groups impart both new ion-exchange selectivities and polarity to the hypercrosslinked skeleton, allowing the selective extraction of desired analytes, even from complex samples, and providing much cleaner chromatograms than in the case of HXLPP-polar. In the search for selective sorbents, the application of these mixed-mode sorbents is one of the potential methods to fulfil this requirement, even using a UV-Vis detection at 210 nm. As is well known, this is the area of greatest absorption of a wide range of organic

compounds, which may result in lower selectivity. In our case, previous extraction using HXLPP-WCX or HXLPP-SAX sorbents with an effective clean-up step provided clean chromatograms, allowing the quantification of target analytes. This is also a great advantage when detecting with selective detectors such as MS to prevent ion suppression/enhancement. The mixed-mode sorbents, with an additional clean-up step, can reduce the levels of residual matrix components from complex samples, leading to a significant reduction in matrix effects [2,7,8]. This is one of the reasons why these sorbents are starting to be applied in different fields such as in environmental analysis [9, 10].

From the data obtained in the studies above, we can conclude that hypercrosslinked sorbents, thanks to their properties, can effectively extract target analytes from complex water samples, providing reproducible results and compare favourably with commercially available sorbents. Since the previous application of HXLPP-WAX sorbents was successfully used in on-line SPE [5], further improvements can be expected by application of these mixed-mode HXLPP-WCX and HXLPP-SAX sorbents in on-line SPE.

Although efforts have been undertaken in the development of new mixed-mode sorbents, there is still much ongoing research that employs hypercrosslinked resins with other species in the ion-exchange family, such as strong cation-exchangers. In further research, a series of hypercrosslinked sorbents modified with sulphonic acid groups (HXLPP-SCX) [11] has been recently synthesised and successfully applied for the selective extraction of basic pharmaceuticals from complex environmental water samples. Moreover, the results demonstrated that the HXLPP-SCX sorbent provided better results than the commercially available sorbents, such as Oasis MCX, Strata-X-C, Bond Elut Plexa PCX and SampliQ SCX, which can be once again attributed to the enhanced interactions (both RP and cationic) with target analytes.

3.2.4. References

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3.3. Application of imidazolium supported ionic liquid phases for solid-phase extraction materials

In the two studies presented in the previous section, the applicability of the mixed-mode hypercrosslinked sorbents for selective extraction of polar pharmaceuticals from complex water samples was demonstrated. In order to explore the potential of new materials as selective sorbents, in this chapter we include a study related to the use of supported ionic liquid phases (SILPs) as SPE sorbents.

As mentioned in the Introduction, ILs have great potential in green chemistry, separations, spectroscopy, and many other fields. In respect of the current state of green analytical chemistry with a special emphasis on environmental friendly sample preparation techniques, ILs have been found very useful as green solvents in both separation and extraction techniques. ILs have been widely used as extracting media in several extraction techniques, such as LPME, SDME, HF-LPME or SPME [1,2], and more recently, in order to take advantage of their singular properties, ILs have been also immobilised onto solid supports and gradually applied as sorptive media in SPME or SPE techniques [2,3]. Good extraction selectivity and the easy immobilisation on the solid support are among the most significant advantages of ILs. Thus, SILPs as sorptive materials provide a new avenue in the search for additional classes of efficient and selective materials in sorptive extraction techniques [2].

Typically, SILPs consist of imidazolium cations which are immobilised onto fused silica or polymer. Although most of the applications used silica-based ILs, recently the research has also been directed towards employing polymers as a support for ILs [4,5]. So far, several studies have focused on the application of silica-based [6-9] or polymer-based [4] SILPs in SPME [6,7] and SPE [4,8,9] for the extraction of a variety of compounds including short-chain alcohols [6], polar and basic amines, PAHs, alkylphenols, parabens [7] and sulphonylurea herbicides [8], among others. As an example, Tian *et al.* [9] developed new SILPs prepared by the surface chemical modification of the commercial silica and successfully applied it as an SPE sorbent to isolate tanshinones from a plant.

These novel materials provided promising results, and in most of them the authors claim that RP interactions are the most important factor [8-11], but in some of the papers, this kind of interactions is not easy to demonstrate [9-11].

Very few reports have focused on the SPE performance of these materials and investigated different extraction conditions including sample pH or analytes with diverse properties (acidic, basic and neutral) in order to clarify their retention mechanisms. For instance, Vidal *et al.* [12] have worked on the preparation and application of silica-based imidazolium sorbent for the selective SPE of acidic organic contaminants, demonstrating a predominantly anion-exchange character. Similarly, Bi *et al.* [13] applied silica confined imidazolium IL as anion-exchange material in SPE for the separation and determination of lactic acid in fermentation broth, successfully separating the target analyte from interferences with 92% recovery.

With respect to polymeric SILPs, Row's research group is currently intensively investigating applications of new polymer-based SILPs, including IL-based monoliths [11,14] in the SPE of alkaloids [10], flavonoids [11], phytosterols [14] and monosaccharides [15], demonstrating its great potential in the extraction of bioactive compounds from natural plants. However, similarly to the silica-based SILP applications reported by this group [9], the retention mechanisms of these SILPs remain unclear.

Our group has recently introduced immobilised IL based on imidazolium trifluoroacetate salt $[MI^+][CF_3COO^-]$, as an SPE sorbent [4] for the selective extraction of acidic pharmaceuticals, in which the SILP acted as a strong anion-exchange sorbent (SAX). Because of the proven high extraction selectivity of this alternative material, we were encouraged to perform a study with the aim of comparing different SILPs as SAX sorbents in the selective extraction of a group of acidic analytes. This chapter reports the preparation and application of two SILPs as SPE materials to selectively extract acidic pharmaceuticals from aqueous samples. Moreover, the SPE performance is compared to the commercially available mixed-mode polymeric Oasis MAX sorbent.

The paper discussing the results obtained in the presented study has been submitted for publication to the *Journal of Separation Science*.

3.3.1. Comparison of different imidazolium supported ionic liquid polymeric phases with strong anion-exchange character for the extraction of acidic pharmaceuticals from complex environmental samples

COMPARISON OF DIFFERENT IMIDAZOLIUM SUPPORTED IONIC LIQUID POLYMERIC PHASES WITH STRONG ANION-EXCHANGE CHARACTER FOR THE EXTRACTION OF ACIDIC PHARMACEUTICALS FROM COMPLEX ENVIRONMENTAL SAMPLES

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Abstract

Two imidazolium supported ionic liquid phases (SILPs) containing different anions, trifluoromethanesulphoate $[CF_3SO_3]^-$ and tetrafluoroborate $[BF_4]^-$, were synthesised and evaluated as solid-phase extraction (SPE) sorbents for extracting acidic pharmaceuticals from aqueous samples under strong anion-exchange (SAX) conditions, which include an effective clean-up of the sample.

The best SILP material $[MI^+][CF_3SO_3^-]$ was selected and successfully applied to the determination of acidic pharmaceuticals in different types of water samples (river water and effluent wastewater). The results were then compared to the commercially available Oasis MAX sorbent.

Keywords: *Supported ionic liquid phases; strong anion-exchange sorbent; solid-phase extraction; acidic compounds*

1. INTRODUCTION

Ionic liquids (ILs) have been known for many years, but research on this class of salts has expanded dramatically over the last few years. In practical terms, the most valuable development in IL-based chemistry is the usefulness of ILs as ecofriendly solvents with multiple applications in synthesis, catalysis and separation science [1-3].

ILs are known to be polar and nonvolatile, but one of their interesting features is that their physical and chemical properties depend on the type and nature of the ions employed [4,5].

ILs in common use involve cations such as alkylimidazolium, alkylpyrrolidinium, alkylphosphonium and isoquinolinium, in addition to anions such as $[BF_4]^-$, $[CF_3COO]^-$, $[CF_3SO_3]^-$, $[PF_6]^-$ and $[AlCl_4]^-$ [6].

Current research on ILs has attracted interest in different fields of analytical chemistry [4-10], especially as relates to techniques such as chromatography and capillary electrophoresis; these applications have been reported in numerous studies [1,6,9,10]. Due to their physicochemical properties, ILs can be used in many extraction techniques, in particular liquid-liquid extraction (LLE) [11,12], liquid phase microextraction (LPME) [13,14], and mainly single-drop microextraction (SDME) [15,16]. Much attention has been paid lately to supported ionic liquid phases (SILPs). This new class of advanced materials combines the properties of ILs with the advantages of a solid support. The properties of SILPs can be tuned by varying the cations and anions, and many SILPs have been applied in sorptive extraction techniques such as solid-phase extraction (SPE) [17,18] and SPME [13,19], where the development of new materials that promote the capacity and selectivity of the technique is a continuous field of research. SILPs have recently been used as SPE sorbents to enrich or isolate analytes from complex biological or environmental samples [17,18,20,21]. The first studies have reported the applications of ILs immobilised onto silica sorbent for the extraction of tanshinones from medical plants [18], lactic acid from fermentation broth [20] and sulphoylurea herbicides from environmental water and soil samples [21]. In the last year, Row *et al.* have reported the applications of different ILs immobilised onto polymer sorbent for the extraction of matrine and oxymatrine [22], β-sitosterol [23] or tanshinones [24] from medical plants.

In a previous study [17] of our group $[MI^+][CF_3COO^-]$ immobilised onto polymeric sorbent demonstrated to be a promising material for extracting acidic compounds from complex samples. These good results obtained with $[MI^+][CF_3COO^-]$ encouraged us to prepare and evaluate other SILPs immobilised onto polymer sorbent with different anions as SPE sorbents and compare them to commercially available polymeric mixed-mode sorbents.

2. EXPERIMENTAL

2.1. Reagents and standards

The reagents used for the SILP syntheses were divinylbenzene (DVB), vinylbenzyl-chloride (VBC), N-methylimidazole (MI), fluoroboric acid (HBF_4), triflic acid (CF_3SO_3H) and trifluoroacetic acid (CF_3COOH). All were supplied by Sigma-Aldrich (Steinheim, Germany). The VBC was purified by vacuum distillation prior to polymerisation. The DVB was extracted with 2 M sodium hydroxide (NaOH), washed few times with water and dried over sodium sulphae. The other reagents were used as received.

The analytes selected to initially evaluate the sorbents were the following pharma-ceuticals: salicylic acid, carbamazepine, antipyrine, trimethoprim, metoprolol, naproxen, fenoprofen, diclofenac, ibu-profen and gemfibrozil (all obtained from Sigma-Aldrich). Standard solutions of 1000 mg L^{-1} of each compound were prepared in methanol.

The mixture of all the compounds was prepared by diluting the standard solution in Milli-Q water (Millipore, Bedford, MA, USA). Milli-Q water and LC-grade acetonitrile (SDS, Peypin, France) were used to prepare the mobile phase. Hydrochloric acid (HCl) from Probus (Barcelona, Spain) was used to adjust the pH of the mobile phase and the sample prior to SPE. LC-grade methanol was purchased from SDS. The other reagents used in the SPE procedure were: sodium hydroxide (NaOH) from Merck (Darmstadt, Germany) and formic acid (HCOOH) from Sigma-Aldrich.

2.2. Resin preparation and characterisation

Polymer particles were obtained by suspension polymerisation of 97.5 g of VBC (98 wt.%) and 2.5 g of 80% pure DVB (2 wt.%) in the presence of 20 wt.% of toluene. The reaction was catalyzed by 0.5 wt.% of benzoyl peroxide and was carried out at 60 °C for 1 h, 70 °C for 1 h, 85 °C for 2 h and finally at 95 °C for 5 h.

After polymerisation, beads were washed with hot water (to free them from stabiliser and salt), room-temperature water, and acetone (to remove most of the organic impurities such as initiator, unreacted monomers, and solvents), and then dried, pre-swollen in toluene and Soxhlet-extracted in the same solvent.

The modification of the VBC/DVB resin was done by refluxing dry polymer with the excess of MI. First, 15.0 g of dry polymer was placed in a round-bottom flask and refluxed with the excess neat MI for 17 h. Next, the polymer was placed in the column and subjected to conditioning with water, 1 M HCl, water, 1 M NaOH and finally with water. Then, the 0.1 M HBF_4 solution and neutral-pH water were used to obtain $[MI^+][BF_4^-]$. To prepare imidazolium triflate $[MI^+][CF_3SO_3^-]$, a solution of 0.1 M CF_3SO_3H and neutral-pH water was used. Figure 1 presents the structures of $[MI^+][CF_3SO_3^-]$ and $[MI^+][BF_4^-]$.

The chlorine content for these polymers was determined using the Volhardt method after the samples (ca. 20 mg) had been burned in oxygen and the fumes absorbed in 20 ml of H_2O_2 solution. The nitrogen content of dry polymers was measured using the Kjedahl method.

2.3. Chromatographic instruments and conditions

The chromatographic experiments were performed with an HP 1090 LC equipped with an injection valve with a 20 µL loop and a 1200 Series UV spectrophotometric detector (Agilent Technologies, Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C18, 5 µm (Teknokroma, Barcelona, Spain). The mobile phase was Milli-Q water adjusted to pH 3 with HCl and acetonitrile (ACN). The flow rate was 1 mL min^{-1} and the temperature of the column oven was set to 35 °C. The gradient profile initially went from 20% to 40% ACN in 4 min, next to 60% ACN at 11 min, and then to 100% ACN at 20 min (held 5 min), after which time the mobile phase was returned to the initial conditions (20%) in 2 min. The wavelength used to detect all the compounds was set at 210 nm throughout the analysis.

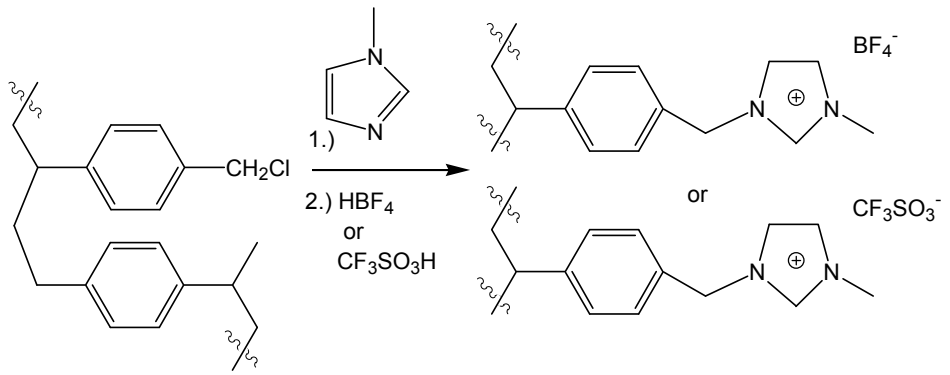

Fig. 2. Scheme of the synthesis of the SILPs studied.

2.4. Solid-phase extraction

The cartridges were packed with 500 mg of the synthesised sorbent, $[MI^+][CF_3SO_3^-]$ or $[MI^+][BF_4^-]$, in a 6 ml polypropylene syringe. The sorbent was retained by two polyethylene frits with a pore size of 10 µm. A vacuum manifold (Teknokroma, Barcelona, Spain) was used to perform the SPE process. The retention capabilities of the SILPs were compared to those of the commercial cartridge Oasis MAX 500 mg/6 ml (from Waters). The cartridges were reused many times.

The SAX procedure was the same for all sorbents. The cartridge was activated with 5 mL of MeOH followed by 5 mL

of Milli-Q water. The sample (250 - 1000 mL), adjusted to pH 7, was then loaded at a flow rate of 5 mL min^{-1}. Next, the cartridge was washed with 10 mL of MeOH. Finally, the compounds were eluted from the cartridge using 15 mL of 10% HCOOH in MeOH. Before injection, the eluate was evaporated until dryness and redissolved in 1 mL of MeOH:H_2O (1:1) prior to LC analysis.

2.5. Sample collection procedure

Environmental water samples such as water from the Ebre River and effluent water from a wastewater treatment plant were adjusted to pH ~3 with HCl and kept in a refrigerator at 4 °C. Before SPE, the samples were adjusted to neutral pH and filtered through 0.22 μm nylon membranes (Supelco, Bellefonte, PA, USA) to eliminate the particulate matter of real samples.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterisation of the SILP

Two SILPs with different anions, $[CF_3SO_3]^-$ and $[BF_4]^-$, were synthesised. The polymer-immobilized imidazolium salts were prepared in a simple reaction of VBC-DVB copolymer with MI. Figure 1 shows a scheme of the synthesis of the SILPs. These sorbents have expanded gel-type structure because the DVB content in them is 2 wt.%, which is too low to provide permanent porosity. Therefore, the specific surface area is negligible. The reason for choosing this level of crosslinking was to ensure good swelling of VBC resin in MI during the modification and to provide enough mechanical strength of the beads for the SPE process. The high water content (21% and 25%, respectively) ensures good kinetics of solute diffusion within the polymeric beads.

The elemental analysis data shows that the modification leading to the immobilized imidazolium salt proceeds with high yield and that the final material contains almost the maximum possible amount of immobilised salt. The elemental analysis gave 5.23 mmol of N per g of dry material, which corresponds to 2.61 mmol of imidazolium groups per g. For $[MI^+][BF_4^-]$, the elemental analysis gave 5.91 mmol of N per g of dry material, which corresponds to 2.95 mmol of imidazolium groups per g.

3.2. Optimization of the SPE procedure

Initially, for the optimisation of the SPE protocol, just $[MI^+][CF_3SO_3^-]$ was selected. This SILP material was tested under SAX SPE protocol because, our previous experience in evaluating SILPs in SPE indicated that this must be the most likely mechanisms of interactions for this type of SILPs [17]

A group of pharmaceuticals with different characteristics (basic and acidic) was initially selected to evaluate these sorbents for extraction purposes. The SPE method was then optimised to

obtain a selective extraction with the highest recoveries of the selected group of acidic compounds. Some parameters that can affect the efficiency and selectivity of the extraction-such as the loading, washing and elution steps-are discussed below.

3.2.1. Loading sample conditions

The extraction performance of SILP sorbents may depend on the pH. The influence of pH on the efficiency of analyte binding was examined with a view to improving the performance of SILP in the SPE determination of pharmaceuticals in water samples. At neutral pH, basic and neutral pharmaceuticals, in neutral form, should be retained on the SILP by reversed-phase (RP) mechanism interactions while the acidic pharmaceuticals, in deprotonated form, should be retained by ionic interactions.

The extraction performance of SILP sorbents may depend on the pH. At neutral pH, basic and neutral pharmaceuticals (in neutral form) should be retained on the SILP by reversed-phase (RP) mechanism interactions while the acidic pharmaceuticals (pK_a < 5) (in anionic form) should be retained by ionic interactions. Initially, 5 mL of the sample spiked at 2 mg L^{-1} were tested at different pH levels (pH 3, 5 and 7), and, according to the SAX protocol, the retained analytes eluted using 10 mL of 5% HCOOH in MeOH.

As expected, the extraction recoveries obtained for all acidic analytes under neutral conditions (pH 7) were 25-40% higher than those obtained at pH 3.0 or 5.0. Therefore, the sample was adjusted at pH 7.

It should be pointed out that under these conditions the basic polar analytes tested, which were retained by RP interactions, were slightly retained after the loading step (%R 20-30%), and therefore, they were discarded for the rest of the study.

Once the loading conditions had been optimised, we tested a higher sample volume in order to evaluate the influence of the different variables for a higher volume such as 250 mL. Since the recoveries were similar to those obtained at 5 ml, this sample volume was used for further optimisation.

3.2.2. Washing step

The aim of the washing step was to reduce the level of matrix interferences bound to the sorbent through RP mechanisms while retaining the acidic compounds bound to the sorbent through anion-exchange interactions. We tested 5 ml of MeOH or ACN as solvents in the washing step. We selected MeOH because it provided slightly better results (~5% improvement in terms of %R) than ACN.

We then studied the volume of MeOH, and with 10 mL all acidic analytes were retained without any loss during the washing step and recovered in the elution step (%R 80-100%). As the volume of MeOH was increased further, compounds such as gemfibrozil and

ibuprofen were partially eluted in the washing step and their elution was completed in the elution step (i.e. ionic interactions between these analytes and the sorbent may be weaker). Ultimately, 10 mL of MeOH was selected as the optimum washing volume.

3.2.3. Elution solution

Elution solvent and elution volume are essential parameters to be investigated in SPE procedure. In order to protonate the acidic analytes and disrupt the ionic interactions with the positively charged SAX sorbent, the elution solvent had to be acidified. To select the suitable solvent for the elution step, we tested 10 mL of 10% of HCOOH in MeOH (we had already tested with 5% of HCOOH) and 1% HCl in MeOH in order to completely elute the most acidic compounds with the minimum volume of solvent. The results obtained with 10% HCOOH in MeOH (%R was close to 100% for all acidic analytes) was better than with 5% HCOOH in MeOH (%R 50-90%). The 1% HCl in MeOH failed as an elution solvent for acidic analytes (%R 20-88%). Thus, we found that 10% HCOOH solution in MeOH provided the best results.

To establish the optimum elution volume, we tested volumes from 10 to 20 mL of 10% HCOOH in MeOH. We found that 15 mL was the minimum volume of solvent to ensure the complete elution of all the acidic compounds and achieve satisfactory extraction yields (%R > 95%).

The optimised conditions for $[MI^+][CF_3SO_3^-]$ were then applied for $[MI^+][BF_4^-]$, and the recoveries were also satisfactory (%R > 90%), which indicated that the SPE protocol is also suitable for $[MI^+][BF_4^-]$.

3.2.4. Sample volume

The optimised conditions for further experiments were as follows: sample pH 7; washing: 10 mL of MeOH; elution: 15 mL of 10% HCOOH in MeOH. The sample volume was tested using both SILP materials ($[MI^+][CF_3SO_3^-]$ and $[MI^+][BF_4^-]$) by percolating increasing volumes (250-1000 mL) of sample. Table 1 shows the recovery values obtained after percolation of 1000 mL of Milli-Q water spiked at 2 μg L^{-1} of each analyte for both of the SILPs tested. The percolation of 1000 mL of Milli-Q water through the $[MI^+][CF_3SO_3^-]$ sorbent allowed satisfactory recovery of the acidic analytes, but percolation through $[MI^+][BF_4^-]$ did not. Strong interactions took place between the $[MI^+][CF_3SO_3^-]$ sorbent and acidic pharmaceuticals, thereby allowing the preconcentration of high sample volumes with good recoveries and demonstrating the good performance of the synthesised material. These results can be explained by the difference in anionic charge. The $[BF_4]^-$ seems to exhibit a higher charge density since single negative charge is distributed over five atoms, what leads to stronger interaction with the imidazolium sites in the resin and may thus complicate the ion-exchange

between analytes and sorbent. Moreover, the more dispersed charge in the anionic structure of $[CF_3SO_3]^-$ causes weaker interactions with the MI cation. Thus, it is possible that, when $[MI^+][BF_4^-]$ was used to extract the acidic analytes, the ionic interactions were not as strong as the partition ones (which played a major role), and that the target analytes were more retained by ionic interactions when $[MI^+][CF_3SO_3^-]$ was used.

From the chemistry of ILs, it is also known that $[BF_4]^-$-based ILs are not water-stable compounds because they hydrolyse [25]. The fact that ILs tend to aggregate in aqueous solutions [26] might perturb ion exchange between sorbent and analytes, which results in low extraction efficiency. Such behavior may also be related to the neutral nature of the $[BF_4]^-$ [27].

Since $[MI^+][CF_3SO_3^-]$ provided significantly better results, this sorbent was selected for the further research.

Table 1. Recovery values (%) of the analytes when the $[MI^+][CF_3SO_3^-]$, $[MI^+][BF_4^-]$ and Oasis MAX sorbents were applied in SPE for preconcentration of 1000 mL of Milli-Q water sample spiked at 2 μg L^{-1} with the analyte mixture.

Analytes	pK_a	Recovery [%]					
		$[MI^+][CF_3SO_3^-]$		$[MI^+][BF_4^-]$		Oasis MAX	
		Wash	Elution	Wash	Elution	Wash	Elution
Salicylic Acid	3.0	0	105	0	50	0	85
Naproxen	4.8	0	81	0	32	0	83
Fenoprofen	4.5	0	100	0	65	0	87
Diclofenac	4.2	0	75	41	28	0	94
Ibuprofen	4.4	0	83	0	31	0	76
Gemfibrozil	4.7	0	101	27	16	0	95

For the experimental conditions, see text. %RSD (n=3) were lower than 17%

3.2.5. Comparison to other materials

In order to compare the performance of $[MI^+][CF_3SO_3^-]$ resin to commercial sorbent, the same experiments were carried out using commercially available Oasis MAX. Oasis MAX is a mixed-mode macroporous sorbent based on a [poly(vinylpyrrolidone-DVB)] skeleton with quaternary amine moieties that acts as a SAX sorbent. Oasis MAX has a macroporous skeleton with a specific surface area of up to 800 m^2 g^{-1}, so it can outperform SILPs in terms of interaction points with the analytes to be extracted. When 1000 mL of Milli-Q water spiked at 2 μg L^{-1} was percolated through the Oasis MAX cartridge, most of acidic analytes gave recovery values similar to those obtained with $[MI^+][CF_3SO_3^-]$ (see Table 1).

These results were also compared to those of the $[MI^+][CF_3COO^-]$ sorbent used in a previous study [17], which provided effective extraction of acidic

analytes from different water samples. When 1000 mL of Milli-Q water was analysed, $[MI^+][CF_3COO^-]$ performed like a SAX sorbent and the recovery values (%R) for acidic analytes such as fenoprofen, naproxen and salicylic acid (101%, 103% and 89%, respectively) were comparable to those obtained with $[MI^+][CF_3SO_3^-]$ (100%, 81% and 105%, respectively), which may indicate that $[MI^+][CF_3SO_3^-]$ and $[MI^+][CF_3COO^-]$ have similar characteristics and demonstrate similar behaviour.

3.3. Analytical performance and applications

To evaluate the applicability of the optimised SILP procedure to environmental sample analysis, the method was applied to Ebre river water and effluent wastewater. Due to the presence of complex natural organic matter in these matrices, we decided to decrease the sample volume. Further experiments with river water were performed by percolating 500 mL samples of Ebre river water spiked at 2 μg L^{-1} of each analyte and thereafter following the same SPE procedure. After the SPE process, the eluate was evaporated to improve the overall sensitivity of the method and thereby allow quantification of the analytes at the concentration levels typically encountered in environmental water samples.

The results for all acidic compounds when SILP was used in river water (see Table 2) are comparable to those obtained in Milli-Q water; however, as expected, due to matrix complexity, the retention of the compounds was slightly affected. Naproxen, fenoprofen and ibuprofen were partially eluted in the washing step (%R < 15%) and showed little fractionation with the SILP sorbent tested, and recoveries for naproxen, fenoprofen and ibuprofen decreased slightly to 79%, 72% and 65%, respectively. Gemfibrozil, however, showed higher fractionation, being 80% washed out in the washing step, and just 20% recovered during the elution step. This behaviour may occur due to the weak ion-exchange interaction of gemfibrozil with the SILP resin.

For comparison purposes, we performed the same experiment using Oasis MAX (see Table 2). The recoveries obtained for the most acidic analytes when Oasis MAX was used in river water were similar to those obtained with $[MI^+][CF_3SO_3^-]$, except for gemfibrozil.

When effluent wastewater was analysed using $[MI^+][CF_3SO_3^-]$ and Oasis MAX, the loading volume of sample had to be decreased due to the complexity of the matrix. Instead of using a 500 mL sample as in the previous case, 250 mL of effluent wastewater, the usual volume in samples of this type, was percolated through the cartridge. The recoveries for naproxen, fenoprofen and salicylic acid were in the 76-95% range with both sorbents tested. However, ibuprofen showed a higher level of fractionation; that is, for $[MI^+][CF_3SO_3^-]$, 41%

recovered in elution (with the rest appearing during washing); and for Oasis MAX, 59% recovered in elution (with the rest appearing during washing). Only when gemfibrozil was loaded onto the $[MI^+][CF_3SO_3^-]$ sorbent was almost eluted in the washing step, similarly to river water. Indeed, gemfibrozil had already presented retention problems in river water, and these problems may increase in more complex matrices, since natural organic matter and other compounds present in wastewater samples give rise to increased competition for binding to the sorbent.

Table 2. Recovery values (%) when the $[MI^+][CF_3SO_3^-]$ and Oasis MAX sorbents were applied in SPE for preconcentration of 500 mL of Ebre river water sample spiked at 2 μg L^{-1} with the analyte mixture.

Analytes	pK_a	Recovery [%]			
		$[MI^+][CF_3SO_3^-]$		Oasis MAX	
		Wash	Elution	Wash	Elution
Salicylic Acid	3.0	0	90	0	110
Naproxen	4.8	13	79	3	81
Fenoprofen	4.5	10	87	0	94
Diclofenac	4.2	0	72	0	85
Ibuprofen	4.4	14	65	0	94
Gemfibrozil	4.7	80	19	10	91

For the experimental conditions, see text. %RSD (n=3) were lower than 16% for %R >15.

The results presented for $[MI^+][CF_3SO_3^-]$ in effluent wastewater were similar to those reported in previous research [17]. The recoveries were slightly higher for fenoprofen and naproxen (93% and 101% for $[MI^+][CF_3COO^-]$ compared to 76% and 77% for $[MI^+][CF_3SO_3^-]$, respectively) and slightly lower for salicylic acid (55% for $[MI^+][CF_3COO^-]$ compared to 95% for $[MI^+][CF_3SO_3^-]$).

Figure 2 shows the chromatograms obtained with $[MI^+][CF_3SO_3^-]$ and Oasis MAX after SPE of a 250 mL sample of effluent wastewater with the addition of 5 μg L^{-1} of analyte mixture followed by the washing out of interferences and the elution of acidic compounds. The chromatogram profile for $[MI^+][CF_3SO_3^-]$ (Fig. 2a) had a smaller initial hump in the baseline than did the profile for Oasis MAX (Fig. 2b), which may be useful for properly quantifying the analytes that appear at low retention times.

It is worth mentioning that the cleanliness of the chromatograms was significantly improved, as a non-selective detector (UV) was used. Using this selective extraction procedure for matrix clean-up may be a way to reduce the ion suppression/enhancement effects in assays based on mass spectrometry detection with an ESI interface.

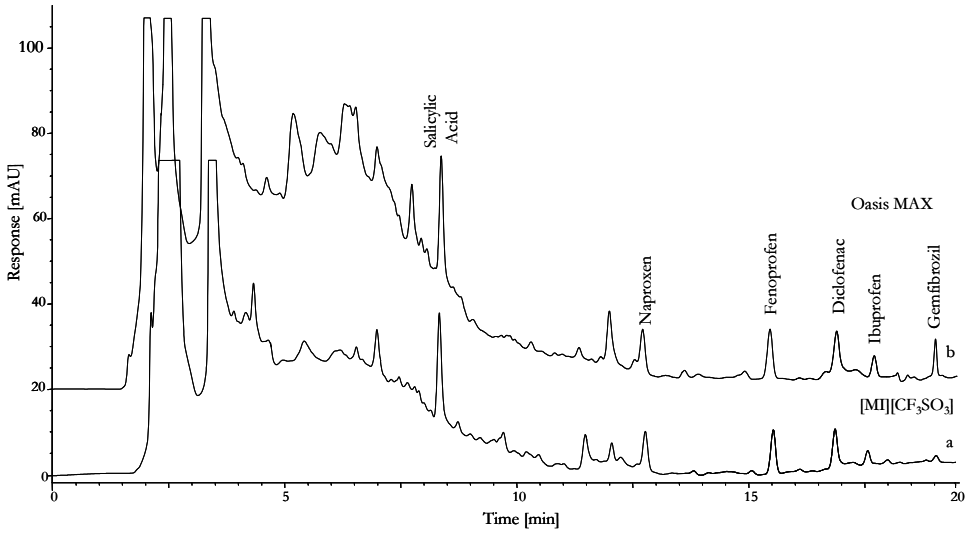

Fig. 4. Chromatograms obtained after off-line trace enrichment with $[MI^+][CF_3SO_3^-]$ (a) and Oasis MAX (b) of 250 mL of effluent wastewater sample with addition of a 5 $\mu g l^{-1}$ level of analyte mixture followed by the elution of acidic compounds.

The results of this investigation indicate that SILPs performed much like conventional sorbents without losing their exceptional features. Thus, the potential of SILPs as alternative packing sorbents in SPE has been demonstrated.

4. CONCLUDING REMARKS

Two SILPs based on 2% VBC/DVB modified with N-methylimidazole containing $[CF_3SO_3]^-$ and $[BF_4]^-$ were synthesised and their application in the analytical field was demonstrated.

The two SILP materials performed as SAX sorbents in selective SPE to extract a group of acidic pharmaceuticals. The best results, obtained with $[MI^+][CF_3SO_3^-]$, were comparable to those of a representative mixed-mode SAX sorbent (Oasis MAX).

The application of $[MI^+][CF_3SO_3^-]$ in SPE of acidic pharmaceuticals from environmental waters demonstrated high selectivity and capacity. This sorbent has therefore been proven as an alternative material for SPE.

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3.3.2. Discussion of results

The results presented in this section demonstrate the applicability of new SILPs as sorbents with SAX character for the extraction of a group of acidic analytes. The added feature of SILPs as SPE sorbents, using SAX protocol, is their selectivity.

As mentioned in the introduction, SILPs can be used as RP sorbents [8], but there are some reports in which the authors did not clearly specify which interactions are involved in the extractability of the SILPs [9-11]. However, after taking into account the SPE protocols used in these applications, it suggests that the extractability of these SILPs may arise from ion-exchange interactions. To the best of our knowledge, this is the first time that SILPs based on $[MI^+][CF_3SO_3^-]$ and $[MI^+][BF_4^-]$ were prepared and applied as SPE sorbents, and the comparative study presented here demonstrates that these phases can be applied for the selective extraction of acidic analytes from real water samples. The present paper proposed a novel SILP-based material that was used as a SAX sorbent for the enrichment of acidic analytes in environmental aqueous samples.

It is also worth mentioning that the typical sample volume used in the applications published of SILPs is relatively low (<1 mL), and in most cases [9-11], the authors used the SILPs as a clean-up material. In order to demonstrate the applicability of the SILPs for selective extraction of pharmaceuticals in environmental analysis, we used significantly higher sample volumes ranging from 250 mL to 1000 mL.

Regarding the washing step, most of the protocols using SILP-based materials include a small volume (<2 mL) of water or organic solvent [9,14,15]. However, in this case we decided to reinforce the washing step using 10 mL of MeOH, in order to enhance the selectivity of the whole extraction process and no losses were observed. From the results obtained in the experimental part when comparing both SILPs, $[MI^+][CF_3SO_3^-]$ showed far higher retention of target analytes than $[MI^+][BF_4^-]$, which indicates that the nature of the anion plays a significant role in influencing the extractability of ILs. In comparison to the commercially available macroporous Oasis MAX sorbent based on N-vinylpyrrolidone and DVB which contains quaternary ammonium moieties, it can be seen that $[MI^+][CF_3SO_3^-]$ has comparable SPE performance. The high

selectivity of the SILPs evaluated is corroborated by the fact that 15 ml of 10% HCOOH in MeOH was required to elute the acidic analytes completely, which is a relatively high volume of elution solvent, due to the strong ionic interactions between acidic analytes and imidazolium cation.

Comparing the SPE performance of $[MI^+][CF_3SO_3^-]$ and the results to those derived from the use of the $[MI^+][CF_3COO^-]$ sorbent [4], it can be noted that similar volumes of complex samples can be loaded providing quantitative results. The volume of washing solvent required to effectively remove the interferences retained by RP interactions using the $[MI^+][CF_3SO_3^-]$ sorbent (10 mL of MeOH) was lower than that required when extracting the acidic analytes (20 mL of MeOH) with the $[MI^+][CF_3COO^-]$ material [4]. However, the volume of elution solvent to elute the target analytes completely using $[MI^+][CF_3SO_3^-]$ was slightly higher and stronger (15 mL of 10% HCOOH in MeOH) than that used for elution from the $[MI^+][CF_3COO^-]$ sorbent (10 mL of 5% HCOOH in MeOH), which indicates stronger ionic interactions between the target analytes and the $[MI^+][CF_3SO_3^-]$ material.

On the basis of the research presented and discussed, it can be concluded that the anion in SILPs considerably influences the retention of analytes onto the sorbent. It should also be pointed out that the extraction performance of SILPs is quite complex, due to the sorbent's characteristics. Therefore, theories on the extraction mechanism need to be confirmed by further experiments in the future.

As mentioned previously, the majority of the new applications used silica-based SILP as the sorbent, compared to relatively few SPE applications using polymeric-based SILP. Some authors claimed that the retention mechanism in these materials may be based on RP interactions. However, there are also few examples that indicate strong anion exchange. Vidal *et al.* [12] have recently published a comprehensive study on issues surrounding the applications of 1-alkyl-3-(propyl-3-sulfonate) imidazolium-functionalised silica for the selective SPE of variety of analytes. It was concluded that anion exchange was the main interaction, but hydrophobic, π-π as well as hydrogen bonding interactions also participate in the extraction process. An additional washing step using 1 ml of water only was included to the SPE protocol. However, no significant

improvement was reported. The extraction efficiencies for most organic acids, when using a mixture of 10 % of CH_3COOH in MeOH as the elution solvent, ranged from 87% to 110%. However, bearing in mind the amount of sorbent used for application (100 mg), the sample volume uploaded onto cartridge was rather low (0.5 mL). The extraction efficiencies obtained for amines and aldehydes were significantly lower, because they were not retained through ionic interactions. The authors also compared the SPE performance of the SILPs under study with two commercially available silica-based SAX materials (HyperSep) and one polymer-based mixed-mode sorbent (Oasis MAX). As in our case, the results arising from the use of SILPs were not markedly different from those obtained with the polymeric mixed-mode sorbent. However, the extraction efficiencies of aromatic compounds provided by the SILP materials were much better than those arising from the use of commercial silica-based SAX, indicating that π-π interactions make an important contribution to the extraction.

Although the use of polymeric SILPs is still in its infancy, there are a few examples of their applications for the extraction of drugs from natural plants among other applications [12-15]. When comparing the SPE performance of polymeric SILPs to commercial silica sorbents, higher extraction efficiency was provided using SILPs [15]. It was also found that the additional washing step, using acetonitrile, allowed the effective removal of interferences and provided high selectivity [14,15]. These results indicate that SILPs are very promising materials that may be a potential tool for future extraction techniques.

When comparing the capacity of the strong anion-exchange SILPs to the HXLPP-SAX sorbent, the ability of HXLPP-SAX to retain basic analytes through RP interactions becomes apparent, arising from its morphological properties. In contrast, SILP materials do not have this ability. However, in terms of selectivity towards acidic pharmaceuticals, these two approaches were comparable, allowing effective clean-up prior to the extraction of target analytes. Therefore, further research should be focused on increasing the capacity of SILPs and enabling their application in other analytical fields.

To summarise, polymeric SILPs are very promising in the search for new approaches and directions of sorptive extraction techniques, since they facilitate

retention through mechanisms that usually involve multiple interactions, such as hydrogen bonding, hydrophobic, and anion-exchange interactions that can significantly increase the capacity and selectivity (by anion-exchange) of IL-based materials towards organic acids. As can be noted, recent developments in the field of SILPs have led to an increase in their use in analytical applications, and more IL-based sorptive materials with enhanced capacity and high selectivity are expected to appear, not only for SPE but for also other extraction techniques.

3.3.3. References

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3.4. Synthesis and application of new polar coatings for stir bar sorptive extraction

The studies discussed so far in this Doctoral Thesis have presented the development of new sorbents for the SPE of polar compounds. Among the sample preparation techniques, SPE has been the most widely used for extracting a variety of analytes from environmental waters [1]. Besides SPE, other important sample preparation techniques are solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE). As mentioned in the Introduction, the SBSE technique has been widely used for the preconcentration of organic compounds from liquid samples in the environmental, food and biomedical fields, owing to the greater sample capacity than SPME. However, as mentioned previously, one of the main problems was that, until very recently, the only commercially available extracting phase for SBSE techniques was polydimethylsiloxane (PDMS), which significantly limited its application to apolar and semi-polar compounds. Therefore, in recent years, efforts have been made to develop new materials for the SBSE of polar compounds. Currently, new commercial stir bars with more polar phases, such as ethylene glycol (EG)-PDMS, and another based on polyacrylate (PA), currently at the pilot stage, have been introduced by Gerstel due to increasing demand for suitable materials for extracting polar compounds. With respect to the in-house prepared stir bars, different approaches have been employed. The most commonly-used preparation techniques include sol-gel technology and monolithic materials [2].

With regard to monolithic phases, when designing a stir bar coating, several factors should be taken into consideration, including the mechanical stability of the material and chemical properties related to the analytes to be extracted, among others. The first and most important factor that must be obtained is the mechanical and chemical resistance. As an example, Huang *et al.* [3,4] recently prepared a series of different monolithic phases with good mechanical resistance and applied them for the SBSE extraction of compounds with different polarities from different matrices.

Although our research group has experience in the development of hydrophilic materials for SPE, the challenge was to design a stir bar device with new properties for SBSE capable of extracting polar analytes. The first stage of the research was focused primarily on the design and further exploration of new

monolithic extracting phases which can provide the capacity and selectivity required for the efficient extraction of polar analytes from complex samples.

To meet the goal of this research, we designed a stir bar supported on a spring, in order to improve mechanical resistance, and coated it with two different monolithic extracting phases with different polarities. In both studies, the main parameters affecting the monolith preparation were optimised. The monomers and the optimal ratio used for preparation of the stir bar coatings were carefully selected considering the polarity of the monomers and their potential sorptive properties with respect to the obtained materials, as well as their mechanical stability.

Depending on the target analytes and their physico-chemical features, such as volatility, thermal stability and polarity, a suitable desorption mode and analytical technique must then be selected. Whereas in GC, the injector provides the means for thermal desorption of compounds (volatile and thermally stable analytes) from the stir bar, no such situation exists for LC. Hence, when using LC (for non-volatile, thermally labile or polar analytes), the compounds are desorbed using a small amount of solvent.

Generally speaking, the aim of the studies reported here was to prepare polar and mixed-mode coatings for stir bars to be used for SBSE, followed by liquid desorption, and evaluate them based on the extraction efficiency of the target analytes as well as to compare their SBSE performance to commercially available stir bars. To go into more detail, the first study reported on preparation of a stir bar based on N-vinylpyrrolidone and divinylbenzene poly(VPD-*co*-DVB), and the second study described the preparation of a polar coating based on methacrylic acid and divinylbenzene poly(MAA-*co*-DVB). Since MAA has a carboxylic moiety, its incorporation into polymer may provide weak cation-exchange ability, as can be seen in Section 3.2.1. for SPE material. With regard to the parameters affecting extraction efficiency, the optimisation of several variables related to the extracting equilibrium and desorption steps was required.

The studies presented in previous sections of this Thesis were carried out using the LC-UV technique. In contrast, the studies reported here were performed

using LC-MS/MS with a triple quadrupole analyser (QqQ) and an electrospray ionisation, as this technique has proven to be very convenient for the determination of a variety of compounds at low concentration levels [5-7].

Finally, we developed SBSE-liquid desorption-LC-(ESI)MS/MS methods for the simultaneous extraction of contaminants with different polarities from environmental water matrices

The results obtained from the first study were published in the *Analitica Chimica Acta* 706 (2011) 135-142. An article that presents the results of the second study has been submitted for its publication in *Journal of Chromatography A*.

3.4.1. Development and application of a polar coating for stir bar sorptive extraction of emerging pollutants from environmental water samples

DEVELOPMENT AND APPLICATION OF A POLAR COATING FOR STIR BAR SORPTIVE EXTRACTION OF EMERGING POLLUTANTS FROM ENVIRONMENTAL WATER SAMPLES

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Abstract

In the present study, a stir bar coated with hydrophilic polymer based on poly(N-vinylpyrrolidone-co-divinylbenzene) was prepared for the sorptive extraction of polar compounds. The main parameters affecting the polymerisation of the coating were investigated.

The new stir bar was applied successfully in stir bar sorptive extraction with liquid desorption followed by liquid chromatography-mass spectrometry in tandem with a triple quadrupole for the determination of a group of polar pharmaceuticals and personal care products (PPCPs) in environmental water matrices. Different variables affecting extraction and desorption such as agitation speed, temperature, ionic strength and extraction time were optimised. The results showed that the stir bar is able to enrich the selected analytes effectively.

The developed method was applied to determine a group of PPCPs in different complex environmental samples, including river, effluent and influent waste water.

Keywords: *stir bar sorptive extraction (SBSE); monolithic polar material; pharmaceuticals and personal care products (PPCPs); environmental water samples*

1. INTRODUCTION

One of the research fields in analytical chemistry is the development of methods to determine contaminants in environmental samples. Due to the low concentration of these contaminants in these kinds of samples, a sample preparation technique together with a high sensitive instrumental technique is required.

For aqueous sample preparation, commonly used techniques include solid-phase extraction (SPE), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE), among others [1-3]. SPE is still the most commonly used technique due to the variety of sorbents commercially available which cover the extraction of compounds with a wide range of physico-chemical properties. In the recent years, SBSE has been proven to be a promising technique for the extraction of liquid samples [1,4] due to the larger coating compared to the one in SPME [3].

In SBSE, the magnetic stir bar encapsulated in a polymeric coat is introduced in the aqueous samples and after stirring for a certain time, the analytes are desorbed thermally or with a solvent. The main factor that determines the extraction efficiency is the partition coefficient of analytes between the phases.

Since the stir bar has a larger volume of polymeric phase than SPME fibres, higher extraction efficiency can be obtained [4]. During the last few years, it has been clearly demonstrated that a wide range of compounds, such as pesticides, steroids, fatty acids, drugs, and so on can be extracted by SBSE [4-6], and this technique has been applied successfully to trace analysis in biomedical [5], pharmaceutical [6] and environmental samples [1].

However, the application of these techniques to polar compounds is still a challenge due to the characteristics of the extraction materials. The only comercially available sorptive extraction phase is polydimethylsiloxane (PDMS).

Nevertheless, since PDMS is a non-polar phase, it is suitable for extracting less polar compounds. Correspondingly, polar analytes are not well recovered and the applicability of PDMS-coated stir bars is reduced to the extraction of the non-polar or semi-polar compounds [6-9].

To overcome this limitation, polar coatings based on different materials prepared by sol-gel technology [10], monolithic approach [11], polyurethane foam [12] or activated carbons [13] have been tested as SBSE coating. Recently, some monolithic polar coatings have been developed to enrich different analytes by SBSE [11,14-17].

For instance, poly(vinylimidazole-divinylbenzene) was successfully applied to extract polar aromatic amines from water samples [17] or vinylpyrrolidone-divinylbenzene (VPD-co-DVB) was applied to the extraction of metals, phenols, anilines, hormones and polycyclic aromatic hydrocarbons (PAHs) [14,15] .

The objective of the present work was the preparation of an improved stir bar with polar coating based on poly(VPD-co-DVB) for SBSE and its application followed by liquid chromatography-mass spectrometry in tandem (LC-MS-MS) for the determination of a broad range of compounds that include polar pharmaceuticals and personal care products (PPCPs) from complex environmental matrices.

2. EXPERIMENTAL

2.1. Reagents and standards

The reagents used for the polymer coating syntheses were divinylbenzene (DVB) (80% grade) supplied by Aldrich (Steinheim, Germany) and N-vinylpyrrolidone (VPD) (99% grade) supplied by Fluka (Buchs, Switzerland). DVB and VPD were freed of polymerisation inhibitors by passing them through short columns packed with neutral alumina. The cyclohexanol (99%) and 1-dodecanol (98%) used as porogens were purchased from Aldrich. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck, Darmstadt, Germany).

The analytes selected to evaluate the sorption characteristics of the stir bars were: paracetamol, caffeine, antipyrine, propranolol, carbamazepine, ibuprofen, diclofenac, methylparaben, ethylparaben, propylparaben, triclocarban, 2,4-dihydroxybenzophenone (DHB), 2,2´-dihydroxy-4-methoxybenzophenone (DHMB) and benzophenone-3 (BP3); all were obtained from Aldrich. The chemical structure, pK_a and log K_{ow} of each analyte are shown in Fig. 1.

Standard solutions for each compound at 1000 mg L^{-1} were prepared in methanol (SDS, Peypin, France). The mixture of all the compounds was prepared by diluting the standard solution in Milli-Q water (Millipore, Bedford, MA, USA).

LC-grade acetonitrile (SDS) and Milli-Q water were used to prepare the mobile phase. Formic acid (Prolabo, Bois, France), hydrochloric acid (Probus, Barcelona, Spain) and sodium hydroxide (Panreac, Barcelona, Spain) were used to adjust the pH of the mobile phase or of the sample. Sodium chloride was obtained from Aldrich.

2.2. Stir bar preparation

In a typical synthesis of a monolith, the monomers DVB (85%, w/w) and VPD (15%, w/w) (40% w/w total monomer in feed relative to solvent) and AIBN (1% mol relative to polymerisable double bonds) were added to the porogen (10% w/v 1-dodecanol in cyclohexanol). The monomer mixture, initiator and porogen were mixed ultrasonically, and the homogenous solution poured into a glass tube with a defined diameter (4 mm i.d.). A stir bar (10 x 3 mm i.d.) was inserted into the middle of a spring (3 mm i.d.) and then immersed vertically into the polymerisation solution. Before polymerisation, the monomer solution was placed on an ice-bath, sparged with oxygen-free nitrogen for 5 min to remove oxygen and then sealed under a nitrogen atmosphere. The sealed glass tube with the stir bar inside was then left in a water bath set at 60 °C for 36 h. After this time, the glass tube was crushed carefully and the glass removed to reveal the polymer monolith.

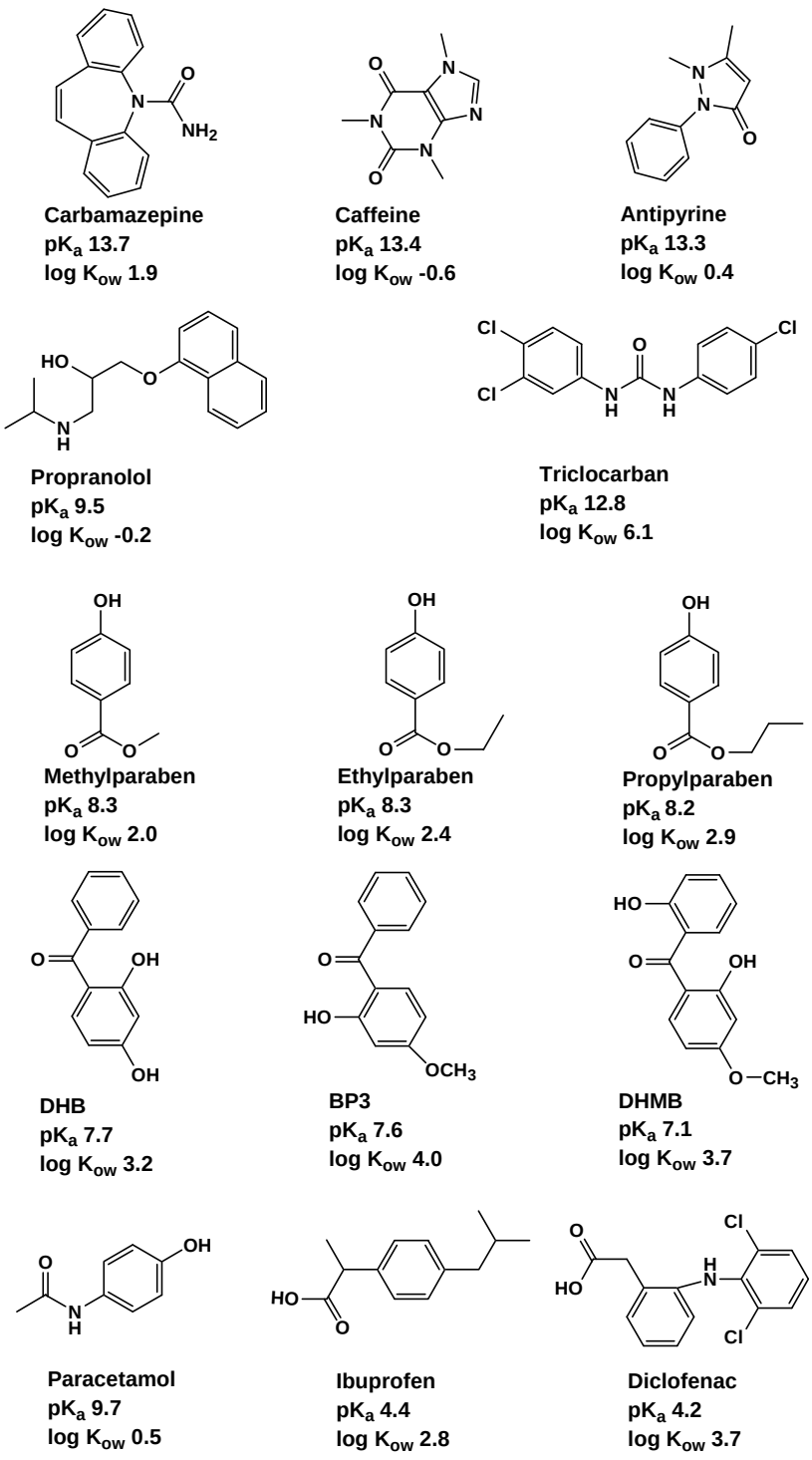

Fig. 1. Chemical structures, pK_a and $logK_{o/w}$ of the analytes studied.

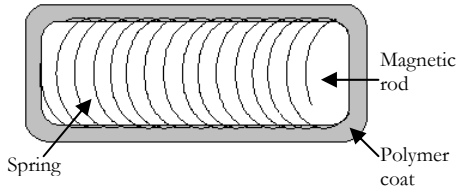

Fig. 2. Assembly of the synthesised stir bar.

A smooth, solid monolith with good dimensional stability was obtained. Fig. 2 schematises the obtained stir bar assembly. The monolithic material on the bar was Soxhlet-extracted with MeOH for 24 h to remove residual monomers and initiator, soluble oligomers and porogen. The final dimensions of the poly(VPD-co-DVB) stir bar was as follows: length: 10 mm; polymer thickness: 0.5 mm corresponding to a volume of polymer of about 70 µL.

The monolithic poly(VPD-co-DVB) were then characterized. The specific surface area of the monolith was measured using a BET treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The carbon, hydrogen and nitrogen content of the polymer were determined with elemental microanalysis using a Carlo-Erba EA 1106 Instrument. FTIR spectroscopy measurements were performed using a Perkin-Elmer Spectrum One FTIR Spectrometer.

2.3. LC-(ESI)MS-MS analysis

The chromatographic experiments were performed with an Agilent HP 1200 liquid chromatograph coupled to a triple quadrupole mass spectrometer with electrospray interface (ESI) from Agilent Technologies (Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column packed with Kromasil 100 C_{18}, 5 µm (Teknokroma, Barcelona, Spain) and the volume injected was 50 µL. The mobile phase was acetonitrile and Milli-Q water adjusted to pH 3 with formic acid. The flow rate was 1 mL min^{-1} and the temperature of the column oven was set at 30 ºC.

The gradient profile was initially from 20% to 100% ACN in 15 min (held 5 min), after which time the mobile phase was returned to the initial conditions (20% ACN) in 3 min.

The (ESI)MS-MS parameters were optimised by direct injection of each analyte. Nitrogen was used as collision, nebulising and desolvation gas. The ESI interface conditions were optimised for each analyte and were: a nebuliser pressure of 45 psi, capillary voltage of 4000 V, desolvation (drying gas) N_2 flow was set at 12 L min^{-1} and desolvation temperature was 350 ºC.

Analyses were performed under multiple reaction monitoring (MRM) mode and MRM transitions for each analyte are summarised in Table 1.

2.4. SBSE assays

The procedure with home-made poly(VPD-co-DVB) stir bar was as follows: the stir bar was activated with 1 mL of MeOH and stirred for 5 min.

Table 1. ESI mode and MRM conditions used for LC-(ESI)MS-MS of target analytes.

Analyte	ESI Mode	Cone Voltage (V)	Precursor Ion (m/z)	Product Ions (m/z)*	Collision Energy (V)
Paracetamol	+	100	152	**110**	15
				93	25
Caffeine	+	125	195	**138**	15
				110	25
Antipyrine	+	100	189	**145**	30
				115	30
Propranolol	+	125	260	**116**	15
				183	15
Methylparaben	-	80	151	**92**	15
				136	5
Carbamazepine	+	150	237	**193**	35
				179	35
Ethylparaben	-	100	165	**137**	15
				92	5
Propylparaben	-	100	179	**92**	15
				136	5
DHB	-	130	213	**135**	15
				169	15
DHMB	-	80	243	**93**	15
				123	5
Diclofenac	-	75	294	**250**	10
				214	20
Ibuprofen	-	75	205	**161**	5
				69	5
BP3	+	130	229	**151**	15
				105	15
Triclocarban	-	130	313	**160**	5
				126	15

* Bold values denotated those ions used for quantification

After drying with lint-free tissue, the stir bar was placed into a flask with 50 mL of water sample at pH 5.0, with 10% of NaCl and then the sample was stirred at 900 rpm for 4 hours at room temperature. After the extraction, the stir bar was removed with tweezers, dipped briefly in Milli-Q water and dried with a lint-free tissue. In order to desorb the analytes with a solvent, stir bar was placed into a vial with 5 mL of MeOH stirred at 900 rpm for 15 minutes.

Then, the stir bar was magnetically removed and the extract was evaporated to dryness under gentle stream of nitrogen. The dry residues were redissolved in 1 mL MeOH:water (1:1) and transferred to a vial, closed with a seal using a hand crimper, and placed into the automatic liquid sampler tray for LC-(ESI)MS-MS analysis.

The retention capability of the poly(VPD-co-DVB) stir bar was compared to the commercially available

PDMS-coated stir bar (Twister), obtained from Gerstel (Mulheim Ruhr, Germany). It consists of a 10 mm long glass-encapsulated magnetic stir bar, externally coated with a layer of 0.5 mm thick, corresponding to a volume of 24 µL of PDMS. Prior to the first use, the stir bar was placed in a vial containing ACN and conditioned for 24 h. Although there are two sizes of PDMS-coated stir bar commercially available, this smaller size was selected, since it provided high sorptive capacity for water sample volumes range from 1 to 50 mL [4].

Reconditioning of stir bars was done after the extraction in MeOH for poly(VPD-co-DVB) stir bar or methylene chloride/MeOH (1:1) for PDMS-coated stir bar [18]. Stir bars were conditioned by placing them in vials with the solvent for 20 min. Then the solvents were refreshed and the procedure was repeated three times. Subsequently, the stir bars were dried with a lint-free tissue, and kept in a vial for the next analysis.

2.5. Sample collection

The river water samples were collected from Ebre River. The waste water samples were collected from the effluent and influent of two sewage treatment plants (STPs).

To ensure proper conditions for keeping the samples, all the real water samples (Ebre river, effluent and influent waste water from STPs) were adjusted to pH~3 with HCl and stored at 4 ºC before analysis.

They were filtered through 0.45 µm nylon membranes (Supleco, Bellefont, PA, USA) before the stir bar extraction to eliminate the particulate matter of real samples.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterisation of the stir bars

With the aim of improving the efficiency of polar compounds by SBSE with commercially available PDMS, a polar group should be introduced in the monolithic coating of the stir bar.

Due to the good results provided by vinylpyrrolidone as polar group in the commercial OASIS HLB sorbent for SPE [19] we selected it as a potential polar monomer. In fact, in a recent study vinylpyrrolidone was already tested as polar monomer in the stir bar coating and results for phenols were better than those obtained with PDMS stir bar [14].

However, a limitation of monolithic stir bars is the mechanical stability along the extractions.

In order to overcome this limitation, we proposed a new design which includes a spring covering the stir (figure 2) in order to increase the mechanical resistance, the number of extractions to be done with the same stir bar, and therefore the robustness of the analytical method.

To do this, a stir of 10 x 3 mm was covered by the spring (3 mm i.d.) and introduced in the glass vial (4 mm i.d.) in which the polymerisation mixture was then poured.

In this case, the presence of the spring made it possible to obtain a thinner coating (0.5 mm) with similar mechanical properties. To test this new design, the starting polymerisation conditions were based on those previously reported for polar monolithic coatings [11,14], and we optimised some parameters in the polymerisation procedure in order to further enhance the properties of the final stir bar.

Some important parameters to take into account in the synthesis of a monolith are: the porogen, that is responsible for the porous formation during the polymerisation; the crosslinker monomer/polar monomer ratio in order to introduce polarity on the coating (*via* monomer) and rigidity (*via* crosslinker); the ratio between total monomers and porogen, and polymerisation temperature and the type of polymerisation initiation.

As porogen, a mixture of 1-dodecanol and cyclohexanol was used since this mixture of solvents had been demonstrated to be useful for imparting porosity into such type of monolithic materials [11,20], because the types of porous formed allow the analytes to penetrate the polymer and also provide enough surface area. From the different ratio tested, 10/90 (w/v) proved to be a convenient porogen mixture.

Regarding the monomers ratio, it was fixed at 85/15 (w/w) of DVB to VPD (this led to a polymer monolith with a specific surface area of 600 $m^2 g^{-1}$), since it was found that the use of lower amounts of DVB (75%) in the monomer feed led to a slight decrease in the specific surface areas of the product (560 $m^2 g^{-1}$). These specific surface areas are as expected, taking into account the high % of DVB and the non-porogen solvent used [19].

The ratio of total monomer to porogenic solvent was set at 40/60 (w/w), because higher monomer ratio led to soft polymers which were broken easily during later stirring operations.

A further variable which was examined was the nature of the initiation step in the polymerisation (thermal *versus* photochemical initiation). With this in mind, thermally-initiated and photochemically-initiated polymerisations were compared. The UV-initiated polymerisations, which were carried out at room temperature, yielded soft and fragile materials consistent with incomeplete cure, whereas those materials produced by thermally-initiated polymerisations were hard and had good dimensional stability, consistent with efficient cure.

As a consequence, thermal initiation was the method of choice in all subsequent polymerisations.

Finally, it was found that the polymerisation temperature had some influence upon the mechanical properties of the monolithic material.

Polymers synthesised at 70 ºC were less mechanically stable than polymers synthesised at 60 ºC, therefore a polymerisation temperature of 60 ºC was employed in all subsequent polymerisations, which were run for 36 hours.

Although the optimum polymerisation conditions were quite similar to those found by Huang *et al.* [15], there are some differences as regards the total monomer/porogen ratio, polymerisation time and temperature, the format and size.

FTIR spectroscopic analyses of the poly(VPD-co-DVB) coatings confirmed that DVB and VPD had been copolymerised effectively into the monoliths. Diagnostic bands consistent with aromatic residues (from DVB) and amide groups (1690 cm^{-1}, from VPD residues) were observed.

Elemental microanalysis of the poly(VPD-co-DVB) monolith gave the following results: carbon (83.0%), hydrogen (7.7%) and nitrogen (2.8%), which are similar to the theoretical values, and provides evidence that both monomers were incorporated into the monolith.

3.2. LC-(ESI)MS-MS optimisation

Once the chromatographic separation was optimised, the best MS-MS conditions were selected and they are shown in section 2.3 and detailed in Table 1. For each analyte, two fragmentations of $[M-H]^+$ or $[M-H]^-$ were acquired. The most intense transition was selected to quantify the analytes.

Paracetamol, caffeine, antipyrine, propranolol, carbamazepine and BP3 were determined under positive ionisation mode; whereas ibuprofen, diclofenac, parabens, DHB, DHMB and triclocarban exhibited a higher response in the negative mode.

All selected analytes showed good linearity ($r^2 > 0.997$) by direct injection of standards and the linear range was 0.5-100 μg L^{-1} for all the analytes, except for methylparaben (0.1-100 μg L^{-1}) and for antipyrine (1-100 μg L^{-1}).

The limits of detection (LODs) calculated as a signal-noise ratio (S/N) ≥3 were 50 ng L^{-1} for the majority of compounds, apart from paracetamol, antipyrine, ethylparaben, DHMB and ibuprofen with a LOD of 100 ng L^{-1}.

3.3. Optimisation of SBSE procedure

In order to test the extraction efficiency of the novel polar stir bar, a group of PPCPs with different polarity were selected.

During the development of the presented procedure, several parameters that influence the SBSE process were evaluated.

Thus, systematic assays were performed to optimise parameters which can influence the SBSE procedure during both extraction and liquid desorption steps.

3.3.1. Liquid desorption conditions

In the first approach, we tested the best liquid desorption conditions, which ensured complete back extraction of selected analytes from the stir bars. Thus, two organic solvents: MeOH and ACN were examined as a desorption solvent. To do this, we initially used the following experimental conditions: 50 mL of the sample spiked at 1 μg L^{-1}, 1 hour of extraction time, 750 rpm of agitation speed at room temperature, and for desorption, 5 mL of organic solvent for 15 min at the same agitation speed, based on previous experience from our group [18].

From the data obtained, MeOH was chosen as the back extraction solvent due to the slightly higher ability to desorb polar analytes from the stir bars (recoveries increase in ~10%). As for desorption volume, we tested 5 mL of solvent in order to immerse the stir bar completely and ensure proper desorption. Further increasing of MeOH volume (10 mL) did not improve the desorption results.

The influence of temperature (25ºC, 35ºC, 40ºC) on desorption in SBSE process was also investigated. The results obtained seem to indicate that desorption was not significantly affected by temperature.

Consequently, room temperature was selected for the further experiments. In order to investigate the time necessary to ensure complete desorption of the analytes from the stir bar, a series of experiments were performed by comparing several periods of time (10, 15, 30 and 60 min) to achieve the best liquid desorption conditions. The results indicated that stirring for 15 min is enough to desorb all analytes of interest from the stir bar and additional desorption did not show presence of the analytes. Consequently, a period of 15 min was selected for desorption process. This desoption time is quite shorter than the one previously used (*i.e.* 2 h) for a similar stir bar [14,15], although in that case, the speed was lower (400 rpm) and stir bar was thicker (1 mm thickness).

3.3.2. Extraction conditions

SBSE is a balanced technique and the analytical process is considerably influenced by agitation speed, temperature, ionic strength, pH and extraction time involved. Starting with the stirring rate, this factor may influence the mass transfer of the analytes during the extraction process. In the present paper, three agitation levels (600, 900 and 1250 rpm) were tested to achieve the best stirring conditions. The response obtained with 900 rpm was higher than that obtained with 600 rpm, and further increases in the stirring rate may damage the monolithic phase. As the agitation speed of 900 rpm provided better recovery of all analytes under study, it was selected for further experiments. This agitation speed was also tested for liquid desorption step and since no significant differences were observed with the initial 750 rpm, 900 rpm was the

selected speed in both extraction and desorption steps. It is noteworthy that higher stirrer speeds could be applied in the present work than were used in previous studies (400 rpm) [14,15]; in the present case, the metal spring acts to reinforce and strengthen the stir bar coating and allow higher stirrer speeds to be realised without attrition of the coating.

The influence of temperature (25 ºC, 35 ºC, 40 ºC) in SBSE process was also studied. It was found that the recoveries of the extractions at 25 ºC and 35 ºC were very similar for most of analytes and recoveries slightly decreased (~10%) at 40 ºC. As a result, 25 ºC was selected for the further experiments.

The effect of sample pH on the extraction efficiency was investigated by adjusting Milli-Q water at the pH values in the range from 3.0 to 11.0.

Fig. 3 shows the influence of the sample pH on SBSE efficiency of a representative group of analytes, and it can be seen that the pH value significantly affects the extraction efficiency of some analytes. The results showed that the extraction recoveries for most of analytes improved with increasing the pH from 3.0 to 5.0 and slowly decreased (except for propranolol) at pH 7. At higher pHs the effect of pH depends on the pK_a (included in Fig. 1) of the studied analytes. For example, the recovery for the analytes with pK_a values lower than 8.3 decreases at the highest pHs tested; however, compounds with high pK_a values such as carbamazepine, caffeine or antipyrine showed no significant changes. In the case of propranolol, the recovery improved under basic conditions (maximum at pH 9.0), due to its pK_a value. Considering these results, pH 5.0 was chosen for further experiments.

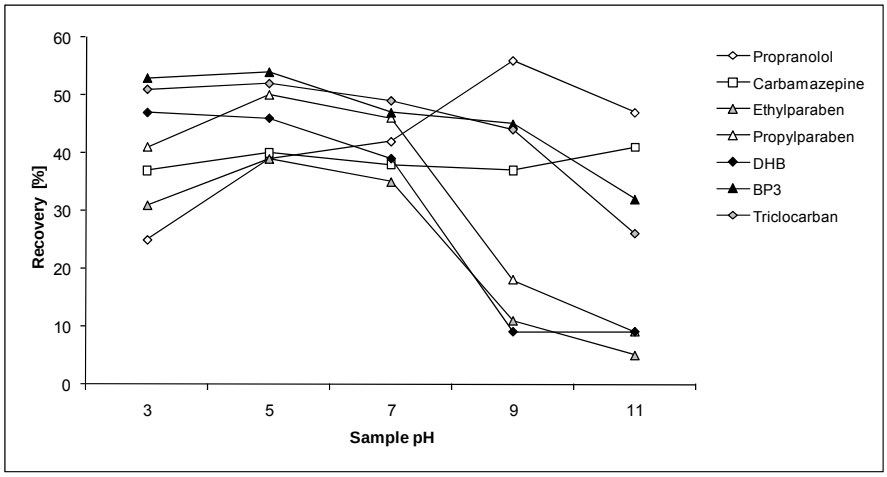

Fig. 3. The effect of sample pH on extraction recovery for a representative group of analytes.

As it is well known, the extraction efficiency in SBSE might depend on the ionic strength in the sample matrix [11]. In the present study, the effect of ionic strength in the recovery of the compounds under study was performed by addition of NaCl from 0 to 15% (w/v) to the aqueous samples.

Fig. 4 shows the effect of salt addition on extraction efficiency for the represent-tative group of analytes. It can be seen that for the same analytes, the recoveries decreased with 5% NaCl addition, and then increased to the maximum with 10% NaCl addition.

This effect may be attributed to the salting-out effect and the electrostatic interaction between polar molecules and salt ions in the solution [21]. With a higher percentage (*i.e.* 15%) of NaCl, the recoveries decreased. As 10% addi-tion of NaCl in the samples provided the best recoveries, this addition was selected in the following studies.

The effect of sample volume on the recovery was also investigated. In order to select the optimum sample volume, different sample volumes (25, 50 and 100 mL) of standard solution of the analyte mixtures were extracted. The results were similar when both 25 mL and 50 mL of the Milli-Q water sample were analysed. When 100 mL of a standard solution was extracted, the recoveries decreased significantly (10-30 % recovery decrease). This result was expected because the higher the volume, the higher the extraction time needed to reach the equilibrium [22]. For this reason, 50 mL of sample was selected for further analysis.

The extraction time is also a very significant parameter. In order to select the best conditions, different extraction periods (1, 2, 4, 6 and 12 hours) were tested.

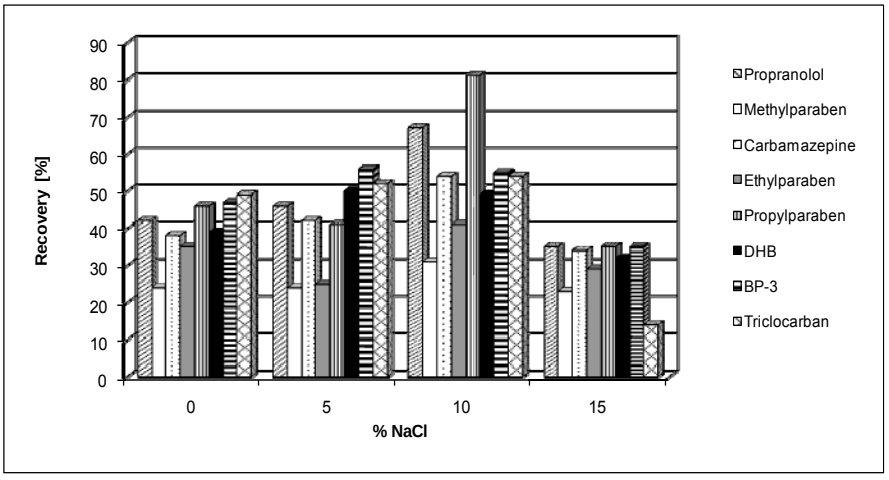

Fig. 4. The effect of % NaCl addition to the sample on extraction recovery for a representative group of analytes .

Fig. 5 shows representative time extraction profiles for a representative group of analytes, where it can be seen that the extraction efficiency increased when the extraction time increased from 1 to 12 hours. However, in order to reach a balance between analysis time and extraction efficiency, 4 hours was selected as the extraction time.

After the optimization procedure, the conditions used to investigate recoveries from environmental samples were as follows: 50 mL of the sample adjusted at pH 5.0 with 10 % NaCl addition and agitated at 900 rpm at room temperature for 4 hours for the extraction and 5 mL of MeOH stirred at the same speed for 15 min for liquid desorption.

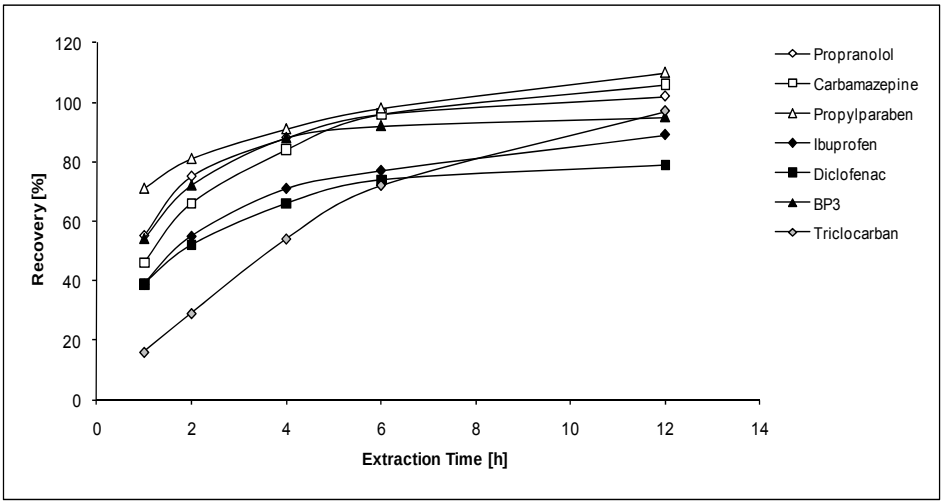

Fig. 5. The effect of extraction time on extraction recovery, for a representative group of analytes.

With the optimum conditions, the recovery for each analyte from the standard solution, including the evaporation step, is shown in Table 2. These recoveries were calculated from the calibration curve by direct injection. The poly(VPD-co-DVB) stir bar method attained very good performance and presented high recoveries, between 80% and 100%, for most analytes and only presented lower recovery for paracetamol, caffeine and antipyrine, whose results were expected since they are very polar compounds (see $\log K_{o/w}$ in Fig. 1).

These results are very satisfactory, taking into account the polarity of these compounds and the results achieved with a similar stir bar [14,15] for other compounds with a broad range of polarity, such as phenols, aromatic amines, and PAHs.

Table 2. Recovery (%) obtained when the poly(VPD-co-DVB) and PDMS stir bars were used in SBSE of 50 mL of Milli-Q water spiked at 50 ng L^{-1} of each analyte.

Analyte	Recovery (%)	
	poly(VPD-co-DVB)	PDMS
Paracetamol	9	-
Caffeine	20	-
Antipyrine	42	-
Propranolol	87	-
Methylparaben	91	-
Carbamazepine	83	-
Ethylparaben	79	2
Propylparaben	89	10
DHB	50	-
DHMB	93	43
Diclofenac	80	4
Ibuprofen	110	11
BP3	92	85
Triclocarban	81	95

% Relative standard deviations (RSDs) (n = 3) were lower than 6% for %R >20%.
For the experimental conditions, see text.

3.3.3. Comparison to a commercial stir bar

The SBSE performance of the poly(VPD-co-DVB) stir bar was compared to the commercially available stir bar based on PDMS.

The SBSE results arising from the use of the PDMS-coated stir bar are also included in Table 2. It can be seen that under the same conditions, the analyte recoveries were higher for most of the compounds with poly(VPD-co-DVB) than with PDMS. Moreover, PDMS was not able to retain any of the more polar compounds while the poly(VPD-co-DVB) stir bar showed good retention, with higher recoveries for most of the analytes. Those results clearly indicate that the selected analytes, having different polarity, presented better affinity for poly(VPD-co-DVB) phase rather than for PDMS. These results can be easily explained by the polar character of the monomer used in the synthesis of the new stir bar coating, while PDMS is an apolar phase. Another significant contribution was that the specific surface area of poly(VPD-co-DVB) monolith was 600 m^2 g^{-1} whereas for PDMS-coated stir bar, the specific surface area is expected to be low from its chemical structure. In any case, it is evident that the poly(VPD-co-DVB) stir bar gives higher recoveries for all of the target analytes than the commercially available PDMS stir bar.

3.4. Analysis of real samples

Given the promising SBSE data obtained with poly(VPD-co-DVB) stir bar when the procedure was applied to Milli-Q water, the same protocol was applied to the analysis of water from the Ebre river and effluent and influent water from a waste water treatment plant (WWTP), where PPCPs had already been detected [23-25].

It is known that electrospray ionisation may give ion suppression/enhancement when complex samples are analysed and this effect was studied by spiking with the analytes the SBSE extracts of river water, effluent and influent WWTP water samples and quantifying the recoveries taking into account the unspiked SBSE extracts. The resulting ion suppression/enhancement ranged between 0 and 22%. Therefore, this effect was not significant.

To calculate the recoveries, the different water samples spiked at different levels of each analyte and the unspiked samples were analysed. Table 3 shows the SBSE results for the different water samples. The data obtained for river water samples were good, with recovery values ranging from 50 to 100% for most of the compounds and similar to the values obtained for extractions from Milli-Q water taking into account the ion suppression and therefore demonstrating the satisfactory ability of this monolithic material to retain both polar and semi-polar compounds even in the presence of the matrix. As can be seen, the data for effluent and influent WWTP water is also good, with recovery values ranging from 50% to 100% for the effluent WWTP sample and 40% to 100% for the influent WWTP sample for most of the compounds.

Then, the method was validated with river water. When the linear range was tested in a 6 concentration levels by duplicate, the analytes exhibited good linearity ($r^2 > 0.993$): between 20 and 2000 ng L^{-1} for carbamazepine and diclofenac; between 100 and 2000 ng L^{-1} for paracetamol and DHMB; and between 50 and 2000 ng L^{-1} for the rest of the compounds. The LODs, calculated using S/N≥3, were between 10 and 20 ng L^{-1} for all the compounds, with the exception of paracetamol and DHMB (50 ng L^{-1}). The repeatability and reproducibility between days of the method, expressed as the relative standard deviation (%RSD) of three analyses of 50 mL of water from the Ebre river spiked at 100 ng L^{-1} were lower than 7% and 15%, respectively, for all compounds. In any case, the good performance of the poly(VPD-co-DVB) stir bar has been demonstrated.

It should be highlighted that between 30 and 40 samples can be extracted with the same stir bar without changes in the recovery, which is quite satisfactory taking into account the thickness (0.5 mm) of the bar. The bar-to-bar reproducibility (%RSD, n=3) were lower than 10% for all the analytes.

In the river samples analysed, propylparaben (88-129 ng L^{-1}), BP3 (52-

103 ng L^{-1}) and ibuprofen (LOQ-72 ng L^{-1}) were present. In one sample, caffeine and methylparaben were also found at 61 ng L^{-1} and 99 ng L^{-1}, respectively. These values are comparable to those found in the samples from the same river [18,23].

Table 3. Recoveries (%) obtained when 50 mL of river water sample spiked at 100 ng L^{-1} with the analyte mixture and 50 mL of effluent and influent WWTP samples spiked at 500 ng L^{-1} with the analyte mixture were SBSE using the poly(VPD-co-DVB) stir bar.

Analyte	Recovery (%)		
	River	Effluent WWTP	Influent WWTP
Paracetamol	8	8	n.d.
Caffeine	14	15	n.d.
Antipyrine	34	35	29
Propranolol	56	64	49
Methylparaben	66	65	23
Carbamazepine	99	85	74
Ethylparaben	86	87	58
Propylparaben	105	103	72
DHB	45	51	40
DHMB	60	65	55
Diclofenac	75	74	67
Ibuprofen	70	73	n.d.
BP3	72	55	29
Triclocarban	50	60	55

% RSD (n=3) were lower than 7% for river water, 13% for effluent water and 17% for influent water when %R> 20%.
n.d. not determined because of the high concentration in the blank sample.

Several samples of effluent WWTPs were also analysed. Some analytes such as antipyrine, propanolol, carbamaze-pine, propylparaben, diclofenac and BP3 were present in the effluent WWTP water samples analysed. Table 4 includes the concentrations found in one effluent sample analysed in triplicate as an example. In another effluent samples we also found caffeine, methylparaben and DHB, whose chromatogram is shown in Figure 6, as an example. Several papers [23,26,27] also reported the presence of these analytes in this kind of sample. Table 4 also shows the concentrations of the analytes found in an influent water sample analysed. As can be seen, analytes such as paracetamol, caffeine, methylparaben and ibuprofen were found at high concentration and therefore these samples had to be diluted to be quantified. As expected, most of these analytes were found at higher concentration than in effluent due to the waste water treatment.

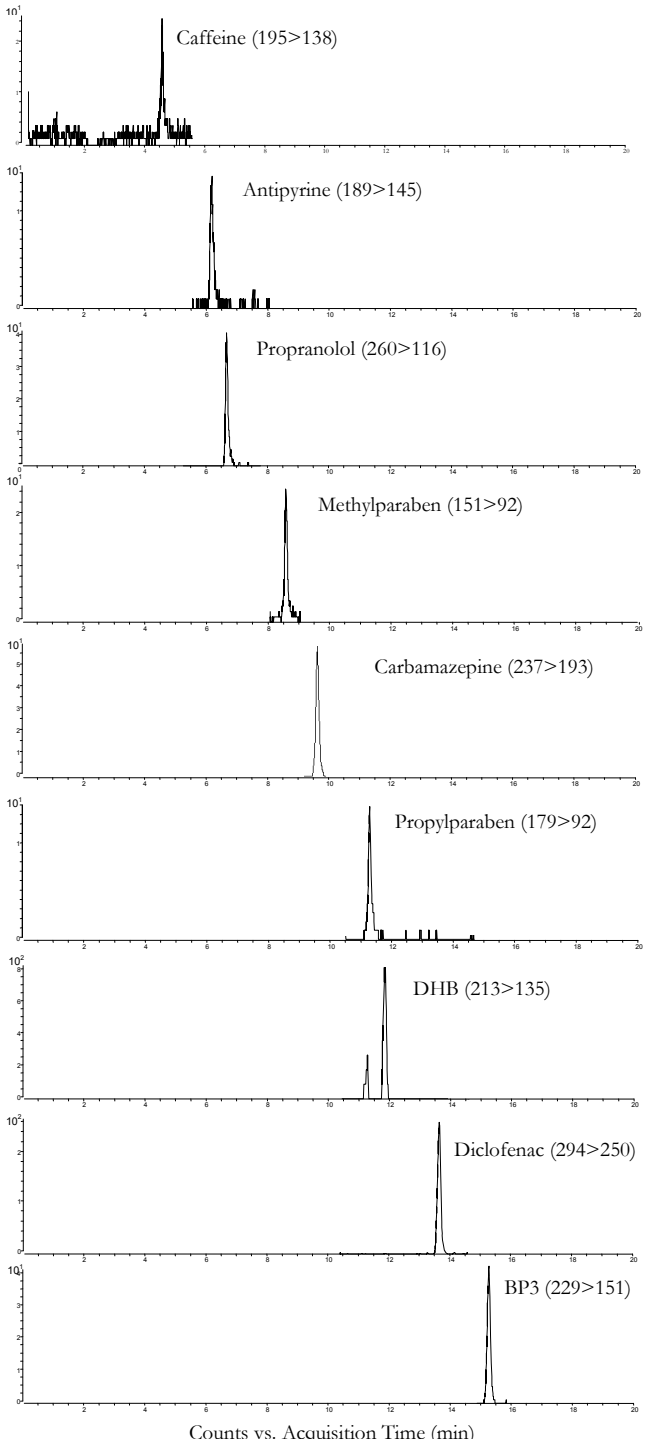

Fig. 6. MRM chromatograms of an effluent WWTP sample. For experimental conditions see the text.

Table 4. Concentrations (ng L^{-1}) of analytes found in effluent and influent WWTP samples (n=3, RSD<17%).

Analyte	Concentration (ng L^{-1})	
	Effluent WWTP	Influent WWTP
Paracetamol	-	21650
Caffeine	-	15803
Antipyrine	118	50
Propranolol	53	<LOQ
Methylparaben	<LOQ	3883
Carbamazepine	232	173
Ethylparaben	-	775
Propylparaben	79	1889
DHB	-	<LOQ
DHMB	-	-
Diclofenac	501	238
Ibuprofen	<LOQ	15005
BP3	50	735
Triclocarban	-	-

4. CONCLUSIONS

In this study, a stir bar coated with poly(VPD-co-DVB) monolithic material was developed by optimising the conditions of the polymerisation to obtain good chemical and mechanical properties of the stir bar.

The poly(VPD-co-DVB) stir bar was successfully applied for extraction of group of analytes with polar characteristics and showed significantly higher retention for the polar analytes compared with the commercially available PDMS stir bar. The combination of SBSE and liquid desorption with LC-(ESI)MS-MS provided an efficient, simple and sensitive method for determination of PPCPs in complex environmental samples at low levels.

Acknowledgements

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3.4.2. Preparation of a polar monolithic stir bar based on methacrylic acid and divinylbenzene for the sorptive extraction of polar pharmaceuticals from complex water samples

PREPARATION OF A POLAR MONOLITHIC STIR BAR BASED ON METHACRYLIC ACID AND DIVINYLBENZENE FOR THE SORPTIVE EXTRACTION OF POLAR PHARMACEUTICALS FROM COMPLEX WATER SAMPLES

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Abstract

A monolithic, hydrophilic stir bar coating based upon a copolymer of methacrylic acid and divinylbenzene [poly(MAA-*co*-DVB)] was synthesised and evaluated as a new polymeric phase for the stir bar sorptive extraction (SBSE) of polar compounds from complex environmental water samples.

The experimental conditions for the extraction and liquid desorption in SBSE were optimised. Liquid chromatography-mass spectrometry in tandem with a triple quadrupole (LC-MS/MS) was used for the determination of a group of polar pharmaceuticals in environmental water matrices.

The extraction performance of the poly(MAA-*co*-DVB) stir bar was compared to the extraction performance of a commercially available polydimethylsiloxane stir bar; it was found that the former gave rise to significantly higher retention of polar analytes than the commercial product.

Keywords: *Stir bar sorptive extraction (SBSE); hydrophilic monolithic coating; pharmaceuticals; environmental water samples.*

1. INTRODUCTION

In recent years, different sampling techniques have been developed to extract organic compounds from aqueous samples. One of these techniques, stir bar sorptive extraction (SBSE), is designed specifically for extractions involving liquid samples. In SBSE, organic compounds are enriched from aqueous samples by direct sorption onto a sorbent phase/coating which encapsulates a magnetic stir bar. Once sorption is complete, the bound compounds can be thermally or liquid desorbed from the sorbent.

The technique has been applied successfully in environmental analyses, biomedical analyses and food analysis, *inter alia* [1-4].

The extraction mechanism of the SBSE technique is similar to solid-phase microextraction (SPME), which is an equilibrium extraction technique based on sorption. The working principle of SPME and SBSE involves the partitioning of analytes between the sample matrix and the extracting phase on the fibre or stir bar. The sorption efficiency depends primarily upon the characteristics of the selected sorbent, as well as on the type of analytes being sorbed [1,5].

Generally speaking, in terms of the sensitivity of determination of apolar analytes at trace levels in complex matrices, SBSE is recognised to be superior to SPME [4,6]. A number of different extracting phases, such as polydimethylsiloxane (PDMS), polyacrylate (PA), carboxen, carbowax-divinylbenzene (CW-DVB), poly(ethylene glycol) (PEG), amongst others, have been commercialised for SPME, however a PDMS coating is the only coating commercially available for SBSE, which sets significant limits upon the applicability of the technique. Although an excellent performance by SBSE for the sorption of apolar analytes is usually obtained [7-10], most polar analytes present in samples are retained poorly by PDMS [11,12]. Therefore, efforts have been directed at developing new sorbents for implementation in the SBSE technique [13-19], such as monolithic materials [14-16], polyurethane foams [18] or those based on sol-gel technology [19], in order to increase the efficiency of extraction of polar analytes.

Concerning the monolithic approach, several polar coatings for stir bars have been reported. Recently, Huang *et al.* prepared a series of polymeric phases for SBSE with different polarities, such as a vinylimidazole-divinylbenzene copolymer [poly(VIm-co-DVB)] [14] a vinylpyridine-ethylene glycol dimethacrylate copolymer [poly(VP-co-EGDMA)] [15], and a methacrylic acid-3-sulfopropyl ester potassium salt-divinylbenzene copolymer [poly(MASPE-co-DVB)] [16] for the extraction of polar and apolar compounds. The utility of the aforementioned materials was evaluated using different matrices (water, urine and honey), and gave rise to promising results. Recently, our research group disclosed methods for the preparation of a new design of stir bar based on a monolithic vinylpyrrolidone-divinylbenzene copolymer [poly(VPD-*co*-DVB)] coating, and its application towards the extraction of a group of polar and apolar analytes from complex water samples [17].

In view of the encouraging results arising from our previous study [17], we prepared a monolithic stir bar coating incorporating a polar monomer with a ionisable functional group, since this introduces mixed-mode character into the material. The selection of the

monomer was based on previous experience within our group in the use of mixed-mode sorbents in SPE [20], where a mixed-mode SPE sorbent incorporating methacrylic acid residues was applied successfully to the selectively extraction of basic analytes (the methacrylic acid residues impart weak cation-exchange character). With all of this in mind, we set about the design and synthesis of a stir bar based on a copolymer of methacrylic acid and divinylbenzene, and this is what is disclosed in the present paper. Post-synthesis, the sorptive capacity of the material was investigated, and the material evaluated for the SBSE of polar pharmaceuticals from complex environmental samples. The results were compared to extraction data derived from experiments with a commercially available, PDMS-coated stir bar.

2. EXPERIMENTAL

2.1. Reagents and standards

Methacrylic acid (MAA) (98% grade) and divinylbenzene (DVB) (80% grade) were supplied by Sigma Aldrich (Steinheim, Germany). DVB and MAA were freed from stabilizers by distillation under reduced pressure and by passing through a short column filled with neutral alumina (Aldrich), respectively. The cyclohexanol (99%) and 1-dodecanol (98%), both from Aldrich, were used as porogens. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator (BDH, Poole, UK) was recrystallised at low temperature from acetone (Merck, Darmstadt, Germany) prior to use.

Paracetamol, caffeine, antipyrine, propranolol, carbamazepine, naproxen and diclofenac (from Aldrich) were the analytes selected to evaluate the sorptive properties of the stir bars.

Standard solutions at 1000 mg L^{-1} of each compound were prepared in MeOH. These solutions were stored at 4 ºC. A standard mixture solution was prepared by diluting each individual standard solution in ultra pure water (Veolia Water Solutions & Technologies, Barcelona, Spain).

LC-grade methanol (MeOH) and acetonitrile (ACN) were supplied by SDS (Peypin, France).

Formic acid (Prolabo, Bois, France), hydrochloric acid (Probus, Barcelona, Spain), sodium hydroxide (Panreac, Barcelona, Spain) and sodium chloride (from Aldrich), were used to adjust the pH of the mobile phase or the sample prior to SPE.

2.2. Stir bar preparation

Polymerisation conditions were selected based on previous experience from related synthetic work [17]. Purified monomers, at a ratio of 75% (w/w) DVB and 25% (w/w) MAA (50% (w/v) total monomer in feed relative to solvent), AIBN (1 mol% relative to polymerisable double bonds) and porogen (10% w/v 1-dodecanol in

cyclohexanol) were placed in a glass tube. The monomer mixture and porogen were mixed ultrasonically into a homogenous solution, then the monomer solution purged with N_2 at 0 ºC for 5 min. Subsequently, the monomer solution was poured into a glass tube of defined diameter (6.5 mm i.d.). A magnetic stir bar (12 x 4.5 mm o.d.) was introduced into the middle of a spring (4.5 mm i.d.) and then immersed vertically into the monomer solution. The glass tube was sealed with a septum and incubated at 60 ºC for 48 h. Once the polymerisation was complete, the glass tube was cut off carefully to deliver a rigid, coherent monolith. The monolithic material which encapsulated the stir bar was then Soxhlet-extracted with methanol for 24 h to eliminate residual monomers, porogen and initiator. The final poly(MAA-*co*-DVB) stir bar obtained had the following dimensions: length: 14 mm; polymer thickness: 1 mm, which corresponds to a polymer volume of around 350 μL.

Nitrogen sorption porosimetry measurements were performed on an ASAP 2010 Micromeritics Instrument (Norcross, GA, USA), and the specific surface areas calculated using the BET method. The carbon, hydrogen and nitrogen contents of the polymeric materials were obtained by elemental microanalysis using a Carlo-Erba EA 1106 Instrument. FTIR analyses were performed using a Perkin-Elmer Spectrum One FTIR Spectrometer (Birmingham, UK).

2.3. LC-(ESI)MS/MS analysis

The extracts were analysed by an Agilent 1200 liquid chromatograph coupled to a 6410 triple quadrupole mass spectrometer with an ESI interface, an automatic injector, a degasser, a quaternary pump and a column oven from Agilent Technologies (Waldbronn, Germany).

The chromatographic column used for analyses was a 100 mm × 4.6 mm i.d. stainless-steel column packed with Kinetex 100 Å C_{18}, with 2.6 μm superficially porous shell particles (Phenomenex, Torrance, CA, USA). The analyses were performed at 35 ºC and the injection volume was 50 μL. A binary mobile phase with a gradient elution was used. The mobile phase consisted of ultra pure water adjusted to pH 3.0 with formic acid, and acetonitrile, and the flow-rate was set at 0.6 mL min^{-1}. The applied gradient was as follows: 10% to 15% ACN in 5 min then to 100% ACN in 5 min and kept constant for 5 min, and then decreased to the initial conditions in 2 min.

Triple quadrupole operating conditions were studied in order to work in multiple reaction monitoring mode (MRM). The instrument operated in positive and negative modes and the ESI parameters were as follows: drying gas flow 12 L min^{-1}, desolvation temperature 350 ºC, nebulizing gas pressure 45.0 psi, and capillary voltage 4000 V. Nitrogen was used as collision, nebulising and drying gas. The MRM transitions, the cone voltage, the collision energy as well

as the pK_a values are summarised in Table1.

2.4. Stir Bar Sorptive Extraction

The SBSE procedure was as follows: the stir bar was activated with 5 mL of MeOH and stirred for 5 min. After drying with lint-free tissue, the stir bar was inserted into a flask with 100 mL of sample adjusted to pH 3.0. Samples were stirred with the stir bar at 750 rpm for 4 hours at room temperature (25 ºC). Following the extraction, the poly (MAA-*co*-DVB) stir bar was removed magnetically from the sample solution, dipped briefly in ultra-pure water (to remove adsorbed impurities) and dried using lint-free tissue.

Table 1. ESI mode and MRM conditions used for LC-(ESI)MS/MS of target analytes.

Analyte	pK_a	ESI Ionisation Mode	Cone Voltage (V)	Precursor Ion (m/z)	Product Ions (m/z)	Collision Energy (V)
Paracetamol	9.7	+	100	152	**110**	15
					93	25
Naproxen	4.8	-	50	229	**185**	5
					170	30
Diclofenac	4.2	-	75	294	**250**	10
					214	20
Caffeine	13.4	+	125	195	**138**	15
					110	25
Antipyrine	13.3	+	100	189	**145**	30
					115	30
Propranolol	9.5	+	125	260	**116**	15
					183	15
Carbamazepine	13.7	+	150	237	**193**	35
					179	35

Bold indicates the quantifier ion.

For the liquid desorption of analytes, the stir bar was introduced into a vial with 5 mL of MeOH and agitated at 750 rpm for 20 minutes. Then, the stir bar was removed magnetically and reconditioned. The extract was evaporated to dryness under nitrogen and the dry residues redissolved in 1 mL MeOH:water (20:80). The extracts were analysed by LC-(ESI)MS/MS.

The results obtained with the poly(MAA-*co*-DVB) stir bar were compared to a commercially available PDMS-coated stir bar, obtained from Gerstel (Mulheim Ruhr, Germany). From the two sizes of commercially available stir bars, the larger stir bar was chosen, since it provides high sorptive capacity for sample volumes greater than 50 mL. It consists of a 20 mm long glass-encapsulated magnetic stir bar, coated externally with a 1 mm thick layer, corresponding to a PDMS volume of 126 μL. Before their first use, the stir bars were introduced into a vial containing acetonitrile and conditioned for 24 hours [21].

The poly(MAA-*co*-DVB) stir bar can be reused and the life time of a single stir bar was found to be between 30 to 40 extractions, depending on the matrix. The stir bars were reconditioned by inserting them into vials containing MeOH for a period of 20 min; then, the MeOH was refreshed and the procedure was repeated three times. Finally, the stir bars were dried using a lint-free tissue, and stored in a vial until the next analysis.

2.5. Sample collection

The river water samples were collected from the Ebre River. The effluent wastewater samples were collected from two sewage treatment plants (STPs).
All the environmental water samples were adjusted to ~ pH 3 using HCl and stored at 4 ºC prior to analysis. They were filtered through 0.45 μm nylon membranes (Supelco, Bellefont, PA, USA) before the stir bar extraction.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterisation of monolith

Different variables which can affect the polymerisation and the sorptive properties of the monolith, such as the monomer type, the crosslink density, the volume and nature of the porogen and the initiation mode in polymerisation were investigated. These parameters can influence the porous character of the monolithic material, and this is significant because it is the pore network which allows the analytes to penetrate into the polymer structure and which also provides the accessible surface area for sorption processes.
In previous, related work within our group [17], a spring was used to stabilise/scaffold the monolithic material when in contact with the magnetic stir bar, to enhance the dimensional stability. This design was very successful and was therefore implemented in the present study as well. Since the incorporation of the polar monomer VPD into the polymer structure facilitated the extraction of polar analytes [17], we decided to increase the polarity yet further and simultaneously attempt to introduce selectivity into the extraction. Although the earlier results using a poly(VPD-co-DVB) sorbent were satisfactory, there was no selectivity in the extraction of the analytes, therefore we intended to make further improvements by introduction of mixed-mode character [20]. Concerning the monomer selection, MAA was identified as a suitable comonomer due to its polar and ionisable nature, and DVB was selected as a suitable crosslinking agent. Different monomer feed ratios (75/25, 50/50, 25/75 and 15/85 w/w of MAA/DVB) were tested. It was found that those monoliths derived from lower DVB contents in the monomer feed (25 and 50 w/w) were of low rigidity and could be damaged easily (*i.e.*, they had relatively poor mechanical/dimensional stability). In contrast, when higher proportions of DVB were used in the

monomer feed, the monolithic products were mechanically and dimensionally stable, and well-suited for their intended use in SBSE. To ensure that the monoliths had polar character as well as mechanical and dimensional stability, the monomer feed was fixed at 25/75 w/w of MAA to DVB.

We selected a mixture of cyclohexanol and 1-dodecanol as porogen, which has been demonstrated previously to be convenient in monolith material synthesis [14,15,22]. The ratio of total monomer to porogenic solvent was fixed at 50/50 (w/w); a similar ratio has been used previously in the preparation of different monolithic stir bar coatings [15,22].

FTIR spectroscopic analyses of the poly (MAA-*co*-DVB) coatings confirmed that the comonomers, DVB and MAA, had been copolymerised into the monolithic structures. The spectra showed the characteristic bands ascribed to O–H stretching (broad band at around 3450 cm^{-1}) and C=O stretching (1705 cm^{-1}) of MAA residues. The region below 1600 cm^{-1} confirmed the presence of the aromatic DVB residues (stretching of C-C bonds in the rings).

Elemental microanalysis of the poly (MAA-*co*-DVB) monolith gave the following results: carbon (80.0%), hydrogen (7.8%), nitrogen (0.1%) and oxygen (12.1%, calculated by difference). These values are in agreement with the values expected based upon statistical incorporation of the monomers from the feed, which indicates yet again that the copolymerisation was successful.

The specific surface area of the monolith was determined to be 500 $m^2\ g^{-1}$ using Brunauer-Emmett-Teller (BET) method, thus the monolith had a well-developed pore structure which ought to facilitate sorption processes.

3.2. LC-(ESI)MS/MS analysis

A chromatographic column packed with superficially porous shell particles was used to obtain high speed and chromatographic efficiencies of sub-2 μm particles while maintaining lower backpressures and reducing solvent consumption. Under the optimum conditions, the separation of the analytes was achieved in less than 13 min.

Analysis was performed either in positive or negative ionisation mode, depending on the response of each compound. The optimum MS-MS conditions were optimised as is shown in Table 1. Two fragmentations of $[M+H]^+$ or $[M-H]^-$ were acquired for all selected analytes. To quantify the analytes, their most intense transitions were chosen.

All selected analytes presented good linearity (r^2>0.999) and linear range, by direct injection of the standard solutions with concentrations between 0.2-50 $\mu g\ L^{-1}$, for most of analytes except naproxen (5-100 $\mu g\ L^{-1}$).

3.3. Optimisation of SBSE procedure

Since the poly(MAA-*co*-DVB) monolith was expected to have mixed-mode character [20], a group of basic and acidic pharmaceuticals (pK_a detailed in Table 1)

was selected to examine the potential selectivity of the monolith.

The poly(MAA-*co*-DVB) monolith can be expected to retain charged basic compounds by ionic and reversed-phase (RP) interactions arising from the presence of carboxylic acid moieties, while the remainder of the compounds are retained by RP interactions alone. Ideally, in the washing step the acidic analytes can be eliminated, while the basic analytes remain bound but can be eluted subsequently in the elution step. Therefore, the parameters affecting SBSE were tested with these ideas in mind; several important variables affecting the extraction and desorption steps, including sample pH, ionic strength, desorption solvent, extraction and desorption time, were studied in detail to optimise the SBSE conditions.

The SBSE procedure was optimised using initial conditions to promote ionic interactions in the mixed-mode materials. The conditions were as follows: 100 mL of the sample at pH 7 (to ensure the deprotonation of the carboxylic acid and protonation of the basic compounds), agitated at 750 rpm during 1 hour, and desorption with 5 mL of 2% TFA in MeOH stirred at 750 rpm for 20 minutes. All the experiments were performed at ambient temperature (25 ºC). The % recoveries under these preliminary conditions ranged between 10 - 60%.

Before the optimisation of these parameters, preliminary tests were performed to establish the weak cation-exchange character of the coating. For this we included, supplementary to the previous conditions, a washing step (brief immersion in 1 mL of solvent) and several solvents, including MeOH, ACN, ethyl acetate, CH_2Cl_2 and 5% NH_4OH in MeOH were tested. Here, the aim was to remove acidic analytes and interferences, which were retained by RP interactions, while maintaining basic analytes retained by ionic interactions. However, we observed that all the compounds were partially or completely eliminated during the washing step, which implies that the ionic interactions were not strong enough to retain basic analytes under the conditions of study. Thus, whilst the poly(MAA-*co*-DVB) phase was found to be suitable for the extraction of polar analytes, its weak cation-exchange properties could not be exploited for these analytes under the specific conditions used for the sorption experiments. Thereafter, the SBSE conditions were therefore optimised by taking into account the requirements for the RP interactions.

Huang *et al.* [16] reported the preparation of a mixed-mode MASPE-DVB-based coating, where, according to the authors, the presence of sulphoic acid groups (deprotonated) in the monolithic material allowed retention of fluoroquinolones (protonated amino groups at pH 5.0), through the combination of hydrophobic and cation-exchange interactions. However, no washing step was performed in this study to demonstrate unequivocally the selectivity which can arise with mixed-mode materials.

3.3.1. Liquid desorption conditions

In methods using SBSE, thermal desorption (TD) of the analytes is usually combined with analysis by gas chromatography-mass spectrometry (GC-MS) [13]. However, due to the fact that a group of polar analytes was extracted, liquid desorption followed by LC was selected to back extract and analyse the polar pharmaceuticals under study. The influence of the liquid desorption conditions on efficiency were optimised.

To ensure the complete elution of target analytes, several different solvents were tested: MeOH and ACN, and MeOH containing different percentages of CH_3COOH or TFA. Considering the size of the stir bar and suitable vials to perform the desorption step, we used a 5 mL solvent volume to guarantee the complete immersion of the coated stir-bar and ensure a proper desorption process. The results using ACN and MeOH with either CH_3COOH or TFA were marginally poorer than when using MeOH. Therefore, MeOH was selected as the desorption solvent due to the slightly higher ability to desorb polar analytes from the stir bars (~5-10% improvement in % recovery). Increasing the MeOH volume further (to 10 mL) did not improve the desorption results. Finally, 5 mL of MeOH was selected for the optimal back extraction of the target analytes.

In the next step, the desorption time of the poly(MAA-*co*-DVB) sorbent was varied from 10 to 30 minutes using an agitation speed of 750 rpm. The level of analytes desorbed from the stir bar increased when the desorption time was extended from 10 to 20 minutes, however a yet further increase in the desorption time did not improve the recoveries. Consequently, 20 minutes for the desorption was selected as optimal for the remaining investigations.

3.3.2. Extraction conditions

Once the desorption conditions were optimised, variables affecting the extraction process were examined. These included sample pH (3.0, 7.0 and 9.0), ionic strength (5, 10, 15 and 20% of NaCl w/v), stirring rate (600 rpm, 750 rpm and 900 rpm), sample volume (50 and 100 mL) and extraction time (1 to 8 hours).

The effect of sample pH on the extraction efficiency was examined in the range from 3.0 to 9.0. The pH is an important parameter in an extraction process as it determines the protonation state of ionisable groups in the polymeric sorbent and analytes, and consequently influences their retention and extraction efficiency. As shown in Fig. 1, under initial conditions the pH value significantly affected the extraction efficiency of the poly(MAA-*co*-DVB) stir bar for selected analytes. The results indicated that the extraction efficiency for acidic pharmaceuticals improved considerably when the pH value was set at 3.0 (at this pH they are in neutral form), but decreased when the pH value was higher. Only the recovery of propranolol (pK_a~9.5) was found to be improved under higher pH conditions.

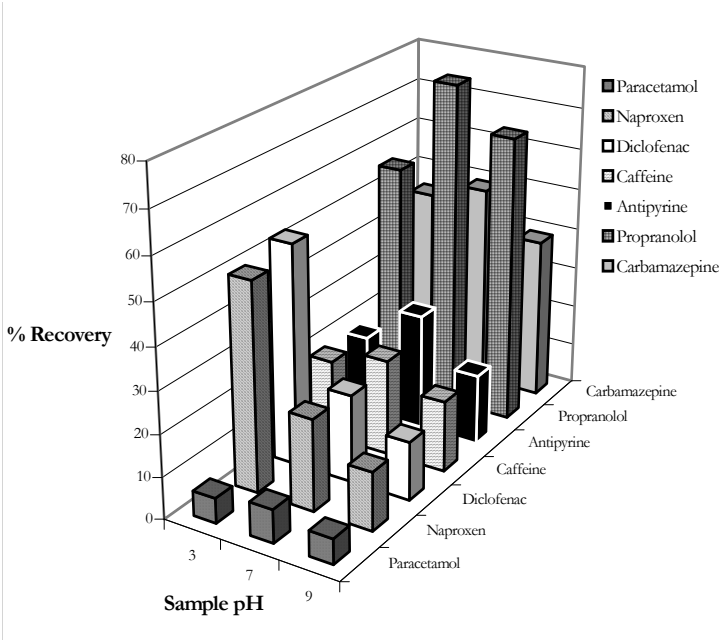

Fig. 1. The effect of sample pH on extraction recovery.

In view of these results, pH 3.0 was selected for further research.

The effect of ionic strength on the extraction efficiency was also investigated. The influence of ionic strength on recoveries of the target analytes was performed by addition from 0 to 20 % of NaCl (w/v) to the aqueous samples. However, the results showed that an increase in ionic strength did not enhance significantly the extraction efficiency. To simplify the extraction procedure, we therefore did not add any salt in the subsequent experiments.

It is well-known that agitation speed can affect the mass transfer of the analytes during the extraction process. Three agitation rates (600, 750 and 900 rpm) were tested to optimise the stirring conditions. The results obtained with an agitation rate of 750 rpm were better than those obtained at 600 rpm; however a further increase in the agitation rate to 900 rpm may shorten the lifetime of the monolithic coating. Since a stirring rate of 750 rpm gave rise to efficient extractions without detriment to the physical integrity of the stir bar, it was selected for further study.

When we varied the sample volume, the results were comparable when both 50 mL and 100 mL of ultra pure water samples were analysed. Therefore, 100 mL of sample was selected for further analysis.

Finally, the extraction time was varied from 1 to 8 hours. The extraction efficiency increases rapidly with an increase in the extraction time from 1 to 4 h, and then changes slowly with further increases in the extraction time. Since a compromise between the extraction time and efficiency was necessary, 4 hours was selected as the extraction time in the following studies.

Overall, then, the optimum SBSE conditions were as follows: 100 mL of sample at pH 3.0 extracted at 25 ºC by agitating at 750 rpm for 4 hours; liquid desorption: 5 mL of MeOH stirred at the same speed for 20 min. The recovery values (listed in Table 2) obtained in the extraction of the analytes from ultra-pure water (spiked at 100 ng L^{-1} with the analyte mixture) for most of the analytes were in range 60% to 100%, except for paracetamol (%R only 13%) and caffeine (%R 45%), which may be due to their weak hydrophobic interactions.

The bar-to-bar reproducibility of poly(MAA-*co*-DVB) monoliths was also studied by comparing the extraction efficiency of target analytes. The bar-to-bar reproducibility %RSD (n=3) was less than 11% for all analytes under study. It is also worth noting that no damage to the stir bar coatings was observed during extractions. The good reproducibility and stability indicates that poly(MAA-*co*-DVB) monoliths are eminently suitable as materials for stir bar coatings.

3.4. Comparison to the other stir bars

Taking into account the SBSE data arising from use of the poly(MAA-*co*-DVB) stir bars, we decided to compare their SBSE performance to the commercially available stir bars based on PDMS. The results obtained using a PDMS-coated stir bar are shown in Table 2.

Table 2. Recovery values (%) obtained when the poly(MAA-co-DVB) and PDMS-coated stir bars were applied in SBSE of 100 mL of an ultra-pure sample spiked at 100 ng L^{-1} with the analyte mixture.

Analyte	Recovery (%)	
	Poly(MAA-co-DVB)	PDMS
Paracetamol	13	-
Naproxen	107	-
Diclofenac	101	23
Caffeine	45	3
Antipyrine	61	-
Propranolol	101	-
Carbamazepine	95	1

% Relative standard deviations (%RSDs) (n = 3) were lower than 10% for %R>15%. For the experimental conditions, see text.

It can be noticed that when using the same SBSE conditions, the recoveries of target analytes were higher for all selected compounds on the poly(MAA-*co*-DVB) monolith. These results can be explained easily by considering the polar nature of the poly(MAA-*co*-DVB) coating, comparing to the apolar PDMS phase. The poor performance of PDMS-coated stir bars in the extraction of polar pharmaceuticals has been reported previously [17,23].

Upon comparing the new results to the results obtained in previous research work with a monolithic coating based on poly(VPD-*co*-DVB) [17], it is clear that in spite of the fact that higher sample volumes and a larger amount of extracting phase were used in the present study, the recovery values for most of the analytes were comparable, except in the case of caffeine and antipyrine where the recoveries with the poly(VPD-*co*-DVB) monolith were 20% and 42%, respectively, whereas with the new poly(MAA-*co*-DVB) stir bar the recoveries were significantly higher (45% and 61%, respectively). Thus, poly(MAA-*co*-DVB) stir bar outperforms the previously synthesised poly(VPD-*co*-DVB) stir bar.

3.5. Application to environmental water samples

Since the analytes under study are contaminants which can be found in river water and effluent wastewater from a treatment plant (WWTP), these sample matrices were selected to perform the study.

In order to improve the overall sensitivity of the method, the 5 mL extract from the liquid desorption was evaporated until dryness and reconstituted with 1 mL of MeOH/H_2O (20/80). No losses of analytes in the evaporation step were observed.

A common drawback when quantifying LC-MS with an ESI source is the ion suppression or enhancement effect arising from a number of organic and/or inorganic compounds present in the matrix sample. The resulting ion suppression or enhancement effect ranged from 0 to 17% for river water samples, and from 3 to 45 % (only for some analytes) for effluent WWTP samples. The analytes most affected by matrix effects were propranolol and antipyrine. Although some authors reported that the matrix effect is less when using SBSE compared to other sorptive extraction techniques, such as SPE [24,25], in this case, due to the polarity of the sorbent material, the suppression was quite significant. Several approaches are typically applied to deal with matrix effects in quantitative analysis, such as sample dilution, improvement of the sample pre-treatment and the chromatographic separation, or the use of stable-isotopically labeled internal standards [26].

From these approaches we selected to dilute the effluent WWTP samples (1:1) with ultra-pure water. The resulting ion

suppression or enhancement effect ranged between 1 to 21%. Therefore, this effect became less significant upon sample dilution.

Table 3 lists the recovery values for the different water samples. The data obtained for river water samples was good, with recoveries ranging from 50 to 100% for most analytes (except paracetamol and caffeine), and similar to the values obtained for extractions from ultra-pure water, demonstrating the satisfactory ability of this monolithic material to retain both acidic and basic pharmaceuticals even when in the presence of matrix interferences. Only propranolol showed a decrease in retention (%R ~50%) when river water samples were analysed, possibly due to the complexity of the matrix and competition between the analyte and the other components from the sample matrix for access to those sites of the polymer where the retention takes place.

Table 3. Recovery values (%) obtained when the poly(MAA-co-DVB) coated stir bar was applied in SBSE of 100 mL of river and effluent WWTP samples spiked at 100 ng L^{-1} and 200 ng L^{-1}, respectively with the analyte mixture.

Analyte	Recovery (%)	
	River	Effluent WWTP
Paracetamol	11	10
Naproxen	92	85
Diclofenac	90	62
Caffeine	35	31
Antipyrine	52	45
Propranolol	50	35
Carbamazepine	90	86

%RSD (n = 3) were lower than 20%. For the experimental conditions, see text.

In comparison to the performance of the poly(VPD-*co*-DVB) coating [17] in the SBSE of environmental samples, the recoveries of analytes were slightly better.

Thereafter, the method was validated with Ebre river water; the linear range with matrix calibration ranged from 10 to 500 ng L^{-1} for antipyrine, propranolol and diclofenac, and from 20 to 500 ng L^{-1} for the remaining analytes, except for naproxen (100-500 ng L^{-1}), with regression coefficients (r^2) greater than 0.999. The limits of detection (LODs), calculated using a signal to noise ratio of ≥3, were 10 ng L^{-1} for most of the compounds, with the exception of naproxen (50 ng L^{-1}). The repeatability and reproducibility of the method, expressed as the relative standard deviation (RSD) of three analyses of 100 mL of Ebre river water spiked at 100 ng L^{-1} were lower than 15% for all compounds.

We also demonstrated the applicability by analysing different environmental sam-ples from a river and effluent WWTP using the SBSE-LC-(ESI) MS/MS method. When analysing three river water samples, analytes such as diclofenac (31-48 ng L^{-1}) and caffeine (<LOQ-33 ng L^{-1}), were found in the samples analysed. The concentration value of carbama-zepine found in river samples was below the LOD. Moreover, in one sample propranolol was found at a concentration of 19 ng L^{-1}. These values are comparable to those found in the samples from the same river [27].

Three samples of effluent from WWTPs were also analysed. Certain analytes, such as diclofenac (63-106 ng L^{-1}), caffeine (129-461 ng L^{-1}), antipyrine (76-125 ng L^{-1}), propranolol (31-48 ng L^{-1}), and carbama-zepine (67-86 ng L^{-1}) were found in the effluent WWTP samples analysed. As an example, Fig. 2 shows represen-tative MRM chromatograms from the analysis, obtained under optimum condi-tions from one of the effluent WWTP samples. The presence of these pharma-ceuticals in similar samples was reported earlier [27-29], and the levels found here are in good agreement with previous reports.

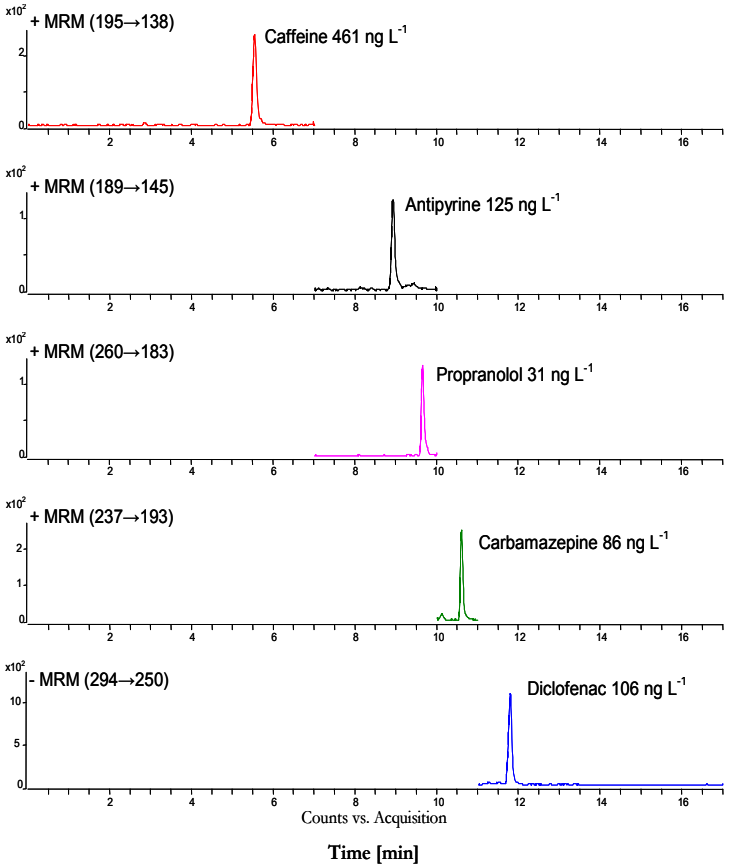

Fig. 2. MRM chromatograms of an effluent WWTP sample. For experimental conditions see the text.

4. CONCLUSIONS

A new poly(MAA-*co*-DVB) monolithic material was prepared as a bespoke coating for magnetic stir bar and served as an extractive polar phase in SBSE.

The poly(MAA-*co*-DVB) coated stir bar was applied successfully to the extraction of polar pharmaceuticals from complex aqueous samples; the results were superior to those obtained with a commercially available PDMS-coated stir bar.

The combination of SBSE and liquid desorption with LC-(ESI)MS/MS provided an efficient, simple and sensitive method for the determination of polar pharmaceuticals present at low levels in complex environmental samples.

The optimised and validated SBSE-LC-(ESI)MS/MS method allowed the detection and quantification of the majority of the compounds studied.

Use of a poly(MAA-*co*-DVB) stir bar can be considered to be a promising alternative to conventional PDMS-coated stir bars in analytical applications.

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3.4.3. Discussion of results

As can be seen in these studies, two monolithic stir bar coatings, newly designed and supported on a spring, were successfully prepared. In both cases, the developed polar coatings enabled quantitative extraction of the analytes with different polarities from water-based matrices.

When the two developed extracting phases were evaluated by SBSE for a group of polar compounds, their retention properties depended on their morphological properties and their polarity. Table 1 summarises the polymerisation conditions and the main characteristics of these developed monolithic materials and their preparation procedure.

Table 1. Polymerisation conditions and characterisation of the prepared stir bars

Extracting phase	Polymerisation conditions			Stir bar size (mm)	Thickness of coating (mm)	S.S.A ($m^2\ g^{-1}$)
	Monomer ratio	Porogen	Time			
poly(VPD-*co*-DVB)	15/85	10 % w/v dodecanol/ cyclohexanol	36 h	11×4.0	0.5	~600
poly(MAA-*co*-DVB)	25/75		48 h	14×6.5	1	~500

S.S.A. Specific surface area

As Table 1 shows, the stir bars were prepared from varying monomers with different monomer ratios. As can be seen, with the synthetic approach, the time required for the preparation of poly(VPD-*co*-DVB) was 36 hours, while the time necessary to obtain a larger stir bar with mechanically resistant poly(MAA-*co*-DVB) coating was longer. This difference can be explained by the higher content of the crosslinker in the poly(VPD-*co*-DVB) coating and the fact that a smaller amount of extracting phase was prepared. It should also be pointed out that the lower the content of DVB in poly(MAA-*co*-DVB) phase, the lower the specific surface area which could affect extraction efficiency. Moreover, as expected, the specific surface areas of these monolithic materials are in accordance to the DVB content, but also with the fact that a non-porogen solvent was used for their preparation.

Primarily, we prepared the smaller stir bar (11 mm length × 0.5 mm film thickness) based on poly(VPD-*co*-DVB). Then, in an attempt to increase the amount of extracting phase, we prepared a bigger stir bar (14 mm length × 1 mm film thickness) based on poly(MAA-*co*-DVB), in order to extract higher sample volumes.

To ensure efficient extraction, the sample volume was selected according to the amount of extracting phase in each prepared stir bar. Thus, for SBSE using a smaller amount of poly(VPD-*co*-DVB) phase, we fixed a sample volume of 50 mL, while for SBSE using poly(MAA-*co*-DVB) phase, the sample volume was set at 100 mL. These sample volumes, under optimised protocols, provided similar recoveries.

The SBSE using the poly(VPD-*co*-DVB) stir bar with liquid desorption has proven to be an effective tool to determine several PPCPs and provides sufficient efficiency at low concentration levels to be reliably used in determining pollutants in environmental aqueous samples.

With regard to poly(MAA-*co*-DVB), we attempted to reinforce the selectivity of the extracting phase by introducing mixed-mode character with weak cation-exchange, based on previous experience with SPE materials. The introduction of carboxylic acid groups into the polymer provided a certain degree of polarity and ion-exchange ability. However, when applied as a mixed-mode coating to selectively extract the selected group of analytes, the results showed that the ionic interactions generated by the target analytes and carboxylic moieties of the extracting phase are not strong enough to retain them during the washing step. This may be related to the greater thickness of monolith that might make the access of target analytes to the retentive interaction sites more difficult.

As previously mentioned in the paper, Huang *et al.* [3] reported the preparation of a mixed-mode material with cation-exchange character. However no additional washing step was performed to confirm clearly that its selectivity resulted from its mixed-mode character.

When comparing the SBSE performance of these two monolithic extracting phases for a group of polar compounds, the results obtained were similar. In comparison to the commercially available PDMS-coated stir bar, both phases, poly(VPD-*co*-DVB) and poly(MAA-*co*-DVB), exhibited significantly higher retention for the polar analytes than PDMS, but it should be noted that an exact comparison is not possible since the amount of extracting phases as well as the optimised extraction and desorption conditions used in these studies were different.

With respect to the samples from rivers and WWTPs, it was found that using the poly(VPD-*co*-DVB) coating, the presented matrix effects were relatively low. Therefore, these samples could be directly extracted without dilution. As expected, when using poly(MAA-*co*-DVB) material in WWTP effluent, matrix effects were higher than those presented using poly(VPD-*co*-DVB), due to the higher polarity of the material and thus more polar interferences retained on the stir bar. To deal with this problem, the samples were diluted prior to SBSE to avoid possible matrix effects, as those found in similar matrices [8].

The performance of the developed methods was demonstrated by their application to samples from river and regional WWTPs. Several common wastewater contaminants, such as propranolol, carbamazepine and diclofenac among others, were detected in all of the WWTP samples at the ng L^{-1} level.

With regard to SBSE performance in the extraction of pharmaceuticals, such as ibuprofen or diclofenac, when comparing for instance polyurethane foams [9] to a poly(VPD-*co*-DVB) phase, it was found that both phases provided comparable values. However, it should be pointed out that the recoveries reported using polyurethane foams as a sorbent were obtained by the extraction of 25 mL of a water sample stirred 6 hours following ultrasonic treatment with organic solvent for the liquid desorption, whereas in the case of poly(VPD-*co*-DVB), for the extraction of 50 mL of the sample, 4 hours of stirring following liquid desorption was sufficient to obtain similar results. The recoveries for parabens obtained using the SBSE-liquid desorption-LC-(ESI)MS/MS method with poly(VPD-*co*-DVB) stir bar were comparable to those obtained with the SBSE-TD-GC-MS method [10] using the PDMS stir bar with the derivatisation of parabens. Therefore, the present method involves fewer steps in the extraction procedure.

As presented above, a new design of stir bars with polar monolithic coatings has been successfully prepared and applied. Since these new materials have already shown their applicability in the extraction procedure, future research should focus on the development of materials for SBSE that will provide higher selectivity, promoting other interactions such as in mixed-mode or MIP materials.

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CHAPTER 4. CONCLUSIONS

The conclusions derived from the studies included in the present Doctoral Thesis can be summarised as follows:

1. Hydrophilic hypercrosslinked sorbents in the form of polymer microspheres based on poly(HEMA-*co*-VBC-*co*-DVB) precursors have been successfully synthesised. The polarity of the resin depends on the chemical properties of the monomer, its ratio and the polymerisation conditions.

2. It has been confirmed that with hydrophilic hypercrosslinked sorbents, such as HXLPP-polar sorbents, the balance between specific surface area and hydrophilicity favours retention in SPE.

3. When extracting polar compounds using hydrophilic hypercrosslinked sorbents, the recoveries were better than those obtained with commercial macroporous sorbents.

4. The hypercrosslinked mixed-mode resin with weak cation-exchange character was successfully synthesised *via* the hypercrosslinking of polymers precursors based on poly(MAA-*co*-VBC-*co*-DVB) synthesised *via* precipitation polymerisation.

5. Hypercrosslinked polymer resin with carboxylic moieties as weak cation-exchangers showed excellent performance for the SPE of basic pharmaceuticals from complex water samples.

6. The post-functionalisation with quaternary ammonium groups of polymer precursors is another way to obtain mixed-mode hypercrosslinked sorbents that can be successfully applied to the selective extraction of acidic pharmaceuticals from environmental water samples.

7. The HXLPP-WCX and HXLPP-SAX sorbents used for the selective extraction of acidic and basic analytes from water samples compare positively to the commercially available mixed-mode sorbents with macroporous structure.

8. Two polymeric SILPs based on N-methylimidazole containing [$CF_3SO_3^-$] and [BF_4^-] were successfully synthesised and applied for the selective SPE of acidic pharmaceuticals from environmental waters, and [MI^+][$CF_3SO_3^-$] demonstrated better performance with recoveries comparable to those obtained with commercial mixed-mode SAX sorbents.

9. A new design of a stir bar has been developed, and two different monolithic coatings using monomers with different polar characteristics have been successfully prepared, exhibiting good chemical, morphological and mechanical properties.

10. The stir bars coated with monolithic materials based on poly(VPD-*co*-DVB) and poly(MAA-*co*-DVB) were successfully applied for the extraction of a group of analytes with a wide range of polarities, providing considerably higher retention for polar analytes compared with the commercially available PDMS stir bar.

11. The combination of SBSE with the new coatings and liquid desorption with LC-(ESI)MS/MS provided a simple and sensitive method for the determination of PPCPs in complex environmental samples at low concentration levels.

ANNEX

ANNEX I. List of abbreviations

4-VIm	4-vinylimidazole
4-VP	4-vinylpyridine
ACB	Activated carbon
ACN	Acetonitrile
ADS	Alkyl-diol silica
AIBN	2,2'-azobisisobutyronitrile
AN	Acrylonitrile
BF_4^-	Tetrafluoroborate
BM	4,4´-bis(maleimido)diphenylmethane
BPA	Bisphenol A
BTEX	Benzene, toluene, ethylbenzene and xylenes
[C_8MIM]	1-octyl-3-methylimidazolium
[C_6VIM]	1-vinyl-3-hexylimidazolium
C_{18}	Octadecyl
CAR	Carboxen
CE	Capillary electrophoresis
CE-MS	Capillary electrophoresis mass spectrometry
CF_3COO^-	Trifluoroacetate
$CF_3SO_3^-$	Trifluoromethanesulphoate
CFME	Continuous flow microextraction
CNFs	Carbon nanofibers
CNSt	Cyanomethylstyrene
CNTs	Carbon nanotubes
CTBA	Cetyltrimethyl ammonium bromide
CW	Carbowax
DBMA	Dimethylbutylamine
DCE	1,2-dichloroethane
DI	Direct immersion
DMN	Di(methacryloyloxymethyl)naphthalene
DVB	Divinylbenzene
DVN	Divinylnaphtalene
DVPh	Divinylbiphenyl
EDA	1,2-ethylenediamine
EDC	Electron capture detector
EG	Ethylene glycol
EGDMA	Ethylene glycol dimethacrylate
ESI	Electrospray ionisation
FID	Flame ionisation detector
FS	Fused silica
FTIR	Fourier-transform infrared spectrometry
GC	Gas chromatography
GCB	Graphitized carbon blacks

GMA	Glycidyl methacrylate
HEMA	2-hydroxyethyl methacrylate
HF	Hollow-fiber
HF-LPME	Hollow-fiber liquid-phase microextraction
HHSE	Headspace sorptive extraction
HS	Headspace
HXL	Hypercrosslinked
IEC	Ion-exchange capacity
IL	Ionic liquid
IS	Immunosorbent
LC	Liquid chromatography
LD	Liquid desorption
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
MAA	Methacrylic acid
MAN	Methacrylonitrile
OcMA	Octyl methacrylate
MAOMA	2-(methacryloyloxy)ethyl-trimethyl ammonium chloride
MASE	Membrane-assisted solvent extraction
MASPE	Methacrylic acid-3-sulfopropyl ester potassium salt
MEDDE	p,p´-dihydroxydiphenylpropane diglycidyl methacrylic ester
MEMDE	p,p´-dihydroxydiphenylmethane diglycidyl methacrylic ester
MEPS	Microextraction by packed sorbent
MI	N-methylimidazole
MIP	Molecularly-imprinted polymer
MISPE	Molecularly-imprinted solid-phase extraction
MMLLE	Microporous membrane liquid-liquid extraction
MS	Mass spectrometry
NTBSTFA	N-tert-butyl-dimethyl-silyl-*N*-methyltrifluoroacetamide
NTf_2	Bis[(trifluoro-methyl)sulphoyl]imide
NVIm	N-vinylimidazole
OS	Oligosorbents
PA	Poly(acrylate)
PAA	Polar aromatic amines
PAH	Polycyclic aromatic hydrocarbon
PAN	Polyacrylonitrile
PANI	Polyaniline
PCB	Polychlorinated biphenyl
PCP	Personal care product
PDMS	Polydimethylsiloxane

PDPA	Polydiphenyl-aniline
PEG	Polyethylene glycol
PF_6	Hexafluorophosphate
PFBAY	Pentafluorobenzaldehyde
Ph	Phenyl
PLE	Pressurised liquid extraction
PNMA	Poly-N-methylaniline
PP	Precipitation polymerisation
PPCP	Pharmaceuticals and personal care product
PPy	Polypyrrole
PS-DVB	Polystyrene-divinylbenzene
PU	Polyurethane
PVA	Poly(vinylalcohol)
QqQ	Triple quadrupole
RAM	Restricted access material
RP	Reversed-phase
SAX	Strong anion-exchange
SBSE	Stir bar sorptive extraction
SCX	Strong cation-exchange
SDME	Single-drop microextraction
SILP	Supported ionic liquid phase
SLM	Supported liquid membrane
SPE	Solid-phase extraction
SPDE	Solid-phase dynamic extraction
SPME	Solid-phase microextraction
STP	Sewage treatment plants
SVOCs	Semi-volatile organic compounds
TD	Thermal desorption
TEOS	Tetraethoxysilane
TFA	Trifluoroacetic acid
TPR	Templated resins
UHPLC	Ultra-high performance liquid chromatography
UV-Vis	Ultraviolet-visible
VBC	Vinylbenzyl chloride
VP	Vinylpyridine
VPD	Vinylpyrrolididone
VN	Vinylnaphtalene
VOC	Volatile organic compound
WAX	Weak anion-exchange
WCX	Weak cation-exchange
WWTP	Wastewater treatment plant
β-CD	β-cyclodextrin

ANNEX II. List of publications

The papers obtained in this Doctoral Thesis, which are published or submitted for publication in different scientific journals, are listed below.

D. Bratkowska, N. Fontanals, P.A.G. Cormack, D.C. Sherrington, F. Borrull, R.M. Marcé, *"Hydrophilic hypercrosslinked polymeric sorbents for the solid-phase extraction of polar contaminants from water",* Journal of Chromatography A 1217 (2010) 3238-3243 (section 3.1.1).

D. Bratkowska, R.M. Marcé, D.C. Sherrington, F. Borrull, P.A.G. Cormack, N. Fontanals, *"Synthesis and application of hypercrosslinked polymers with weak cation-exchange character for the selective extraction of basic pharmaceuticals from complex environmental water samples",* Journal of Chromatography A 1217 (2010) 1575-1582 (section 3.2.1).

D. Bratkowska, A. Davies, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N. Fontanals, *"Hypercrosslinked polymers with strong anion-exchange character for the selective extraction of acidic pharmaceuticals from environmental water samples",* Journal of Chromatography A (2011)(submitted)(section 3.2.2).

D. Bratkowska, N. Fontanals, S. Ronka, A.W. Trochimczuk, F. Borrull, R.M. Marcé, *"Comparison of different imidazolium supported ionic liquid polymeric phases with strong anion-exchange character for the extraction of acidic pharmaceuticals from complex environmental samples",* Journal of Separation Science (2011)(submitted)(section 3.3.1).

D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, *"Development and application of a polar coating for stir bar sorptive extraction of emerging pollutants from environmental water samples",* Analytica Chimica Acta 706 (2011) 135-142 (section 3.4.1).

D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé *"Preparation and application of a polar monolithic coating based on methacrylic acid and divinylbenzene for the sorptive extraction of polar pollutants from environmental water samples",* Journal of Chromatography A (2011)(submitted)(section 3.4.2).