PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS

Núria Gilart Alzuria

Dipòsit Legal: T 1098-2014

PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS

Núria Gilart Alzuria

DOCTORAL THESIS

Supervised by

Prof. Francesc Borrull and Dr. Núria Fontanals

Departament de Química Analítica i Química Orgànica

UNIVERSITAT ROVIRA I VIRGILI

Tarragona

2014

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

UNIVERSITAT ROVIRA I VIRGILI

Departament de Química Analítica i Química Orgànica

El Dr. FRANCESC BORRULL i BALLARÍN, Catedràtic 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: "PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS", presentada per NÚRIA GILART ALZURIA per optar al grau de Doctor per la Universitat Rovira i Virgili amb menció europea, 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, 14 de febrer de 2014.

Prof. Francesc Borrull i Ballarín

Dra. Núria Fontanals i Torroja

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

L'etapa que avui acaba va començar molt lluny d'aquí, concretament a Hèlsinki, gràcies a un correu electrònic de la Dra. Rosa Maria Marcé, la qual m'oferia la possibilitat d'optar a una beca predoctoral al seu grup de recerca, esdevenint així l'inici d'aquesta etapa. Per tot això i més, li vull agrair la seva confiança i suport dipositats en mi durant aquests anys.

Les meves paraules més sinceres d'agraïments van dirigides al Dr. Francesc Borrull i a la Dra. Núria Fontanals, per deixar-me aprendre al seu costat, per la seva paciència, dedicació, ajuda, amabilitat, comprensió, suport i bons consells. Darrera seu, voldria agrair les Dres. Eva Pocurull, Carme Aguilar i Marta Calull, amb les quals he compartit interessants reunions de grup, consells i divertits moments a l'office i als congressos. A tots els que formen part d'aquest grup de recerca, MOLTES GRÀCIES DE TOT COR. No són menys importants tota la gent que forma part del Departament (Tere, Jaume, Avelina, Olga i Dúnia) que en algun moment o altre m'han ajudat i m'han facilitat la meva etapa aquí a la universitat.

An important part of this stage would not have been possible without the valuable help and knowledge of Prof. Peter A.G. Cormack, who always helped me in the new world of organic synthesis, who always had time to answer my questions and from whom I always received kind words and good advice. Also, I would like to thank all the people who worked in the *Polymer Group* during my stay at the University of Strathclyde in Glasgow: Omer, John, Marco, Yati, Marion and specially Kim and Maitane. They definitely made my stay easier in their research group.

Per mi, totes aquelles persones que han compartit el dia a dia amb mi al laboratori durant tots aquests anys signifiquen molt més que companys del laboratori, són com una família, amb els quals comparteixes millors i pitjors moments, molts riures i alguna que altra llàgrima però sobretot alegria, generositat i amistat. Així, Noelia, Anna, Laura, Carol i Mireia A. gràcies per resoldre'm algun que altre problema de gasos, Irene i Igor gràcies les vostres aventures i per fer-nos més fàcil l'electroferesi, Dominika gràcies per ensenyar-me tot l'après, Mireia N. i Daniela gràcies per la vostra amistat, Paula gràcies pels ànims i alegria rebuts, Pol gràcies per ajudar-me tan aquí com fora, Tatiana gràcies per estar sempre allí, Alejandro G., Cristian i Alejandro M. gràcies per posar diversió, alegria i vitalitat al laboratori, Núria M. gràcies pel teu treball i esforç, Montse tot i la distància gràcies per tots els bons moments, Antonio i Marta gràcies a tots dos per la vostra experiència, ajuda, consells, ànims, amistat i moltes rialles.

El camí fins aquí ha estat molt llarg, molta gent que arriba i se'n va, però les que encara segueixen al meu costat són les meves amigues, Patri, Nun, Lydia, Aida, Lidia, Anna, Ares i Sheila. A totes elles gràcies per estar al meu costat tant en els bons com els mals

moments i espero que així segueixi durant molts anys i que sapigueu que aquesta tesi ha sortit gràcies als vostres ànims i suport.

En aquest punt, recordo com he arribat fins aquí i miro enrere i veig als meus pares. L'esforç amb el que ells van treballar i segueixen treballant perquè arribem allà on volem és el que em queda i el que em fa seguir endavant. Aquesta cadira de tres potes (el pare, la mare i jo) no s'aguantaria sense la Sister, a la qual adoro i admiro. Gràcies a tots tres per confiar i creure en mi, us estimo. Finalment, l'altra persona que ha fet possible que jo arribés fins aquí i pogués acabar aquesta etapa ha estat l'Albert, sense ell això no hagués estat possible, sense el seu suport, comprensió, il·lusió, ganes, esforç i moltíssima energia. A tots vosaltres, moltes gràcies!

INDEX

CHAPTER 1

INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

Nowadays, it is a well-accepted fact that the advances of human society, such as urbanisation, industry and our modern lifestyles, have caused many significant changes in the environment and nature, leading to air, water and soil pollution, which in turn affects human health. As society is mainly responsible for the current environmental problems we face, in recent years, the scientific community has paid special attention to the fate and occurrence of emerging organic contaminants (EOCs) in the environment, with the aim of reducing the impact of such pollution on health [1]. EOCs include groups of unregulated organic compounds, such as pharmaceuticals, steroids and hormones, personal care products (PCPs), illicit drugs or drugs of abuse, brominated flame retardants, industrial additives, disinfection by-products, nanomaterials, surfactants, among others, which have been detected in environmental waters, soils and sewage sludge at low levels of concentration (levels of low ng L^{-1} in waters and low μg Kg^{-1} in sewage sludge). These organic compounds could be added to food, plastic bottles, detergents, cosmetics, toys, pesticides, among others. Their production, usage and application contribute to environmental pollution and health risks for the consumer. Over the years, the impact of EOCs on human and animal life has been observed, causing adverse effects, such as endocrine disruptor activity, carcinogenicity and neurotoxicity, etc. [1,2].

The way that organic compounds enter the environment depends on their usage and mode of application (e.g. disposal of municipal, industrial and agricultural wastes or excretion of pharmaceuticals). As most EOCs come from human use and they are widely consumed, wastewater treatment plants (WWTPs) are the main pathway by which EOCs are introduced into the environment. For this reason, the study of the effectiveness of WWTPs in removing EOCs is crucial [3]. It has been widely demonstrated that, in most cases, the different treatments included in WWTPs (primary, secondary and tertiary (optional)) are not efficient enough to eliminate the majority of EOCs, which are still detected in effluent wastewater samples [1,4-6]. In addition, over recent years, there has been a growing concern regarding the presence of metabolites or transformation products of EOCs, representing a new challenge for environmental analysis. For instance, after drug intake, drugs are generally absorbed by the organism and further subjected to metabolic reactions, in which the chemical structure of the parent compound can be modified before the metabolite leaves the human or animal organism via urine or faeces. After their excretion and release into the environment, both parent compounds and metabolites can undergo structural changes as a result of biotic (biotransformation), non-biotic (photolysis, hydrolysis, etc.) and technical processes (wastewater and drinking water treatments). All compounds transformed by living organisms are considered metabolites, while chemicals resulting from non-biotic or technical transformation are referred to as transformation products (TPs) [7-9]. In

general, these structural changes lead to new compounds with different environmental behaviours and ecotoxicological profiles from their parent compounds. In some cases, the new metabolites or TPs are more active and harmful compounds, representing a critical concern of scientific society [3,9].

Many EOCs reach surface waters (rivers, lakes and estuaries, among others) and groundwaters after resisting degradation. Although EOCs are attenuated at trace levels (ng L^{-1}) by dilution in surface waters, these compounds can persist in the environment and reach drinking water supplies, threatening human health [5,10]. The persistence of some contaminants in the environment and, as a result, their presence in drinking water supplies entails the existence of several contaminants in the water cycle, as shown in a schematic representation in Figure 1.

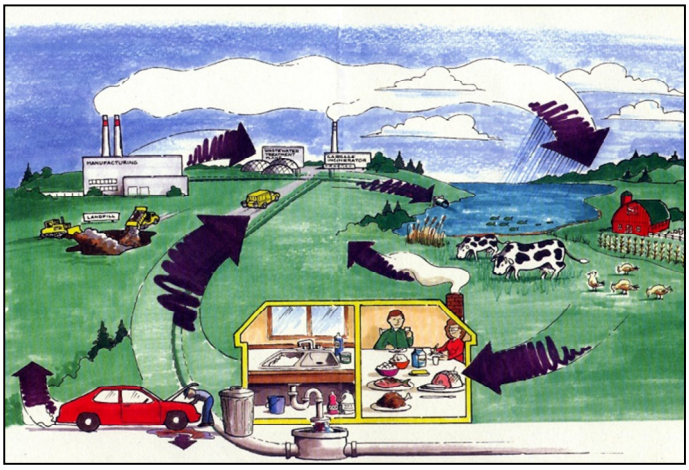


Figure 1. Contaminants in the water cycle.

Currently, the European Commission has several regulations in place to ensure the quality of groundwaters, surface waters and drinking waters [11,12]. For instance, the European Drinking Water Directive 98/83/EC [12] limits the content of a few number of organic contaminants, including pesticides, polycyclic aromatic hydrocarbons (PAHs) and some chlorinated organic solvents. In 2008, the Environmental Quality Standards Directive 2008/105/EC [13] introduced concentration limits for 33 priority substances. This directive included polybrominated biphenylethers (PBDEs), specified PAHs, octylphenols and nonylphenols, and a range of chlorinated hydrocarbons. Despite numerous directives regarding water quality and several studies proving the adverse effects of EOCs on human health, none of the EOCs mentioned above have yet been included. More recently, a revision of two previous directives [11,13] was proposed [14],

in which 15 priority substances were added, stating the maximum allowable concentrations in surface waters and, for some substances, in biota. The additional priority substances included the incorporation of some EOCs, such as a pharmaceutical (diclofenac), two hormones (17β-estradiol and 17α-ethinylestradiol), surfactants (perfluorooctane sulphonic acid and its derivatives (PFOS)) and some pesticides (bifenox, aclonifen and dichlorvos, among others). Although the regulations are continuously being updated with new priority substances being introduced, the number of EOCs included in the current regulations is minimal and more efforts should be made to establish quality standards for a wider range of compounds and, as a result, reduce human exposure to them.

Determining water quality is a priority for human health. The majority of the EOCs that have been determined in surface and wastewaters are found at concentration levels between ng L^{-1} and μg L^{-1}. Therefore, in recent years, selective and sensitive analytical techniques have been required, becoming essential tools for achieving the suitable and reliable assessment of the presence of EOCs in environmental samples [1,2,15]. It is well-known that the accurate quantification of EOCs in environmental samples can be an analytical challenge due to the complexity of the matrices involved, as well as their low levels of occurrence. During the last few years, the increasing demand for monitoring EOCs in environmental samples has led to the use of selective, robust and sensitive instrumental techniques. In this respect, liquid chromatography (LC) and gas chromatography (GC) are the preferred separation techniques for identifying and quantifying EOCs in environmental analysis. GC is the most suitable analytical technique for determining apolar and volatile compounds. However, its applicability can be extended to more polar compounds if a derivatisation step is included prior to the chromatographic separation in order to reduce the polarity and enhance the volatility of the compounds. LC is widely used for the determination of less volatile, apolar and polar or thermolabile organic contaminants. In GC, the determination of many structurally different organic contaminants may involve more than one derivatisation reaction. In contrast, organic contaminants with a wide range of polarities can be separated and determined by LC without the need for a previous derivatisation step, giving LC an attractive advantage with respect to GC. For this reason, LC has become the separation technique of choice for determining a large number of EOCs. It should also be noted that capillary electrophoresis (CE) has also been reported for the assessment of EOCs in environmental waters, but to a lesser extent in comparison to LC or GC, due to smaller injection volumes [3,16].

In order to achieve the goal of the environmental analysis, namely the quantification of many organic contaminants down to ng L^{-1}, chromatographic techniques need to be

coupled to a powerful detection technique to achieve sensitivity and selectivity. These requirements are fulfilled by mass spectrometry (MS), replacing the classical detectors for LC, such as ultraviolet-visible (UV), fluorescence (FD) or electrochemical (ED). Nowadays, several reviews found in literature show the numerous LC-MS applications in environmental analysis for determining pharmaceuticals, drugs of abuse, perfluorinated compounds, polar pesticides and PCPs, among others [2,8,17,18]. A few years ago, LC coupled to MS with a single quadrupole as the analyser gained popularity and became one of the preferred techniques for the quantitative analysis of a large range of polar organic contaminants [18]. Moreover, when the aim of the study was the development of screening methods for target and non-target compounds, the time-of-flight (TOF) mass analyser and Orbitrap were the most desirable options, due to their high resolution and accuracy, but they offered less sensitivity. Later, tandem mass spectrometry (MS/MS) appeared as new solution for the increasing demand of the research into more emerging contaminants with different chemical properties at very low concentration levels in complex matrices, providing higher selectivity and sensitivity and becoming, in combination with LC, the most broadly-used instrumental technique in the quantitative analysis of EOCs. In MS/MS, the ions are subjected to two or more sequential stages of mass spectrometry according to the quotient mass/charge (m/z). Currently, the most commonly-used MS/MS instruments coupled to LC are triple quadrupole (QqQ) [19-23] and ion trap (IT) analysers [24-27], with the former being widely applied in the development of multi-residue methods for trace levels determination of EOCs in environmental analysis [28-32]. It is well known that the QqQ system exhibits excellent performance for quantitative analysis when working in selected reaction monitoring (SRM), also known as multiple reaction monitoring (MRM), in terms of sensitivity and selectivity, due to the selection of two specific SRM or MRM transitions for each analyte, at least, for confirming and identifying it. However, sometimes, no reliable transitions or a second transition with a low intensity can hinder the identification of the analytes at low concentrations [33]. Therefore, hybrid mass spectrometers and high resolution mass spectrometry (HRMS) technologies emerged to tackle to this problem and provide enough analytical information for accurate identification. Examples of these approaches are the quadrupole linear ion trap (QqLIT), quadrupole time-of-flight (QqTOF) and linear ion trap-Fourier transform-Orbitrap (LTQ-FT-Orbitrap). Just like QqQ, QqLIT provides superior performance in terms of sensitivity. Furthermore, QqLIT allows the proper identification of TPs because it has MS^3 capability and sequential fragmentations can be performed [18]. Thus, this hybrid mass spectrometer has been applied for determining a large number of pharmaceuticals, covering various therapeutic groups, in environmental waters (sea, waste and surface waters) [34-38]. Recently, Ferrando-Climent et al. [39] developed a LC-MS/MS (QqLIT) method for the quantification of anticancer drugs in hospital and urban wastewaters as

well as for the screening of human metabolites and transformation products of anticancer drugs. The results showed the QqLIT system allowed the sensitive and suitable quantification and identification of several anticancer drugs in the matrices with method detection limits (MDLs) ranging from 0.8 to 24 ng L^{-1}. Moreover, this study firstly confirmed the presence of three human metabolites of two anticancer drugs (tamoxifen and cyclophosphamide), being detected in hospital wastewaters.

Unlike QqQ and QqLIT, QqTOF and LTQ-FT-Orbitrap are characterised by their high selectivity, becoming powerful confirmatory tools in environmental analysis since they are able to provide fast, sensitive and reliable detection and identification of target compounds and their transformation products, due to their high mass accuracy and mass resolution [16,18]. These systems enable accurate mass measurements to be obtained of a large number of analytes, target and non-target compounds, resulting in a high confirmatory capability and a reduction in false positives. Moreover, excellent full-scan sensitivity is provided by these systems in comparison to QqQ [18,40]. With respect to QqTOF, both quantitative and qualitative analytical methods have been developed to determine a large number of EOCs in environmental samples, obtaining more sensitive and selective analysis than using the single quadrupole or TOF. For instance, screening for hormones, phenols and their metabolites [41], pharmaceuticals and their degradation products [42] or a group of several EOCs (including pharmaceuticals, PCPs and illicit drugs) [43] in environmental waters has recently been performed using LC-MS/MS (QqTOF), due to its great sensitive and specific performance. In addition, there are also studies in which LC-MS/MS (QqTOF) has been used for the accurate quantification pharmaceuticals, industrial additives (benzotriazoles) and illicit drugs, among others, in environmental samples [44-47]. Due to the recent introduction of LC-MS/MS (LTQ-FT-Orbitrap), fewer studies have reported its use for both qualitative and quantitative purposes, such as for industrial additives (benzotriazoles and benzothiazoles) [48], illicit drugs and metabolites [49] or pharmaceuticals and pesticides [50]. All of these studies state that these HRMS techniques coupled to LC are a very powerful combination with excellent capabilities for simultaneous identification, confirmation and quantification of organic contaminants in complex matrices, showing great sensitivity similar to that reported in LC-MS/MS (QqQ) methods. In Table 1, the advantages and limitations of the most common tandem mass analysers are compared.

Despite the increasing popularity of screening methods for non-target compounds through the use of HRMS techniques, LC-MS/MS (QqQ) under MRM mode is still the instrumental technique of choice, due to its outstanding performance in terms of sensitivity and confirmation power when quantification analysis is needed. Nowadays, several reviews regarding the determination of EOCs in environmental waters

[17,33,51,52] reveal the numerous LC-MS/MS (QqQ) applications in environmental analysis.

Table 1. Comparison of characteristics of different tandem mass analysers [18].

	QqQ	IT	QqLIT	QqTOF	LTQ-FT-Orbitrap
Advantages	1.High selectivity and sensitivity in SRM mode	1.Moderate to high sensitivity in full-scan mode 2.Moderate sensitivity in SIR mode 3.Multiple-stage MS	1.High selectivity and sensitivity in SRM mode 2.Moderate to high sensitivity in full-scan mode 3.MS^3 capabilities 4.Higher capacity for ion storage	1.High sensitivity and selectivity in full-scan mode 2.High resolution	1.Moderate to high sensitivity in full-scan mode 2.High resolution 2.Multiple-stage MS 3.Large space charge capacity
Limitations	1.Low sensitivity in full-scan mode 2.Low resolution	1.Limited capacity for ion storage	1.Low accurate nominal mass	1.Less sensitive than QqQ for quantitative purposes	1.Not fully UHPLC compatible at high resolution 2.High cost

LTQ-FT-Orbitrap: linear ion trap-Fourier transform-orbitrap; QqLIT: quadrupole linear ion trap; QqTOF: quadrupole time-of-flight; QqQ: triple quadrupole; SIR: selected ion recording; SRM: selected reaction monitoring; UHPLC: ultra-high pressure liquid chromatography

In MS, apart from the selection of a suitable analyser, the way in which the analytes are introduced into the analyser is also essential. Thus, in LC, the most widely-used ionisation sources are electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI) and, more recently, atmospheric pressure photo-ionisation (APPI). All three ionisation sources are included in the group of soft ionisation interfaces, since the analyte molecule does not suffer excessive fragmentation. In addition, the chemical properties of the studied analytes, such as polarity, are a crucial feature for selecting a suitable ionisation source, resulting in an enhancement of the sensitivity of the whole analytical method [18,53]. In environmental analysis, the most common coupling between LC and MS is performed through ESI, since this ionisation source is desirable and very sensitive for organic contaminants with ionisable functional groups with a

moderate to high polarity. In contrast, APCI is recommended for low polarity compounds with a certain degree of volatility. Both ESI and APCI can be considered complementary ionisation sources [18,51]. Due to the high complexity of the environmental samples, the presence of matrix components can interfere with and modify the ionisation process of the target analytes, resulting in a decrease or increase in their signal response (ion suppression or enhancement, respectively). Therefore, this effect, also known as the matrix effect (ME), depends on the compound, the sample and the ionisation source. It is generally calculated using the formula shown in Figure 2, in which A represents the peak area of the analyte spiked after the extraction and B represents the peak area of the analyte spiked at the same concentration in a neat solution [54]. Thus, values near 100% mean that there is no matrix effect.

$$\mathrm{ME}\ (\%) = \frac{\mathrm{A}}{\mathrm{B}} \times 100$$

Figure 2. Formula for calculating matrix effect.

Moreover, other studies use the concept of ion suppression (decrease of the signal response) or enhancement (increase of the signal response), defined as ((1-A/B) x 100). Therefore, positive values mean that there is a suppression of the signal of the analyte, while negative values mean that an enhancement of the signal of the analytes is found [54,55]. In all of the papers included in this Thesis, the term ion suppression/enhancement was selected to evaluate this effect when LC-MS/MS was used.

Quantitative analysis with the aforementioned ionisation sources (ESI and APCI) can be substantially affected by the ME caused by the presence of matrix components in the sample that can compete with the analytes to be ionised. Some authors have stated that APCI is generally less sensitive to ME than the more commonly-used ESI, as the ionisation mechanisms are different and can affect the efficiency of formation the desired ions in the presence of coeluting compounds [54,56,57]. For instance, several authors studied the effect of using ESI or APCI on the intensity of the target analytes in environmental analysis [58-62]. As an example, Wick et al. [58] studied the ME for a group of EOCs, including biocides, PCPs and benzothiazoles, in environmental waters, when ESI and APCI were tested as ionisation sources in LC-MS/MS. The results showed that ESI was significantly more susceptible to matrix interferences than APCI, resulting in lower absolute recovery values. Although APCI was shown to be less susceptible to ion

suppression, higher background noise was observed, leading to higher MDLs. In the end, the authors decided to use ESI, compensating the ME with the use of internal standards. Another interesting study has been reported by García-Ac et al. [61], in which a comparison between APPI, ESI and APCI was developed for the on-line solid-phase extraction (SPE) followed by LC-MS/MS analysis of five pharmaceuticals from various therapeutic classes in municipal wastewaters. Unexpectedly, better signal response and less ME were observed using ESI in comparison to APCI and APPI. For instance, one of the compounds studied (methotrexate) could be only ionised using the ESI mode. In addition, lower background noise was obtained by ESI and, as a result, ESI could provide a suitable validation for each analyte in wastewater samples with the lowest reported MDLs. To sum up, from the above studies, it can be concluded that the ionisation source can really affect the signal response of the analytes, especially, when real samples are analysed. Moreover, it is worth adding that each analyte presents different behaviour depending on the sample and ionisation source. Therefore, this fact should be taken into account to ensure the highest sensitivity.

In environmental analysis, the capability for quantifying low concentrations levels at which most of the EOCs are usually found is given by the instrumental technique as well as the sample preparation. As detailed previously, many improvements have been performed in terms of detection techniques and their improved sensitivity thanks to tandem mass spectrometers. However, sample preparation also plays a crucial role in the whole analytical procedure, since it enables the clean-up of complex matrices as well as enriching the analytes of interest and enhancing the sensitivity of the method [63-65]. The choice of a sample extraction technique depends on the physical state of the sample (solid, liquid or gas) as well as the chemical properties of the analytes (e.g. polarity). Therefore, the application of suitable sample pretreatment will lead to clean sample extracts, which will help to improve separation and detection [64]. Due to the extensive use of MS or MS/MS in environmental analysis in recent years and their high sensitivity, great importance has been placed on achieving an effective and selective clean-up of the complex matrices in order to avoid or reduce the ME, rather than achieving a high enrichment factor.

To extract organic contaminants from different types of samples, a variety of extraction techniques have been widely used and reported in literature. In particular, for liquid samples, liquid-liquid extraction (LLE) has been the most commonly-used extraction technique. However, the use of large volumes of organic solvents, low degree of automation and, particularly, the tedious and time-consuming steps have become the main disadvantages of this technique, with LLE being replaced by SPE. With similar principles to LC, SPE is a sorption-based extraction technique in which a liquid sample

comes into contact with a solid-phase or sorbent, with the compounds being selectively adsorbed onto the surface of the sorbent. In addition, SPE has a number of advantages over LLE, such as more complete extraction of the analytes, more efficient separation of interferences from the analytes, reduced organic solvent consumption, no emulsion formation and easier automation [66,67]. This sorptive extraction technique has become increasingly popular in environmental, food and biological analysis because of its numerous advantages, including a high enrichment factor, high recovery values, great reproducibility and fast and easy performance in comparison to the conventional LLE. Among these advantages, the high versatility offered by SPE is well known, due to the wide range of commercially available sorbents, extending its application to a variety of analytes with different chemical and physical properties in different complex matrices. In addition, extensive research has been carried out over the years into the synthesis of in-house SPE sorbents (including polar polymeric sorbents, molecularly imprinted polymers (MIPs), mixed-mode sorbents, supported ionic liquid phases (SILPs) or nanomaterials, among others) to enhance the capacity and selectivity towards a wider range of organic contaminants, especially for high polarity compounds [68-70]. More detailed information about SPE is discussed in Section 1.1.

Although SPE is most commonly used in sample preparation, other sorption-based and solvent-based extraction techniques have been proposed for the analysis of liquid samples with the aim of developing more miniaturised and time-saving techniques, reducing or eliminating the consumption of toxic organic solvents and, as a consequence, obtaining more environmentally friendly sample preparation techniques. In terms of green analytical chemistry, solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), microextraction by packed sorbents (MEPS), disposable pipette extraction (DPX) or liquid-phase microextraction (LPME) related techniques have been developed and these will be discussed further. Since 2010, numerous critical reviews have been published regarding sample preparation techniques, including both extraction and microextraction techniques [63,71-74], as well as reviews about new materials for sample preparation techniques [69,70,75-80]. Figure 3 shows a schematic representation of the conventional and modern extraction techniques for the analysis of liquid samples.

With regard to sorption-based extraction techniques, the first attempt was achieved with the introduction of SPME in the early 1990s [81] as a solvent-free, efficient and fast preconcentration technique, in which the analytes are adsorbed onto a fibre coated with a suitable extracting phase, typically polydimethylsiloxane (PDMS) [82]. This technique offers great versatility due to the different sampling modes, such as direct immersion (DI) and headspace (HS), leading to a broad spectrum of compounds being

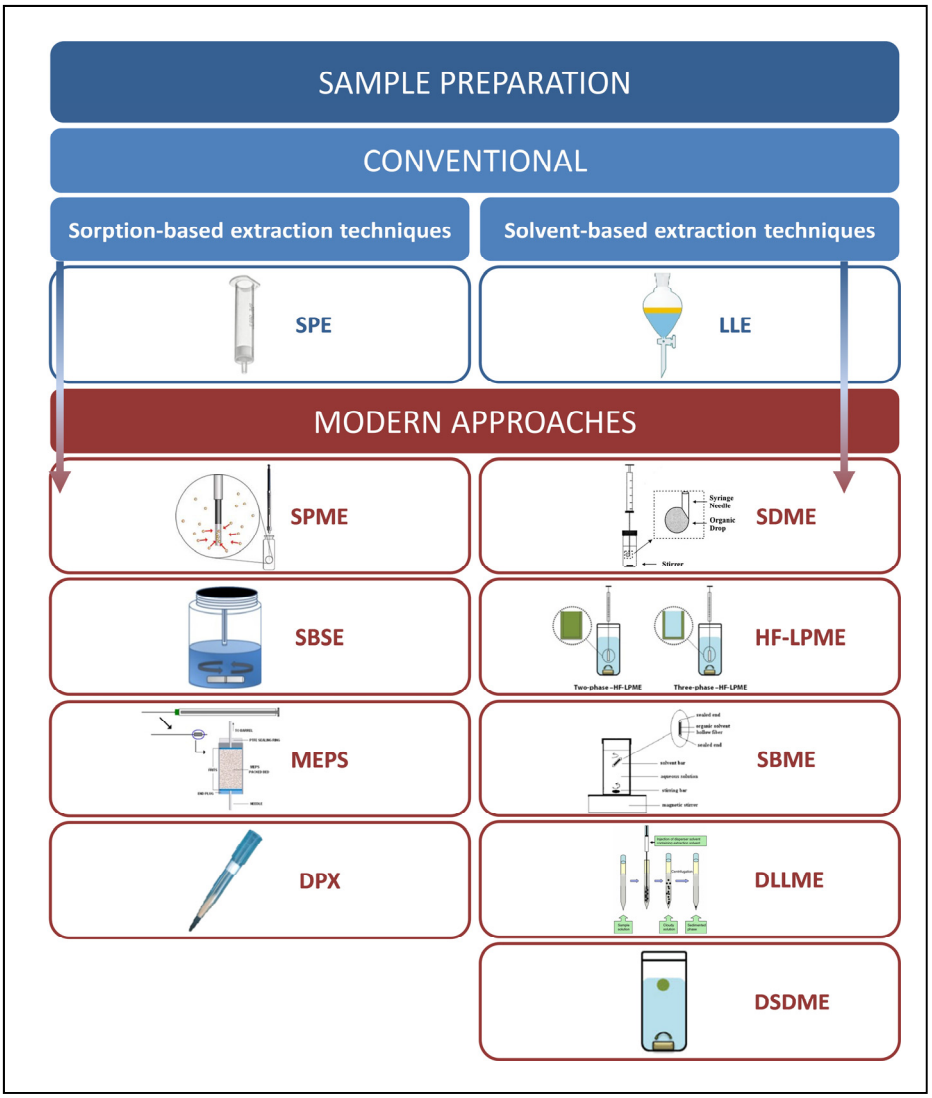


Figure 3. An overview of the most commonly used extraction techniques for the analysis of liquid samples: dispersive liquid-liquid microextraction (DLLME); directly suspended drop microextraction (DSDME); disposable pipette extraction (DPX); hollow-fibre liquid-phase microextraction (HF-LPME); liquid-liquid extraction (LLE); microextraction by packed sorbents (MEPS); solvent bar microextraction (SBME); single drop microextraction (SDME); stir bar sorptive extraction (SBSE); solid-phase extraction (SPE) and solid-phase microextraction (SPME).

extracted, especially volatile and semi-volatile ones, followed by GC. However, SPME has also been coupled, but to a lesser extent, directly with LC in order to determine weakly volatile and thermally labile compounds not amenable to GC [83]. Due to its suitability for extracting compounds with moderate to high volatility, numerous applications have reported the coupling between SPME and GC, which has attracted great interest in terms of preconcentrating organic contaminants, such as pharmaceuticals, volatile organic compounds (VOCs), volatile sulphur compounds (VSCs), PAHs and pesticides, from different types of samples (environmental, food or biological) in just one step. Thus, a large number of SPME applications and new SPME formats and materials have recently been reported in a number of reviews [83-85]. Moreover, several SPME fibres have been commercialised, based on PDMS, polyacrylate (PA), divinylbenzene (DVB), carboxen (CAR), carbowax (CW) or a combination of different polymers (PDMS/DVB, PDMS/CAR or CW/DVB). These coatings cover a small range of compounds with low and moderate polarity. In order to extend the number of extracted analytes, the synthesis of novel and in-house SPME fibres has been developed. A recent review has been published by Xu et al. [75], in which the newest and most commonly-used materials for SPME have been included, such as ionic liquid (ILs), carbonaceous materials, polar polymeric materials and MIPs. Despite the advantages offered by SPME, this technique fails as a result of the low capacity of SPME fibres due to the low volume of extracting phase [86].

Like SPME, SBSE is also a sorptive extraction technique and it consists of a magnetic bar covered with a polymeric material. This technique was first introduced by Baltussen et al. [87] in 1999 in order to improve capacity with an increase in the volume of the extracting phase. In comparison to SPME, the volume of sorptive material in SBSE is between 50 and 250 times higher. Therefore, the incorporation of a high amount of extracting phase and the stirring of the sample in the same device result in higher extraction efficiencies and lower MDLs [88,89]. In SBSE, volatile and non-volatile analytes can be retained onto the coating by HS or DI sampling, respectively, with the latter being simpler and more widely applied when SBSE is used. After the extraction, the stir bar is removed from the aqueous sample and then the analytes are desorbed, usually by thermal desorption (TD) and, to a lesser extent, by liquid desorption (LD). Since TD is the most widely-applied way for desorbing the analytes, no organic solvent is employed, meaning that SBSE in just as environmentally friendly an extraction technique as SPME. This versatile technique has become popular for enriching organic contaminants from environmental, food, biological and pharmaceuticals samples [88,90,91]. Although SBSE has higher extraction capacity than SPME, due to the higher amount of extracting phase covering the stir bar, this technique lacks the commercially available coatings, with PDMS-coated stir bars, known commercially as Twister by

Gerstel, being the only type applied in SBSE for many years. Moreover, the nature of this coating, PDMS, resulted in the extraction of compounds of low or moderate polarity (generally for those with log $K_{o/w}$>3), such as pesticides, VOCs, PAHs and some pharmaceuticals, among others [88]. Since the only commercial stir bar was not completely suitable for the extraction of polar analytes, in recent years, several polar in-house coatings and new commercial coatings for SBSE have been proposed to overcome the limitation of PDMS. More specific information about the recent advances in SBSE coatings and their applications will be discussed in Section 1.2.

Although SPME and SBSE are unique in terms of desorbing analytes from the sorptive material using TD with the absence of an organic solvent, other extraction techniques have been proposed, such as MEPS, DPX and several LPME techniques, in which the miniaturisation of the extraction device led a significant reduction in the volume of organic solvent to elute the analytes to a few µL, resulting in greener analytical performance. MEPS emerged in 2004 as a miniaturisation of SPE in order to reduce organic solvent consumption, as well as the sample volume required, maintaining the same benefits as the conventional SPE. In MEPS, approximately 1-4 mg of solid extracting phase is packed inside the syringe (100-250 µL) barrel as a plug or between the needle and the barrel as a cartridge [92,93]. Small sample volumes (10-1000 µL) and washing and elution volumes (20-50 µL) are usually applied, making this a suitable extraction technique for an on-line coupling to GC, LC or capillary electrochromatography (CEC). Unlike SPE, less commercial sorbents are available for MEPS extraction, such as C_8, C_{18}, C_8/SCX, SAX, SCX and silica. The application of MEPS has been successful in biological and environmental analysis, such as for determining illicit drugs and pharmaceuticals in blood, hair and urine samples or PAHs, pharmaceuticals, PCPs and pesticides in environmental water samples [92]. Although there are several commercially available extracting phases for MEPS, new advances have been reported, such as the synthesis and application of MIPs as sorbents for MEPS. Thus, Prieto's research group has recently published two studies in which two MIPs were synthesised to be applied as MEPS sorbents for selectively extracting oestrogenic compounds [94] and fluoroquinolones [95] from environmental water samples. With respect to MIP for fluoroquinolones [95], the MIP-MEPS procedure was compared to conventional molecularly imprinted solid-phase extraction (MISPE), with the former technique displaying higher recovery values (> 93%) and lower MDLs (0.5-3.8 ng L^{-1}), even using a lower sample volume (only 1600 µL). Due to the low availability of sorbents for MEPS and the great performance achieved by the MIP-MEPS format, particular efforts should be made to develop more selective and polar materials to extend the applicability of MEPS in other research fields.

With regard to DPX, this simple and fast technique is another variant of traditional SPE and consists of a tip (1-5 mL) filled with a dispersible sorbent loosely placed between two frits. In DPX, the sample is mixed with the sorbent and each particle of sorbent comes into contact with the analytes several times, leading to fast and efficient extraction. This technique enables low sample and elution volumes and, as such, less waste is generated. Several DPX tips are commercially available with reversed-phase and ion-exchange sorbents [96]. Since DPX is efficient with smaller sample volumes, this technique is more suitable for biological and food analysis than for environmental analysis because of the low concentrations of the studied analytes. Therefore, some examples of the application of DPX include the determination of illicit drugs in vitreous humour [97], pesticides in fruits and vegetables [98], antibiotics in bovine tissue [99] and illicit drugs in urine [100], among others. The main characteristics of the sorption-based extraction techniques are included in Table 2.

Miniaturisation and, therefore, reduced organic solvent and waste volumes have also been applied in solvent-based extraction techniques, mainly with the aim of improving LLE performance. In the mid-to-late 1990s, this was achieved with the implementation of novel LPME techniques. Using these LPME techniques, a small organic volume is necessary for extracting the analytes of interest. The format or device in which the extracting solvent is located is the key to classifying the different LPME-related techniques. Among them, single-drop microextraction (SDME) has become popular since its introduction in 1997, due to the simplicity of operation and use [101,102], consisting of a drop of a solvent (few µL) being placed at the needle tip of the syringe for the extraction time established. During the extraction, the drop can be immersed in a liquid phase (DI-SDME) or in the headspace (HS-SDME). Then, the drop is withdrawn into the syringe before being injected to GC mainly, or alternatively LC. In DI-SDME, a solvent immiscible with the sample matrix (usually water) is required. Toluene, *n*-octanol, hexane and ethyl acetate are the most commonly used in this SDME mode. In contrast, in HS-SDME, the solvent required needs to be non-volatile to avoid low reproducibility as well as being a good acceptor phase for those volatile and semi-volatile compounds. With this in mind, greener extracting solvents in HS-SDME have been used, such as aqueous solutions or ILs, with the latter being organic salts that are liquid at room temperature and have high boiling points. ILs have high viscosity and low vapour pressure, resulting in a larger and reproducible extraction drop [103-105]. Moreover, both ILs and aqueous solutions as extracting solvents in SDME are mainly applied in CE and LC. Some reviews reveal that the main SDME applications have focused on the determination of pesticides, phenols, pharmaceuticals, PCPs and PAHs, among others, mainly in environmental waters and biological samples [102,106]. Despite the low cost and the simplicity of the equipment, the main disadvantage of

Table 2. The most relevant features for the sorption-based and solvent-based extraction techniques.

Extraction Technique		Main features
SORPTION-BASED	**SPE**	-Extraction of a wide range of compounds, from low to high polarity -High availability of commercial sorbents and formats -High capacity and selectivity -High applicability in many research fields -Consumption of organic solvents
	SPME	-Extraction of volatile and semi-volatile compounds, but with low and moderate polarity -Solvent-free extraction technique -Automated desorption of the analytes -Few amount of extracting phase and a limited availability of different commercial phases
	SBSE	-Extraction of compounds with low and moderate polarities -Higher amount of extracting phase than SPME -The stirring of the samples is integrated in the extraction device. -PDMS has been the only commercially available extracting phase for SBSE for many years. -The desorption of the analytes can be completely automated and solvent-free through TD
	MEPS	-Miniaturisation of SPE -Low amount of sorbent -Less sample volume and organic solvents required -Few commercial sorbents for MEPS
	DPX	-Low amount of sorbent -Less sample volume and organic solvents required -Simple and fast -Few commercial extracting phases -More applicability in bioanalytical research
SOLVENT-BASED	**LLE**	-Large volumes of organic solvents -Tedious and time-consuming -No automation
	SDME	-Simple operation and use -Low volume of solvent (µL) -Instability of the droplet -Low reproducibility -Low cost -No automation
	HF-LPME	-Low volume of solvent -The extracting solvent is protected by a porous hollow fiber -Better reproducibility
	SBME	-Low volume of organic solvent -The bar is covered by a hollow fibre filled with the organic solvent -Enhanced transfer of the analytes due to the movement of the bar in the sample
	DLLME	-Simple and fast procedure -Low cost -Low volume of organic solvents
	DSDME	-Simple procedure -Low volume of organic solvents -Extraction solvent holder is not required -Difficulty in collecting the exact volume of extracting solvent.

DLLME: dispersive liquid-liquid microextraction; DSDME: directly suspended drop microextraction; DPX: disposable pipette extraction; HF-LPME: hollow-fibre liquid-phase microextraction; LLE: liquid-liquid extraction; MEPS: microextraction by packed sorbents; SBME: solvent bar microextraction; SDME: single drop microextraction; SBSE: stir bar sorptive extraction; SPE: solid-phase extraction; SPME: solid-phase microextraction; TD: thermal desorption.

SDME is the difficulty in terms of achieving a stable organic drop. A further improvement in LPME techniques was the introduction of hollow-fibre LPME (HF-LPME). In HF-LPME, the extracting solvent is located in the pores of a hollow fibre and then it is immersed in the sample solution. This microextraction technique can be used in two modes: two-phase HF-LPME and three-phase HF-LPME, using an organic solvent or aqueous solution as extracting solvent, respectively. Therefore, the two-phase HF-LPME is mainly followed by GC, whereas the three-phase HF-LPME is suitable for LC or CE for the extraction of those more polar and water-soluble compounds. Both modes have been applied for determining pesticides, PAHs, illicit drugs, pharmaceuticals and phenols in environmental and biological samples and, to a lesser extent, in food and beverage samples [107,108].

Since 2004, LPME related techniques have been developed to obtain higher enrichment factors and reproducibility, such as solvent-bar microextraction (SBME), dispersive liquid-liquid microextraction (DLLME) or directly suspended droplet microextraction (DSDME). Firstly, SBME is considered a hybrid between HF-LPME and SBSE, which involves a bar covered with a hollow fibre filled with an extracting solvent. The stirring of the sample using a conventional stir bar and the free movement of the SBME device enhances the transfer of the analytes towards the extracting solvent [109]. A few applications of SBME have been reported, such as for extracting clenbuterol from urine samples [110], pharmaceuticals from water samples [111] and PAHs from soil samples [112], among others. In this technique, 1-octanol and toluene are the most commonly-used solvents as acceptor phases. With respect to DLLME, it has recently been proposed as the real miniaturisation of LLE and consisting of the extraction of the analytes from aqueous solutions to an extracting solvent mixed previously in a dispersing solvent. The low cost and the high simplicity of this technique has allowed numerous applications for the extraction of PAHs, metals, pharmaceuticals, and pesticides from environmental, food and biological samples [113]. Lastly, DSDME is a new version of the well-known SDME, in which a drop of the selected solvent is delivered onto the surface of an immiscible aqueous sample agitated by a stirring bar. While one of the advantages of this technique is that extraction solvent holder is not required, its main drawback is the difficulty in taking out the small suspended drop from the solution [76,106]. All of these LPME-related techniques presented above are also considered to be green extraction techniques due to the small amounts of organic solvent used. However, these techniques require the use of hazardous organic solvents, even at small volumes. Moreover, the nature of these solvents, mainly water immiscible and volatile, usually entails the application of these microextraction techniques followed by GC, since an evaporation step has to be included in LC applications. Despite this fact, some reviews have demonstrated the great combination between these techniques and

LC or CE [76,105,106,108]. The main features of the abovementioned extraction techniques are grouped in Table 2.

Nowadays, throughout all the steps included in a whole analytical procedure, improvements in sample preparation are constantly observed in the literature, particularly in order to obtain more environmentally friendly methodologies. In addition, these constant advances result from the increasing number of analytes at low levels of concentration found in more complex samples, requiring more selective and effective extraction techniques to extract more polar analytes without the influence of matrix interferences. Of the aforementioned enrichment techniques, the sorptive extraction techniques, such as SPE, SPME and SBSE, have been increasingly applied to preconcentrate analytes from liquid samples rather than the solvent-based extraction techniques. This fact remains dependent on the high availability of commercial extracting phases as well as the feasibility of the preparation of in-house materials, leading to highly versatile techniques and covering a wide range of analytes and matrices. In light of the above, the following sections into which this Doctoral Thesis is divided present a detailed description and recent advances in SPE and SBSE, particularly highlighting the most relevant approaches for the extraction of polar EOCs from environmental waters.

1.1. Solid-phase extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

Since the emergence of solid-phase extraction (SPE) in the mid 1970s, this technique has been widely used for extracting a broad range of analytes with different chemical properties from liquid samples. The main goals of SPE are analyte enrichment and sample clean-up, providing lesser laborious but more environmentally friendly sample preparation than conventional liquid-liquid extraction (LLE) [66,114]. This sorption-based extraction technique enables the transfer of the analytes from a liquid sample to a solid extracting phase (loading step).Then, matrix interferences can be removed from the sorbent using a washing solvent (washing step) and finally, the analytes of interest are totally released from the extracting phase using an organic solvent (elution step), followed by the instrumental technique. Several reviews and books have been published in which SPE is extensively described and discussed regarding its development, parameters and applications [66,67,114-116].

The design of efficient formats for SPE has developed over the years. Nowadays, sorptive materials are commercially available in different formats, such as SPE cartridges or syringe barrels, disks and 96-well plates. SPE cartridges are the most commonly used, with a certain amount of extracting material packed into a polypropylene barrel between two frits. Depending on the type and the volume of the sample to be percolated, different cartridge weights are commercially available, ranging from 10 mg to 10 g. A more recent SPE format is the 96-well plates, which is the miniaturisation of the SPE in order to process a large number of small samples with a semi-automated handling system, which is suitable for biological and pharmaceutical analysis [66,67,117]. Despite the advantages offered by the 96-well plates for SPE, cartridges are still the most preferred SPE format, due to the extensive availability of sorbents with different chemistries and sizes, as well as their easy operation, covering a wide range of research fields [67,96,115,118-120]. Despite the widespread availability of commercial sorbents for SPE with excellent extraction efficiencies for retaining a large number of organic contaminants, SPE sometimes fails in terms of retaining high polarity compounds. Therefore, the development of novel in-house sorbents for SPE with improved properties, such as higher capacity, more polar functionalities and higher selectivity, has become a novel research field in sample preparation, with the aim of increasing the number of extracted analytes and the range of applications of SPE. In Sections 1.1.1. and 1.1.2., the novel approaches regarding new commercial or in-house materials for SPE are presented.

The versatility of SPE is based on the numerous commercially available sorbents and also due to the performance mode of working: off-line or on-line SPE. Typically, off-line SPE methodologies have widely been performed for years because of the lack of automated processes to connect directly to analysis techniques and the high sample

volumes loaded (up to 1 L). Therefore, the direct and automated coupling between SPE and the chromatographic system was achieved with the introduction of the on-line SPE, consisting of a precolumn filled with the selected sorbent by direct connection to LC and, to a lesser extent, GC systems. Little sample manipulation, no losses during the different SPE steps, more reproducible results and higher enrichment factors and sensitivity than off-line SPE methods are the main advantages of the on-line SPE approach. For instance, Pedrouzo et al. [19] reported low method detection limits (MDLs) for cocaine (0.5 ng L^{-1}) and benzoylecgonine (0.5 ng L^{-1}) after preconcentrating 500 mL of a river water sample by off-line SPE and LC-MS/MS. In contrast, Fontanals et al. [121] only needed 10 mL of river water sample to achieve similar MDLs for cocaine (1 ng L^{-1}) and benzoylecgonine (1 ng L^{-1}) using an on-line SPE system followed by LC-MS. The great success of on-line SPE systems is based on the total introduction of the desorbed analytes from the sorbent to the chromatographic system, resulting in enhanced sensitivity. However, some shortcomings can appear when on-line SPE is applied, such as the incompatibility of the elution solvent with the chromatographic technique (e.g. mobile phase in LC) or the small amount of sorbent in the precolumn, which may lead to low capacity [117,118,122,123].

Of all the parameters to bear in mind during the optimisation of a SPE procedure, appropriate SPE sorbent selection is one of the most critical in order to achieve efficient extraction of the analytes of interest from liquid samples. Nowadays, the broad variety of available sorbents is the reason for the high applicability and versatility of this technique in many research fields. The development of sorptive materials for SPE has become an important issue to be studied and improved. Figure 4 shows the main classification of sorptive materials for SPE depending on their chemical structure. SPE sorbents are mainly classified as inorganic-based and organic-based. In addition, the modification of these inorganic or organic supports with different functional groups has contributed towards enhancing the selectivity of the extractions. With respect to inorganic supports, the most common sorbents are silica (SiO_2), alumina (Al_2O_3), magnesium silicate ($MgSiO_3$ or Florisil) and chemically-bonded silica sorbents. Due to the well-accepted technology of chemically-bonded silica packings for LC columns, developing similar materials in a suitable format for SPE was a simple task. As a result, the modification of silica particles led to the development of many sorbents used for reversed-phase, normal-phase and ion-exchange SPE, such as octadecyl silica (C_{18}), octyl (C_8), ethyl (C_2), cyclohexyl (CH), phenyl (Ph), cyanopropyl (CN), diol (OH), aminopropyl (NH_2), propylbenzene sulphonic acid and trimethylaminopropyl bonded silica sorbents, among others. The interaction mechanisms are based on Van der Waals forces (hydrophobic interactions) as well as hydrogen bonding and dipole-dipole forces [124]. Despite the fact that the silica-based sorbents have been the most frequently used in

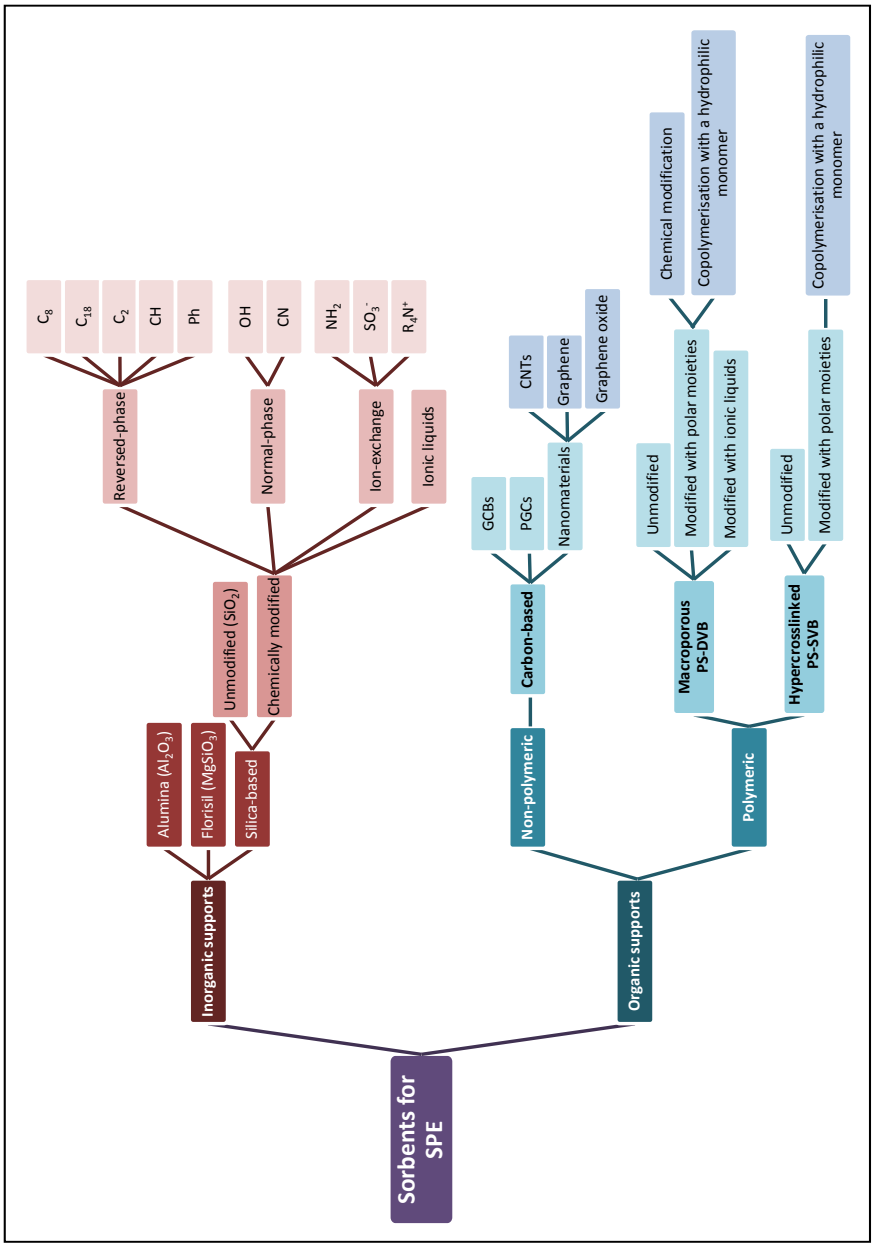


Figure 4. Main classification of the SPE sorbents depending on their chemical properties

SPE applications, due to the numerous modifications and functionalities, their main drawback is the narrow pH stability range and the presence of residual silanol groups that can have a negative effect on the retention of certain analytes [115-118]. While alumina or florisil sorptive materials have mainly been used to retain interferences and elute analytes of interest in food or environmental analysis, the chemically-bonded silica sorbents have focused on extracting organic contaminants (pesticides, PAHs and hormones, among others) from complex matrices [52,67,115,116,125-128].

With regard to organic based sorbents, another generation of sorbents that displayed greater affinity to more polar compounds was the carbon-based SPE sorbents, such as graphitised carbon blacks (GCBs) and porous graphitic carbons (PGCs). The GCBs are non-porous with a moderate specific surface area of about 100 $m^2\ g^{-1}$, and are considered to be both reversed-phase and anion-exchange sorbents, due to the presence of positively charged chemical heterogeneities on their surface. However, GCBs present a high retentive behaviour leading to difficult desorption [114,118,129]. Some commercial GCBs are available, such as Carbopack, Carbograph 4, Envi-Carb, and they have been applied for extracting phenolic compounds, pesticides and their degradation products [128,129]. PGC is a porous graphitic carbon immobilised on a silica substrate with a higher stability than GCBs and with greater retentive behaviour than C_{18} sorbents, with the compounds being adsorbed by both hydrophobic and electrostatic interactions [129,130]. As an example, Hennion et al. [129] reported the extraction of a group of polar pesticides from municipal water samples using C_{18} bonded silica and GCB sorbents under the same SPE conditions. The results demonstrated the low capacity of the C_{18} bonded silica sorbent with low recovery values of the analytes (mostly between 3 and 55%) in comparison with the GCB sorbent, which provided recovery values higher than 75%. Another study was reported by Bruzzonitti et al. [131], who evaluated different SPE cartridges (C_{18} bonded silica, CN bonded silica, Ph bonded silica, polystyrene-divinylbenzene (PS-DVB) and GCB sorbents) for extracting a group of herbicides from drinking waters. After the percolation of 1 L of ultrapure water through all five sorbents, the best results were gained with the C_{18} bonded silica and PS-DVB sorbents due to their ability to establish hydrophobic and π-π interactions, respectively. In this study, GCB sorbent provided excellent recovery values for the more acidic compounds due to the presence of oxygen complexes on the sorbent surface, which enabled stronger interactions. However, the most apolar compounds failed to be retained onto this sorbent. In the end, as a compromise between the recovery values of all the studied analytes, C_{18} bonded silica was the selected sorbent. In relation to carbon-based sorbents, more recently, carbon nanotubes (CNTs), graphene and graphene oxide have attracted a special attention for their unique chemical and physical properties being exploited in biotechnology field for biosensors, energy storage and

industrial manufacturing processes, as well as in sample preparation as advanced materials [132,133]. In the last few years, these nanomaterials have experienced increased usage as sorbents for SPE [132]. CNTs are tubes of graphitic carbon, which could also be described as graphene sheets in the shape of a cylinder. In contrast, graphene and graphene oxide are new forms of carbonaceous materials with a thickness of a single layer or a few layers. These novel carbon-based materials offer several advantages for SPE applications: high specific surface area for their nanostructure, ability to establish π-π interactions, high mechanical, thermal and chemical stability and the possibility of being functionalised. For these reasons, the use of CNTs, graphene and graphene oxide as SPE sorbents has increased for trace analysis of both inorganic and organic compounds in food, biological and environmental samples [132,134,135]. With respect to CNTs, Niu et al. [136] and Al-Degs et al. [137] agreed that multi-walled CNTs outperformed C_{18} bonded silica and GCBs sorbents for the preconcentration of several highly polar compounds (antibiotics, sulphonamides and phenolic compounds) and pesticides from water samples, respectively. In addition, graphene provided better SPE performance than using GCB and C_{18} bonded silica sorbents for the preconcentration of chloramphenicol in aquatic products [138]. The authors highlighted that the singular properties of graphene (high specific surface area) improved the sorption ability of the analyte.

As well as silica- and carbon-based sorbents, porous polymeric resins emerged in an attempt to overcome the disadvantages of previous materials, showing wider pH stability, higher specific surface area, no presence of troublesome silanol groups and reversible adsorption of the analytes of interest. As in the case of silica-based sorbents, reversed-phase, normal-phase and ion-exchange polymeric materials for SPE are available. The conventional polymeric sorbents applied in SPE are macroporous crosslinked resins based on PS-DVB with a hydrophobic structure with a specific surface area up to 500 $m^2\ g^{-1}$, obtained by suspension polymerisation. Their hydrophobic structure interacts with the analytes through Van der Waals forces and π-π interactions of the aromatic rings. In particular, the numerous π-π sites present in the macroporous PS-DVB sorbents leads to greater analyte retention than with silica-based sorbents. With this in mind, one way to increase the capacity of these hydrophobic sorbents was achieved by increasing the content of the crosslinking agent during the synthesis. This resulted in macroporous PS-DVB sorbents with higher specific surface areas (up to 800 $m^2\ g^{-1}$) and, as a result, a large number of π-π interactions. Despite the early improvements in polymeric sorbents for SPE, these hydrophobic macroporous PS-DVB displayed poor capacity and low retention of polar compounds [68,116,139,140]. Since their introduction, several commercial polymeric sorbents have become available: Amberlite XAD-1, Amberlite XAD-2, Amberlite-XAD-4 and Amberlite XAD-16 (with

specific surface area between 100 and 800 m^2 g^{-1}) supplied by Rohm & Haas; Amberchrom 161 (720 m^2 g^{-1}) supplied by Supelco, PLRP-S-10 and PLRP-S-30 (500 and 375 m^2 g^{-1}, respectively) supplied by Polymer Laboratories; Strata SDB-L (500 m^2 g^{-1}) supplied by Phenomenex, Bond Elut LMS (no data) and Bond Elut ENV (~700 m^2 g^{-1}) supplied by Agilent Technologies, AttractSPE™ DVB (600 m^2 g^{-1}) supplied by Polyintell and Isolute 101 (500 m^2 g^{-1}) supplied by Biotage [139]. Although the behaviour of these polymeric sorbents is mainly hydrophobic, they have displayed acceptable capability for extracting low and moderate polar organic contaminants, mainly aromatic (including phenols, pesticides and some PCPs), from complex matrices in comparison to previous SPE sorbents [124,141,142].

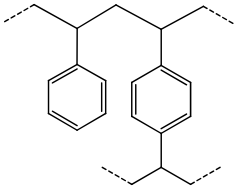
As described above, the specific surface area and extraction capacity of a polymer are closely related: the higher the specific surface area, the more accessible the aromatic rings are to interact with the analytes, resulting in efficient extraction capacity [139,143]. As increasing the content of crosslinking agent was not enough because it resulted in accessibility problems, in 1970, Davankov and Tsyurupa [144] introduced a new polymerisation method to obtain hypercrosslinked networks, consisting of a post-crosslinking of linear or lightly crosslinked polystyrene using a crosslinking agent and a Friedel-Craft catalyst, with a higher specific surface area (up to 1000 m^2 g^{-1}) [145,146]. However, a few years later, Jeřábek et al. [147] proposed the use of a commercial vinylbenzyl chloride-divinylbenzene (VBC-DVB) resin as the starting material, together with appropriate solvent and Friedel-Craft catalyst, to obtain hypercrosslinked resins. With this approach, the hypercrosslinked network contained the original pores from the precursor as well as those resulting from the crosslinking process [147]. Along the same lines, a further step was developed by Sherrington's group [148,149], who suggested the synthesis of structurally well-defined gel-type and macroporous VBC-DVB precursor resins. In addition, they demonstrated that this approach can be applied to different synthetic routes, such as suspension polymerisation [148,149], precipitation polymerisation [150] and non-aqueous dispersion polymerisation [151], resulting in differences in particle size and providing better packing of the sorbent and more efficient interactions between the analytes and the resin. Once the precursor resin was obtained and combined with a suitable Friedel-Craft catalyst, the resulting hypercrosslinked resins presented a highly porous structure with a bimodal distribution of pore sizes. Thus, the smaller the pore size synthesised, the higher the specific surface obtained. The high specific surface area (up to 2000 m^2 g^{-1}) and the high hydrophobicity make the hypercrosslinked resins perfect sorbents for SPE of apolar and moderately polar compounds with a higher sorption capacity than the conventional polymeric sorbents [148,151].

To demonstrate this, hypercrosslinked resins have been commercialised by several manufacturers since they began, such as the Hypersol-Macronet™ sorbents (1200 $m^2 g^{-1}$, Purolite International), Amberchrom GC-161m (900 $m^2 g^{-1}$, Tosohaas), Lichrolut EN (1200 $m^2 g^{-1}$, Merck), Supelclean™ Envi-Chrom P (900 $m^2 g^{-1}$, Supelco), Chromabond HR-P (1200 $m^2 g^{-1}$, Macherey-Nagel), HySphere-SH (>1000 $m^2 g^{-1}$, Spark Holland) and Bakerbond SDB-1 (915 $m^2 g^{-1}$, J.T. Baker), among others. Commercial macroporous and hypercrosslinked resins applied as SPE sorbents are listed in Table 3. All of these hypercrosslinked resins exhibit high specific surface area, becoming desirable for extracting pesticides [152,153], phenols [154-156], pharmaceuticals [27], sweeteners [157,158], UV filters [159] and volatile organic compounds (VOCs) [153], mainly extracted from environmental matrices. Of the abovementioned commercial hypercrosslinked sorbents, Lichrolut EN and Bakerbond SDB-1 have been the most widely applied, due to their high specific surface area and, as a result, their high hydrophobicity. As an example, Culleré et al. [160] studied the selectivity and the efficiency of several SPE sorbents for extracting VOCs from aqueous samples. Several sorbents were evaluated: macroporous polymeric sorbents (Bond Elut LMS, Bond Elut ENV, Bond Elut PPL), hydrophobic hypercrosslinked sorbents (Lichrolut EN, SupelClean™ Envi-Chrom P), hydrophilic hypercrosslinked sorbents (Isolute ENV+, Bond Elut Plexa) and six ion-exchange sorbents. Although this study included acidic, basic and neutral compounds, the authors concluded that interactions between the analytes and the sorbents were mainly hydrophobic, with Lichrolut EN being selected for its high retention capability for nearly all of the analytes. Another study shows the effectiveness of using a hypercrosslinked sorbent for extracting sweeteners from environmental matrices [157]. In this work, the hydrophobic hypercrosslinked sorbent Bakerbond SDB-1 was selected after comparing it with other sorbents, such as ion-exchange and C_{18} bonded silica sorbents, providing good recovery values (56-96%) when 50 mL of tap water was loaded. In other research, the SupelClean™ Envi-Chrom P was used to determine chlorinated phenolic compounds in river waters. Due to the highly crosslinked structure of the sorbent, successful recovery values for these polar compounds (95-100%) were obtained after percolating 50 mL of river water [155].

Apart from commercial hypercrosslinked sorbents, advances in the development of improved hypercrosslinked resins have been achieved in the laboratories. Some examples have been reported by Fontanals et al. [148,161], who synthesised several hypercrosslinked resins with different properties. In one of these studies [161], three hypercrosslinked resins with different specific surface areas (880-1320 $m^2 g^{-1}$) were synthesised by precipitation polymerisation, before being compared to a commercial hypercrosslinked sorbent, Lichrolut EN (1200 $m^2 g^{-1}$, Merck), for on-line SPE of polar pollutants, including phenols and pesticides, from water samples. The results showed

the high extraction efficiency and capacity of the in-house hypercrosslinked resins for the almost complete extraction of the polar compounds from 500 mL of water samples (recovery values between 74% and 105%) in comparison to the commercial Lichrolut EN (recovery values between 41% and 88%). The great success of these hypercrosslinked resins as SPE sorbents was attributed to the small and spherical microspheres with a low micrometre range (~4 μm) of these resins, resulting in better SPE packing and improved extraction efficiencies.

Table 3. Structure, surface areas and suppliers of the most common commercially available macroporous and hypercrosslinked resins with a hydrophobic behaviour used as SPE sorbents.

Structure		Sorbent	Surface area ($m^2 g^{-1}$)	Supplier
MACROPOROUS	 Polystyrene-divinylbenzene (PS-DVB)	Amberlite XAD-1	100	Rohm & Haas
		Amberlite XAD-2	300	
		Amberlite XAD-4	≥750	
		Amberlite XAD-16	800	
		Amberchrom 161	720	Supelco
		PLRP-S-10	500	Polymer laboratories
		PLRP-S-30	375	
		Bond Elut LMS	No data	Agilent Technologies
		Bond Elut ENV	700	
		Strata SDB-L	500	Phenomenex
		AttractSPE™ DVB	600	Polyintell
		Isolute 101	500	Biotage
HYPERCROSSLINKED		Amberchrom GC-161m	900	Tosohaas
		Hypersol-Macronet™ sorbents	~1200	Purolite International
		Lichrolut EN	1200	Merck
		Supelclean™ Envi-Chrom P	900	Supelco
		Chromabond HR-P	1200	Macherey-Nagel
		HySphere-SH	>1000	Spark Holland
		Bakerbond SDB-1	915	J.T. Baker

In light of the above, it has been demonstrated that hypercrosslinked sorbents present good extraction efficiencies for a variety of compounds with different chemistries as well as successful capacity in terms of percolating high sample volumes. The main reason for this success can be attributed to the presence of aromatic rings in the analytes' structures, with π-π interactions being the main mechanisms between the sorbent and the analytes. Even though, with an aromatic ring, the analyte exhibits polar functional groups in its structure, it could only be retained onto the sorbent through hydrophobic interactions. Therefore, the absence of hydrophilic interactions leads to poor retention of highly polar compounds. In addition, their hydrophobic behaviour promoted the retention of organic interferences from complex matrices. For these reasons, more polar and selective sorbents are required for SPE. In the following section, the main approaches for obtaining more hydrophilic SPE sorbents are presented and discussed, including both commercial and in-house types.

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

1.1.1. Hydrophilic polymeric materials for solid-phase extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

To overcome the high hydrophobicity of previous sorbents, the introduction of polarity into polymeric resins has emerged as a new research field in the development of novel materials for solid-phase extraction (SPE) in order to enhance polar interactions between the analyte and the sorbent. To achieve the desired polarity in a polymer, two strategies have been proposed: chemical modification of a hydrophobic polymer with a polar moiety and copolymerisation of monomers containing suitable polar functionalities [68]. The introduction of polarity has been accomplished in macroporous resins and, subsequently, in hypercrosslinked resins. In this section, there is a discussion of these two strategies widely used in the synthesis of both commercial and in-house materials as SPE sorbents and their applications in different research fields.

Chemically modified polymeric sorbents

The first approach for obtaining hydrophilic polymeric sorbents consists of the chemical post-modification of a polystyrene-divinylbenzene (PS-DVB) resin (macroporous or hypercrosslinked) with different polar functional groups, mainly using the Friedel-Craft reaction. A schematic representation of this synthetic approach is detailed in Figure 5. This strategy allows polymeric materials to be obtained with the optimal morphological properties of the precursor, which can be previously optimised. However, a limitation of these resins is the low degree of modification because of the restricted accessibility of the functional group in the crosslinking matrix [68,140].

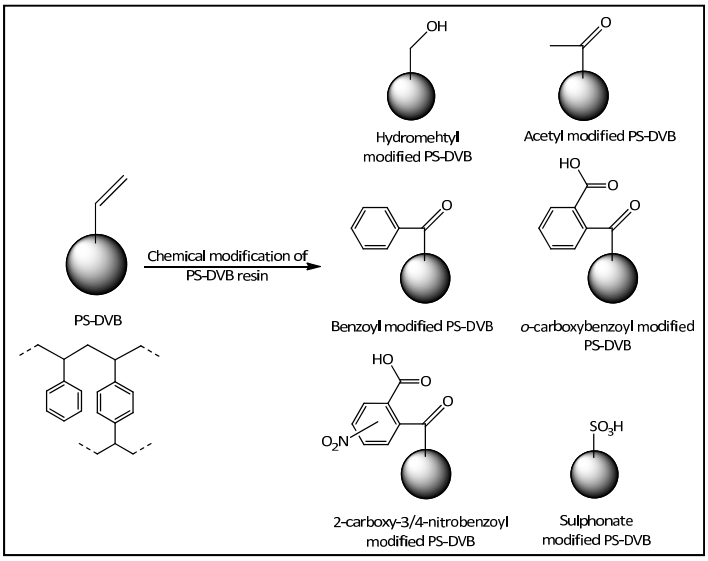


Figure 5. Scheme of some chemical modifications of PS-DVB resin.

Several reviews and books [115,124,162,163] have reported the numerous in-house hydrophilic polymeric sorbents synthesised by our research group and Fritz's group. In their studies, hydrophilic functional groups, such as acetyl, hydroxymethyl, benzoyl, *o*-carboxybenzoyl, 2,4-dicarboxybenzoyl, 2-carboxy-3/4-nitrobenzoyl and sulphonate were chemically introduced into a PS-DVB backbone. As an example, Masqué et al. [164] chemically modified a hypercrosslinked PS-DVB resin (Amberchrom GC-161m, 900 $m^2 g^{-1}$) with a benzoyl group and subsequently compared it to several macroporous (PLRP-S, 375-500m^2 g^{-1}) and hypercrosslinked (Supelclean™ ENVI-Chrom P (900 m^2 g^{-1}) and Lichrolut EN (1200 m^2 g^{-1})) polymeric sorbents for the on-line SPE enrichment of pesticides and phenolic compounds from environmental waters. The results demonstrated the high capacity of the sorbent, loading up to 100 mL of water samples. In addition, as expected, the synthesised sorbent provided higher recovery values than the unmodified macroporous PLRP-S sorbent for its lower specific surface area. However, comparing it to the hypercrosslinked Supelclean™ ENVI-Chrom P sorbent, better results were obtained using the modified sorbent, underlining the presence of a polar functional group which enhanced the retention of the studied analytes. Eventually, even though Lichrolut EN presented the highest specific surface area, similar recovery values were achieved using the synthesised sorbent.

In all instances, these hydrophilic polymeric sorbents showed more efficient extractions of polar compounds than the conventional hydrophobic resins, due to the presence of polar functional groups that allowed hydrophilic interactions, apart from the π-π interactions inherent in the PS-DVB structure.

Another interesting approach in terms of chemically modified resins is introducing polar and, at the same time, selective functional groups, such as crown ethers. Along these lines, Lee et al. [165] chemically modified the macroporous resin Amberlite XAD-4 with 4-carboxybenzo-18-crown-6 for extracting three adrenal hormones and neurotransmitters (dopamine, epinephrine and norepinephrine) from urine, exhibiting great SPE performance (recovery values higher than 86%) when 10 mL of urine sample was percolated. It was demonstrated that the structural selectivity of the crown ether played an important role on the extraction of the studied analytes, as did the polar interactions between the analytes and the functional group, through hydrogen bondings. The chemical modification of the macroporous resin Amberlite XAD-4 with polar functional groups has been used as chelating resin to be applied for the SPE of trace elements from water samples. For instance, Dave et al. [166] chemically modified an Amberlite XAD-4 with a monoaza dibenzo 18-crown-6 ether resin to be evaluated for the preconcentration of rare earth elements (lanthanum (III), neodymium (III) and samarium (III)) from 50 mL aqueous solutions. More recently, Karadas et al. [167] also

functionalised the resin Amberlite XAD-4 with 2,6-pyridinedicarboxaldehyde for the on-line SPE of cadmium(II), cobalt(II), copper(II), lead(II) and manganese(II) ions from 10 mL of environmental waters.

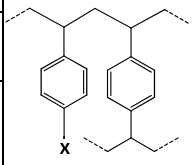
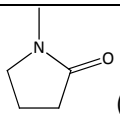
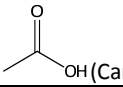
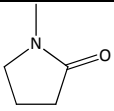
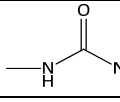
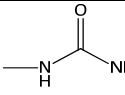
Due to the fact that several studies have reported the synthesis and application of in-house chemically modified resins and the improvement observed in the extraction of polar contaminants, the commercialisation of different chemically modified sorbents for SPE emerged, including both modified macroporous and hypercrosslinked resins: Isolute ENV+ (1100 $m^2 g^{-1}$) developed by International Sorbent Technology (IST), Strata-X (~800 $m^2 g^{-1}$) developed by Phenomenex, Bond Elut Plexa (550 $m^2 g^{-1}$) developed by Agilent Technologies, Supel-Select HLB (~400 $m^2 g^{-1}$) developed by Supelco, Spe-ed Advanta (no data) developed by Applied Separations, Cleanert PEP (600 $m^2 g^{-1}$) developed by Bonna-Agela Technologies, HyperSep Retain PEP (550-750 $m^2 g^{-1}$) developed by Thermo Scientific and ExtraBond UU and EB2 (850 $m^2 g^{-1}$ and 700 $m^2 g^{-1}$, respectively) developed by Scharlab. The main properties of these sorbents are included in Table 4.

Of the abovementioned sorbents, it is worth highlighting the use of Isolute ENV+, Bond Elut Plexa and Strata-X as sorbents for SPE of a large number of polar contaminants from biological, food and environmental samples [139]. In particular, Isolute ENV+, which is hypercrosslinked PS-DVB resin chemically modified with hydroxylated groups, has been applied for extracting polyfluorinated alkyl substances from air [168], polar pesticides from environmental waters [169] and phenolic compounds from environmental samples [170]. In the latter study, it should be noted that Isolute ENV+ was selected as an improved SPE sorbent for phenols in comparison to unmodified PS-DVB and silica-based sorbents. More recently, Metafa et al. [171] developed a comparative study of seven sorbents for SPE of volatile aroma compounds from wine samples [171]. Of the sorbents tested (conventional PS-DVB, C_{18} bonded silica, hydrophilic and ion-exchange polymeric sorbents), Isolute ENV+ was selected, as it yielded satisfactory recovery values for the whole range of the target compounds, with similar results to those using the hydrophilic Oasis HLB sorbent (see the section "Copolymerisation with a hydrophilic monomer" for further details). These results can be attributed to the high specific surface area and the presence of hydroxylated groups in the Isolute ENV+.

With regard to Strata-X, pharmaceuticals, personal care products (PCPs), pesticides, illicit drugs and their metabolites were extracted from environmental and biological samples using this hydrophilic SPE sorbent [20,172-177]. Although Strata-X had a lower specific surface area than Isolute ENV+, the polymeric skeleton with the incorporation of pyrrolidone groups exhibited good retention of polar compounds and high capacity due

to hydrophobic, hydrogen-bonding and aromatic interactions. As an example of the performance of Strata-X, Bjørk et al. [177] proposed fully automated SPE using Strata-X followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the successful quantification of 31 illicit drugs and metabolites in blood. The large number of analytes with different polarities and functionalities demonstrated the high versatility of this sorbent, providing recovery values from 35% to 101%.

Table 4. Structure, surface areas and suppliers of commercially available chemically modified polymeric sorbents for SPE.

Sorbent name	Sorbent structure		Surface area ($m^2 g^{-1}$)	Supplier
	Polymer based	Chemically modified with (X)		
Isolute ENV+		—OH (Hydroxyl)	1100	International Sorbent Technology
Strata-X		 (Pyrrolidone)	~800	Phenomenex
Bond Elut Plexa		—OH (Hydroxylated and amide free surface)	550	Agilent Technologies
Supel-Select HLB		n.d.	~400	Supelco
Spe-ed Advanta		 (Carboxyl) [a]	n.d.	Applied Separations
Cleanert PEP		 (Pyrrolidone)	600	Bonna-Agela
HyperSep Retain PEP		 (Urea)	550-750	Thermo Scientific
Extrabond UU		n.d.	850	Scharlab
Extrabond EB2		 (Urea)	700	

n.d.: no data
[a] Characterised in [178]

Another chemically modified sorbent is Bond Elut Plexa. Despite its commercialisation, this sorbent has been less applied than those mentioned above. As the manufacturers indicate, Bond Elut Plexa has an apolar PS-DVB core with

hydroxylated and amide-free surface. These properties make the sorbent suitable for the enrichment of PCPs from river waters [23], bisphenol-A and its metabolites from rat plasma samples [179] and 11 antibiotics and their metabolites from human urine samples [180]. Although few applications found in literature, it is important to mention that the Bond Elut Plexa has also been included in comparative studies of different sorbents for SPE of sweeteners [181] or a group of emerging organic contaminants (EOCs) (tetracyclines, sulphonamides, analgesics and hormones) [182] from water samples. However, other sorbents included in these studies provided better results than the Bond Elut Plexa.

Supel-Select HLB, Spe-ed Advanta and Cleanert PEP are less commonly applied commercial chemically modified sorbents, the properties of which are unknown. For this reason, few applications report the use of these sorbents. With respect to Spe-ed Advanta, Sirvent et al. [178] used this SPE sorbent to enrich phenolic compounds from water samples (up to 100 mL), providing satisfactory results (recovery values between 70% and 100%), even better than those obtained using Isolute ENV+. In reference to Supel-Select HLB, no detailed information about the hydrophilic functionalities has been reported by the manufacturer. To the best of our knowledge, there is one study, in which Supel-Select HLB is evaluated together with other sorbents for extracting illicit drugs from surface water samples [183]. However, this sorbent was not selected, due to the low recovery values compared to the other sorbents. A possible explanation of the unsuccessful performance can be attributed to its low specific surface area (~400 $m^2 g^{-1}$). Cleanert PEP is also a less commonly applied sorbent, just like the two previously described SPE materials. In the literature, a study reports the determination of organophosphorus pesticides in underground waters (500 mL) by SPE and gas chromatography-mass spectrometry (GC-MS) [184]. When this sorbent was compared with two classical C_{18} bonded silica sorbents, better results were achieved using the Cleanert PEP because it has both hydrophobic and hydrophilic properties. Lastly, to best of our knowledge, no applications have reported the use of HyperSep Retain PEP, ExtraBond UU and ExtraBond EB2 as SPE cartridges.

Copolymerisation with a hydrophilic monomer

This strategy has been widely applied for obtaining both commercial and in-house hydrophilic copolymers as SPE sorbents. The procedure is easy to follow and consists of the copolymerisation of a hydrophilic monomer with a crosslinking monomer, usually divinylbenzene (DVB), which enables a final copolymer to be obtained with both hydrophobic and hydrophilic functionalities that can interact strongly with the analytes

[68,140]. Figure 6 shows some examples of both commercial and in-house hydrophilic copolymeric sorbents for SPE.

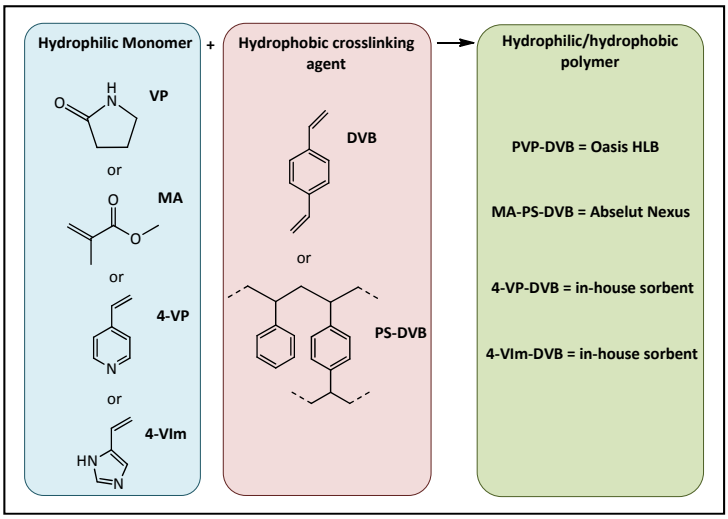
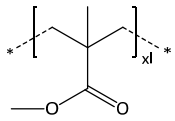
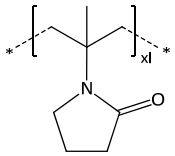
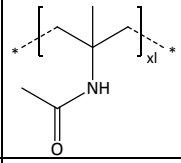


Figure 6. Scheme of some examples of commercial and in-house polar sorbents for SPE obtained by copolymerisation with a hydrophilic monomer.

Since they first emerged, several hydrophilic sorbents for SPE obtained by copolymerisation have been commercialised: Amberlite XAD-7 (450 m^2 g^{-1}) and Amberlite XAD-8 (310 m^2 g^{-1}) supplied by Rohm and Haas, Oasis HLB (830 m^2 g^{-1}) and Porapak RDX (550 m^2 g^{-1}) supplied by Waters, Abselut Nexus (or Bond Elut Nexus, 575 m^2 g^{-1}) supplied by Agilent Technologies, Bakerbond Speedisk H_2O-Philic DVB (no data) supplied by J.T. Baker, Discovery DPA-6S (no data) supplied by Supelco and AttractSPE™ W/O (800 m^2 g^{-1}) supplied by Polyintell. Their principal characteristics are presented in Table 5.

Of these sorbents, the most widely-used commercial hydrophilic sorbent obtained by copolymerisation is Oasis HLB from Waters, which has a macroporous poly(*N*-vinylpyrrolidone-DVB) (PVP-DVB) structure. A large number of studies have demonstrated the extensive applicability and versatility of Oasis HLB in the extraction of several polar EOCs from a variety of complex matrices. Thus, pharmaceuticals, PCPs, sweeteners and illicit drugs, among others, have been extracted from environmental, biological and food samples using Oasis HLB [31,181,185-190]. In addition, not only has this sorbent been used to enrich and clean up the analytes from a matrix, but it has also been used to clean up biological samples and, in particular, environmental solid samples to eliminate interfering compounds [15,37,127,191,192].

Table 5. Structure, surface areas and suppliers of commercially available hydrophilic copolymeric sorbents for SPE.

Sorbent name	Copolymer structure	Surface area ($m^2 g^{-1}$)	Supplier
Amberlite XAD-7	 Methacrylic acid-divinylbenzene (MA-DVB)	450	Rohm & Haas
Amberlite XAD-8		310	
Abselut Nexus (or Bond Elut Nexus)		575	Agilent Technologies
Oasis HLB	 Poly(*N*-vinylpyrrolidone-divinylbenzene) (PVP-DVB)	830	Waters
Porapak RDX		550	
Bakerbond Speedisk H_2O-Philic DVB	n.d.	n.d.	J.T. Baker
Discovery DPA-6S	 Polyamide	n.d.	Supelco
AttractSPE™ W/O	n.d.	800	Polyintell

n.d.: no data

Oasis HLB has also been included in comparative studies, in which its high capacity and retention towards polar compounds have been demonstrated [181,183,193-197]. As an example, Gros et al. [194] developed a multi-residue analytical methodology for determining 29 pharmaceuticals, including lipid regulators, non-steroidal anti-inflammatory drugs (NSAIDs) and psychiatric drugs, among others, from environmental waters. In this study, two hydrophilic polymeric sorbents (Oasis HLB and Isolute ENV+), a C_{18} bonded silica sorbent (Isolute C_{18}) and an ion-exchange sorbent were evaluated. After percolating 500 mL of river water sample, the best SPE performance was achieved using the Oasis HLB, with recovery values ranging from 70% to 110%. Despite its high hydrophobicity, Isolute C_{18} provided slightly lower results than using Oasis HLB. In contrast, due to the fact that many compounds with different functionalities and polarities were analysed, Isolute ENV+ and the ion-exchange sorbent failed because these sorbents are more specific for highly polar and basic compounds, respectively. These results demonstrated the suitable balance between the hydrophobic and hydrophilic interactions that Oasis HLB established with the analytes.

Another comparative study between 11 different SPE sorbents was developed to enrich a group of pharmaceuticals (antibiotics, anthelmintics and glucocorticoids) from 100 mL of surface water sample [197]. In this study, polymeric (Oasis HLB, SampliQ OPT, SampliQ Polymer SCX, Strata SDB-L and Strata-X) and silica-based (Sep-Pak C_{18}, SampliQ C_8, SampliQ C_8/Si-SCX, SampliQ C_{18}, Strata C_8 and Strata C_{18}E) SPE sorbents were tested, being capable of establishing hydrophobic and/or ion-exchange interactions. The comparison of the recovery values obtained using the different SPE sorbents revealed the better performance and retention capacity of the polymeric sorbents in comparison to silica-based sorbents, with the latter displaying low recovery values for certain compounds. Of the polymeric sorbents, better SPE performances were provided by Strata-X (recovery values > 95%), Strata SDB-L (recovery values > 86%) and Oasis HLB (recovery values = 99-104%), which can be attributed to the presence of DVB, which promotes the π-π interactions, enhancing the retention of the studied analytes. Since Oasis HLB was demonstrated to be the most suitable sorbent with the highest recovery values for the selected pharmaceuticals, it was chosen for the method application.

The following study also tested several SPE sorbents for the enrichment of ten polar pharmaceuticals from 1 L of tap water sample [193]. The selected sorbents were Abselut Nexus, Focus, Bakerbond SDB, Bond Elut PPL and Bond Elut ENV as polymeric sorbents, and Bond Elut Certify and Bond Elut C18 LO as silica-based sorbents. Of these sorbents, Bond Elut Certify and Bond Elut C18 LO yielded the lowest recovery values due to the lower retention of sorbents towards polar pharmaceuticals as well as for the absence of π-π interactions. With respect to the polymeric sorbents, Bond Elut PPL and Oasis HLB provided the best results in terms of recovery values in 1 L of real water sample. Although Bond Elut PPL is a hydrophobic PS-DVB polymer modified with a proprietary apolar surface, this sorbent and Oasis HLB allowed similar extraction efficiencies for most of the compounds, 42-104% and 48-106%, respectively. In particular, for some compounds, the results obtained with these two sorbents were complementary, with some compounds being better recovered using Bond Elut PPL than using Oasis HLB, and vice versa. However, Oasis HLB was selected since it was able to recover nearly 100% of most of the studied compounds.

As mentioned above, Oasis HLB has also been applied as a clean-up sorbent when environmental solid samples are analysed, such as soil, sediments or sludge. In the next study, Azzouz et al. [191] presented an analytical methodology for determining 22 pharmaceuticals, PCPs and hormones from different types of environmental solid samples using microwave-assisted extraction (MAE) followed by SPE and then detected by GC-MS. Once the MAE conditions were optimised, the SPE was evaluated to remove as many interferences as possible. In view of this, several SPE sorbents (Amberlite XAD-

2, XAD-4, Oasis HLB, Lichrolut EN and RP-C_{18}) were tested to perform the clean-up of the MAE extracts. Of these sorbents, Oasis HLB provided the best performance in terms of recovery values, breakthrough volumes and reduction of interfering compounds, which could be achieved by loading the MAE extract adjusted to pH 7. The whole MAE/SPE/GC-MS/MS analytical procedure provided successful recovery values (91-101%) for 1 g of each sample extracted.

Apart from Oasis HLB, other commercial hydrophilic polymeric sorbents were also applied in different research fields, but to a lesser extent. For instance, the sorbent Abselut Nexus, which is based on polystyrene crosslinked with 50% divinylbenzene and poly(methylmethacrylate), has demonstrated its ability to extract isoflavones, methadone and benzodiazepines from biological samples [198,199], antibiotics from food samples [200,201] and pesticides from environmental waters [202]. Of the cited studies, the high capacity of Abselut Nexus is demonstrated in the latter study [202], in which large volumes of surface water samples (100 L) were percolated through 8 g cartridges of this polar resin for determining 41 agricultural pesticides. The authors evaluated the SPE performance obtained using Abselut Nexus, C_{18} bonded silica, Amberlite XAD-2 and Bond Elut PPL sorbents. Both Bond Elut PPL and Abselut Nexus yielded similar average recovery values. In the end, Abselut Nexus was the chosen sorbent, due to its high capacity and good and more reproducible recovery values (52-141%). Due to the high sample volume loaded, many water matrix interferences were coextracted. Therefore, the authors applied a subsequent multi-solvent clean-up of the SPE extract using a silica sorbent.

Another sorbent also applied for the extraction of polar contaminants was Bakerbond Speedisk H_2O-Philic DVB. Although its structure is unknown, this sorbent has recently been used as an SPE disk for extracting antibiotics from seawaters (1.5 L), displaying great SPE performance with high recovery values [203]. Porapak RDX (550 m^2 g^{-1}) is another hydrophilic polymeric sorbent, which has the same structure as Oasis HLB (830 m^2 g^{-1}), but it has a lower specific surface area. This fact could explain the few applications found using this sorbent. In contrast, Krogh et al. [204] studied several SPE sorbents (Porapak RDX, Isolute ENV+, Oasis HLB and C_{18} silica-based sorbents) to enrich a group of surfactants from environmental water samples (1 L). After comparing them, the authors concluded that Oasis HLB and Porapak RDX were the most suitable for retaining these analytes. However, they eventually chose Porapak RDX because Oasis HLB tended, in several cases, to clog during loading. Moreover, Porapak RDX was included in a comparative study for preconcentrating phenolic compounds from wastewater samples [205]. In this study, Porapak RDX was compared to Lichrolut EN

(1200 $m^2 g^{-1}$) and Isolute ENV+ (1100 $m^2 g^{-1}$), with the latter being selected due to their higher specific surface area and capacity.

With regard to Discovery DPA-6S, it is based on polyamide with an extremely low specific surface area. Few studies report the use of this sorbent, which has been included in comparative studies, such as for the extraction of phenolic compounds and steroids from water samples [196]. Of the sorbents tested, Strata-X and Discovery DPA-6S provided similar recovery values. However, Oasis HLB was the sorbent of choice, showing the best recovery values (57-118%). Lastly, it is worth noting that Amberlite XAD-7 and XAD-8, based on methacrylate-divinylbenzene, have mainly been applied for extracting metal ions (iron (III), lead (II), chromium (III) and copper (II)), forming complexes, from food and water samples [206,207].

Despite the high availability of commercial hydrophilic copolymeric sorbents, the synthesis of in-house hydrophilic sorbents has attracted great interest from many research groups, with a view to enhancing the specific surface area, polarity and selectivity. In light of this, our research group published several reviews reporting both commercial and in-house polar materials as sorbents for SPE of polar contaminants from complex matrices, up to 2010 [68,139,140,143]. Most of the in-house hydrophilic copolymeric sorbents have been synthesised by our research group and the research groups of Bagheri and Trochimczuk.

Bagheri and co-workers were the first to synthesise several hydrophilic copolymeric sorbents, based on polyaniline (PANI), poly-*N*-methylaniline (PNMA), polydiphenylaniline (PDPA) and polypyrrole (PPy), for application as sorbents for SPE of phenolic compounds and polar pesticides from waters [208-211]. Although these synthesised resins presented low specific surface area (32-48 $m^2 g^{-1}$) and, as a result, low breakthrough volumes, their ability to interact with polar analytes through π-π interactions and hydrogen bondings allowed comparable recovery values to be obtained to those achieved using the commercial Lichrolut EN or Oasis HLB. It is important to highlight that the low specific surface area is due to the absence of a crosslinking monomer, such as DVB, during the synthesis.

Following similar steps, Trochimczuk's research work used different hydrophilic monomers, such as acrylonitrile (AN), methacrylonitrile (MAN), cyanomethylstyrene (CMSt), vinylnaphtalene (VN) and vinylbiphenyl (VPh), combined with a crosslinking monomer (DVB or divinylbiphenyl (DVPh)) [212-215]. These hydrophilic resins displayed higher specific surface areas (300-700 $m^2 g^{-1}$) than those synthesised by Bagheri and co-workers, as a result of the presence of DVB monomer as a crosslinking agent. All of

these resins were evaluated in terms of sorption properties towards phenolic compounds and derivatives in aqueous samples through batch experiments. With respect to AN-DVB, MAN-DVB and CMSt-DVB resins, the authors concluded that polar and highly water-soluble compounds could be strongly adsorbed onto the resins due to the high content of nitrile groups in their structures.

Other hydrophilic copolymeric sorbents were developed by Bielicka-Daszkiewicz et al. [216], including di(methacryloyloxymethyl)naphtalene-DVB (DMN-DVB), 4,4'-bis(maleimido)diphenylmethane-DVB (BM-DVB), *p,p'*-dihydroxydiphenylmethane diglycidyl methacrylic ester-DVB (MEMDE-DVB) and *p,p'*-dihydroxydiphenylpropane diglycidyl methacrylic ester-DVB (MEDDE-DVB), for the extraction of phenol and hydroquinone from water sorbents [216]. The presence of ester, imide and hydroxyl functional groups in the resins' structures allowed suitable SPE performance for the studied compounds. However, the extraction efficiencies of these in-house sorbents were also compared to two commercial sorbents: Strata-X and Bakerbond SDB-1, with the commercial ones providing better SPE performance. This fact was attributed to the low specific surface area of the in-house hydrophilic sorbents (100 $m^2 g^{-1}$) compared to the specific surface area of the commercial sorbents (800-915 $m^2 g^{-1}$).

Improved in-house hydrophilic sorbents in terms of specific surface area and polarity were achieved by our research group. As in previous studies, DVB was also selected as the crosslinking monomer, while the polarity of the final resin was obtained with hydrophilic monomers, such as 4-vinylpyridine (4-VP), N-vinylimidazole (NVIm) and 4-vinylimidazole (4VIm) [217-219], obtaining hydrophilic resins with specific surface areas between 504 and 710 $m^2 g^{-1}$. The on-line SPE of polar contaminants, including phenolic compounds and pesticides, was successfully achieved using the in-house resins, showing comparable recovery values with other commercial sorbents (Oasis HLB, Strata-X and Lichrolut EN) when 100 mL of water samples were analysed. Furthermore, the same research group was the first to introduce polar moieties during the synthesis of hypercrosslinked resins. In this way, Bratkowska et al. [220] synthesised a hydrophilic hypercrosslinked terpolymer based on 2-hydroxyethyl methacrylate (HEMA), *para*-vinylbenzyl chloride (VBC) and DVB with a specific surface area of 850 $m^2 g^{-1}$. In this study, together with Oasis HLB, Strata-X and Lichrolut EN, the in-house resin was applied for off-line SPE of a group of polar contaminants from water samples (1 L). The results showed that the presence of hydroxyl groups in the terpolymer, as well as its high specific surface, could be responsible for obtaining similar and slightly higher recovery values than using the commercial sorbents.

To summarise the SPE performance of the discussed hydrophilic copolymeric sorbents, Table 6 shows the recovery values (%) obtained when different sample volumes of ultrapure water with phenolic compounds were percolated. As mentioned in the previous section, the first conclusion that is drawn is that the higher the specific surface area, the higher the capacity of the sorbent and, as a result, higher recovery values were achieved. A second conclusion is that the presence of polar moieties in the resins' structures really has a positive effect on the retention of phenolic compounds. As an example, for 4-nitrophenol (4-NP), the use of a commercial hypercrosslinked resin with a high specific surface area but no polar moiety (Lichrolut EN) did not provide as satisfactory recovery values as the in-house hydrophilic polymeric resins, such as HEMA-VBC-DVB and NVIm-DVB.

Table 6. Recovery values (%) obtained using different hydrophilic copolymeric resins as SPE sorbents to enrich phenol, 4-chlorophenol (4-CP) and 4-nitrophenol (4-NP) from different volumes of ultrapure water.

RESINS (mg)	Surface area (m^2 g^{-1})	Recovery values (%)			Sample volume (mL)	Ref.
		Phenol	4-CP	4-NP		
PANI (120)	48	0	62	-	200 [a)]	[210]
PNMA (120)	32	32	106	-	200 [a)]	[210]
PDPA (120)	38	0	72	-	200 [a)]	[210]
PPy (35)	40	84	94	-	25 [b), c)]	[211]
BM-DVB (200)	35	85	-	-	50 [a)]	[216]
DMN-DVB (200)	100	90	-	-	50 [a)]	[216]
MEMDE-DVB (200)	70	22	-	-	50 [a)]	[216]
MEDDE-DVB (200)	20	20	-	-	50 [a)]	[216]
4-VP-DVB (40)	710	70	-	85	100 [b)]	[217]
NVIm-DVB (40)	626	88	-	84	100 [b)]	[219]
4-VIm-DVB (40)	504	59	-	87	100 [b)]	[218]
HEMA-VBC-DVB (200)	850	86	-	91	1000 [a)]	[220]
Oasis HLB (200)	830	78	-	86	1000 [a)]	[220]
Isolute ENV+ (200)	1100	92	-	95	1000 [a)]	[220]
Lichrolut EN (200)	1200	79	-	85	1000 [a)]	[220]

-: not included in the study
[a)] Off-line SPE
[b)] On-line SPE
[c)] Results obtained in 25 mL of river water samples.

The main application of hydrophilic polymeric materials has been as classical sorbents for SPE. However, recent advances in the miniaturisation of SPE also have taken advantage of these polar materials. More recently, in 2013, Gao et al. [221] synthesised a hydrophilic copolymer based on HEMA and ethylene glycol dimethacrylate (EGDMA) to be applied as sorbent for an in-house extraction device, known as miniaturised syringe assisted extraction (mini-SAE). In this extraction device, 50 mg of sorbent were packed in the cap of the syringe needle and then the cap was coupled with the tip of the syringe. The mini-SAE was applied for extracting two antibiotics (sulphadiazine and sulphamonomethoxine) in milk. After the pretreatment of the milk by centrifugation, 1 mL of milk solution was drawn to the syringe. Once the elution was performed, successful recovery values were obtained for the two sulphonamides (>85%). It is important to mention that a washing step was included, with several organic solvents and waters being studied. Eventually, 1 mL of water was selected because the organic solvents (methanol, acetonitrile, ethyl acetate and n-hexane) caused significant analyte losses during the washing step. Moreover, this hydrophilic sorbent was compared to three commercial sorbents (Oasis HLB, C_{18} bonded silica and ion-exchange ones). The results revealed that the presence of hydroxyl and ester groups in the in-house sorbent promoted stronger interactions, mainly hydrogen bondings, with the sulphonamide functional groups of the studied analytes than using the commercial sorbents.

Throughout this section, several approaches have been presented for improving SPE performance, particularly for extracting polar contaminants. Thus, novel synthetic routes were proposed for obtaining novel SPE materials with excellent properties: high specific surface and polar functionalities. However, currently, these properties are not enough if selectivity is required. For this reason, the synthesis of more selective sorptive materials have become essential for obtaining cleaner SPE extracts that result in less interferences and, thus, improve sensitivity.

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

1.1.2. Selective materials for solid-phase extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

The current role of analytical scientists is to develop improved methodologies for the determination of organic contaminants at low levels of concentration in complex matrices. As mentioned in the Introduction, improvements in sensitivity have been accomplished with the introduction of tandem mass spectrometry (MS/MS), combining different analysers that enable both quantitative and qualitative objectives to be achieved. Additionally, significant progress has also achieved in terms of sample preparation, especially in the development of novel materials for sorptive extraction techniques. With respect to solid-phase extraction (SPE), the need for more hydrophilic sorbents for SPE with high capacity grew due to the increasing number of moderate and highly polar organic contaminants present in the environment. As demonstrated in the previous section, this demand has been successfully met over the last few years with a wide range of studies regarding the synthesis and the application of novel SPE sorbents. However, the improved morphological (high specific surface areas) and chemical properties (polar functional groups) of these novel sorbents also entailed the retention of numerous compounds with different polarities as well as the retention of interfering compounds. One way for removing organic matter and other interferences from the SPE sorbent is including a washing step, preferably using an organic solvent. However, as the interactions with this kind of sorbents are not specific, a significant decrease in the recovery values of the analytes was observed due to the disruption of these interactions between the target analytes and the sorbent. In most of the cases, the solution involves excluding this step from the SPE procedure and accepting the presence of interferences. For this reason, selectivity has become an additional requirement for the synthesis of extracting materials for SPE, as well as for other sorptive extractions techniques.

The selectivity of an SPE sorbent can be towards a selected target analyte or a group of compounds belonging to the same family. Hence, the target analyte or analytes can strongly interact with the selective sorbent, while matrix interferences will be less retained or even distinguished. To achieve selectivity, all of the parameters involved during the synthesis of a sorbent (e.g. reagents and solvents used, the type of the synthesis, etc.) play an essential role in creating selective and specific functional groups for the selected analyte or analytes. In addition, selectivity can also be enhanced during the performance of SPE. For instance, in the loading step, the adjustment of the sample pH is usually taken into account, since it allows the chargeability of the analytes to be changed (neutral or ionised) and they can then establish specific interactions with the sorbent (e.g. ion-exchange and/or hydrogen bondings). In addition, the great advantage of selective sorbents is their ability to support the clean-up of the sorbent with an organic solvent without analyte losses. The possibility of including a washing step with an organic solvent really helps to obtain elution extracts free from interferences (as much as possible) as well as better signal responses for the target analytes.

Selective SPE is especially required when liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used in order to avoid matrix effects. As detailed in the Introduction section, the matrix effect (ME) is the alteration of the ionisation efficiency of the target analytes by the presence of coeluting substances, which is noticeable if electrospray ionisation (ESI) is used as the ionisation source. Due to the widespread use of LC-(ESI)-MS/MS as the current analytical methodology of choice for quantitative analysis, several reviews have extensively described the term ME, how it can be assessed and different strategies to overcome this effect [54,56,57,222-224]. These reviews show the most common actions to eliminate or reduce the matrix effect, represented in Figure 7, which are the modification of mass spectrometry (MS) and/or chromatographic conditions, changes/improvements in extraction processes and the selection of the most suitable calibration method.

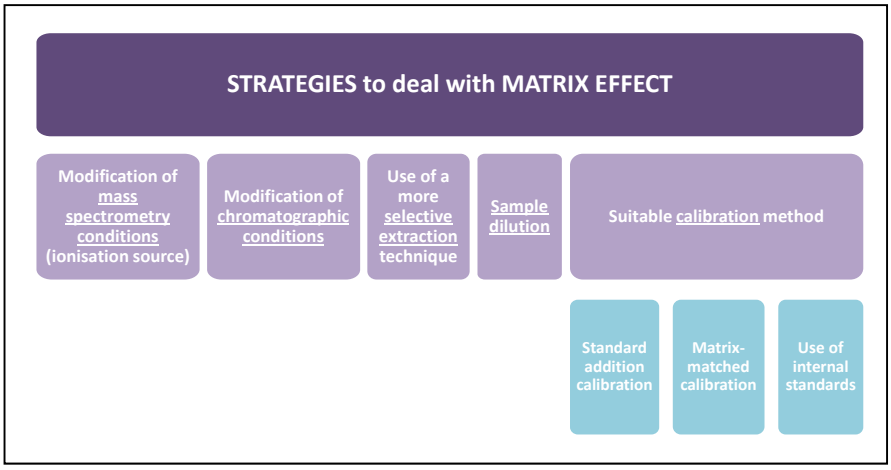


Figure 7. Scheme of the most common strategies to overcome matrix effect.

The modification of MS conditions can result in a lower matrix effect if different ionisation sources are tested. From the literature, it can be concluded that ESI is more subject to the matrix effect than atmospheric pressure chemical ionisation (APCI) because of the different mechanisms they use to produce charged analytes. Nowadays, several manufacturers commercialise multimode ionisation sources, working under ESI or APCI conditions [51]. Therefore, if possible, the effect of the ionisation source type should be evaluated. Another option for combating the ME is changing the chromatographic conditions. Interfering compounds can be eluted during the solvent front (highly polar interferences) and also eluted during the end of an elution gradient (highly retained interferences). Improving LC separation efficiency, the retention of the target analytes can be adjusted between these two areas, reducing the effect of the

matrix interferences [57,224]. Another possibility is the use of hydrophilic interaction liquid chromatography (HILIC). HILIC combines the use of bare silica or polar bonded stationary phases and mobile phases with a high content of a polar organic solvent. As a result, the most apolar interfering compounds can be easily eluted and the sensitivity of the target analytes can be enhanced due to the high presence of organic solvent, making the ionisation process easier. Moreover, its use should be tested for each individual application, since HILIC might be highly distorted by the presence of ions from the matrix [224].

More effective strategies to reduce or eliminate the ME involve the sample preparation. Sample preparation includes not only a preconcentration step but also a purification/clean-up step, with the latter being essential for lower the content of interfering compounds prior to LC-MS/MS determination. As a result, liquid-liquid extraction (LLE), protein precipitation or SPE have become popular options for purifying the extracts depending on the application field, such as environmental, biological or food analysis [224,225]. In particular for environmental analysis (both liquid and solid samples), a subsequent SPE clean-up [21,37,226,227], sample extract dilution [23,228,229] and lower sample volumes [35,181,230] are the most common procedures for tackling the matrix effect. With respect to sample dilution or loading lower sample volumes, these strategies help to overcome this effect easily but their application usually results in less sensitive method performance. In contrast, one way to preconcentrate the analytes, as well as cleaning up the sample, can be achieved by adding an SPE clean-up after the sample extraction technique. With this approach, the analytes are firstly extracted from the matrix and preconcentrated and then the sample extract is cleaned using SPE. An example of this approach was developed by Herrero et al. [226], who evaluated the matrix effect for benzotriazole, benzothiazole and benzenesulphonamide contaminants in environmental waters using SPE followed by LC-MS/MS. The authors proposed Oasis HLB as the SPE sorbent and they observed that the analytes displayed different MEs depending on the sample pH, with pH 7 being selected as optimal in terms of the matrix effect as well as recovery values. In addition, when a clean-up step of the Oasis HLB elution extract was performed through a lab-packed Florisil SPE cartridge, a significant reduction of the matrix effect was observed for all the compounds studied (between 5 and 30% of ion suppression). The Florisil cartridges enabled the retention of many interfering compounds, reducing the matrix effect. However, it did not show any affinity towards the target analytes, maintaining good recovery values. Although the application of a SPE clean-up step after the preconcentration technique usually results in a reduction of the ME, it involves the inclusion of another step in the overall analytical performance, resulting in more time-consuming and expensive methodology.

All of the above mentioned strategies usually help to reduce the ME, but in most of the cases, it cannot be completely eliminated. Consequently, an appropriate calibration method must be used to compensate this effect. The standard addition calibration method is the most effective way to compensate the alterations to the signal responses of the analytes affected by each sample. However, this approach is laborious and time-consuming because a calibration curve must be performed for each sample that needs to be analysed. Another option is the matrix-matched calibration method, in which the calibration curve is performed with spiked samples that have the same or extremely similar composition to the samples to be analysed. This approach is not as lengthy as standard addition calibration and it has been widely used to compensate the ME [222]. Lastly, the use of isotopically labelled internal standards (ISs) has becoming increasingly popular to balance out this effect. In this approach, the matrix interferences are expected to affect both the target analyte and its IS similarly, resulting in the proper correction of this effect. Unfortunately, this strategy presents some disadvantages, such as the high cost of ISs, the use of an IS for each analyte, and the lack of commercially available ISs [56,57,224].

Along these lines, suitable approaches have been presented in order to overcome ME. However, these strategies usually only reduce or compensate the problem. As mentioned at the beginning of this section, a selective sample preparation can be the key to reducing or almost eliminating ME. Thus, a specific and selective SPE sorbents can enrich the analytes and clean the matrix from interferences in the same step, making it an easy, simple, cheap and fast sample pretreatment and approach to tackle the ME. Over the last few decades, the most selective SPE performances have been achieved thanks to the use of molecularly imprinted polymers (MIPs), which present high selectivity towards a target analytes or analytes due to the presence of specific interactions between the analyte and the sorbent. These sorbents were firstly synthesised in the laboratory and caused a revolution in terms of selectivity. Several years later, some manufacturers have started to commercialise them. Another type of sorbent is mixed-mode sorbents, which provide selective SPE due to their dual-phase and ability to establish both ion-exchange and reversed-phase interactions with the analytes. Their polymeric structure with acidic or basic functional groups extends the range of analytes to be extracted from complex samples. The feature in common between MIPs and mixed-mode sorbents is their capacity for removing interferences through a washing step using an organic solvent because the analytes are retained selectively with the sorbent, representing great progress in sample preparation for overcoming ME. This section ends with an extensive review of the performance provided by selective materials for SPE, such as MIPs and mixed-mode sorbents, in environmental analysis. This review has already been published for publication in the

first volume of the journal *Trends in Environmental Analytical Chemistry* by an invitation of the Editor.

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

1.1.2.1. Selective materials for solid-phase extraction in environmental analysis

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

SELECTIVE MATERIALS FOR SOLID-PHASE EXTRACTION IN ENVIRONMENTAL ANALYSIS

Núria Gilart, Francesc Borrull, Núria Fontanals, Rosa Maria Marcé*

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

Abstract

This review describes the recent advances in the development of new sorbent materials for solid-phase extraction for improving the retention of more polar contaminants, as well as removing interferences from the matrix in environmental analysis. This overview mainly focuses on the most commonly used commercial and in-house sorbents for SPE for extracting polar pollutants from environmental samples.
There is a discussion and assessment of the enhancement in terms of selectivity provided by molecularly imprinted polymers (MIPs) and mixed-mode sorbents, and their ability to reduce the matrix effect in environmental matrices. Moreover, recent approaches in MIPs and other materials and formats of sorptive extraction techniques are discussed.

Keywords: *emerging organic contaminants; environmental waters; solid-phase extraction; molecularly imprinted polymers; mixed-mode sorbents; selectivity*

1. INTRODUCTION

The presence of emerging organic contaminants (EOCs) in the environment has mainly been caused by their incomplete removal from untreated water in sewage treatment plants (STPs). This fact has brought about the development of an extensive field of research: environmental analysis. In this area, analytical chemistry plays an essential role since it allows the identification of these contaminants, determining their concentration, establishing degradation pathways and testing their toxicity and possible human health risks [1].
Several reviews have reported the main analytical techniques, such as gas chromatography (GC) and liquid chromatography (LC), mainly followed by mass spectrometry (MS) or tandem mass spectrometry (MS/MS), used for the determination of EOCs, such as pharmaceuticals and personal care products (PPCPs), hormones, illicit drugs, polar pesticides or artificial sweeteners, in environmental samples [1,2].

Despite the availability of powerful separation and detection techniques, the analysis of complex environmental matrices requires an effective and selective sample pretreatment for facilitating the removal of interferences, obtaining cleaner extracts and, as a result, achieving a decrease in terms of method detection limits (MDLs). It is well-known that the high complexity of the analysed matrices may involve the presence of co-eluting compounds, causing matrix effect (signal enhancement or suppression). Therefore, many efforts have focused on sample preparation in order to obtain clean extracts prior to separation and detection, thereby avoiding tedious and time-consuming procedures. Over time, solid-phase extraction (SPE) and, more recently, other sorptive extraction techniques, such as stir bar sorptive extraction (SBSE) or solid-phase microextraction (SPME), have replaced the conventional liquid-liquid extraction (LLE) when environmental liquid samples are analysed. Of these, SPE has become the most popular choice for preconcentrating target analytes and cleaning the matrix. Moreover, the huge variety of commercially available sorbents and formats makes this technique suitable for the extraction of many compounds with different polarities and physicochemical properties [3].

In view of this, the aim of this review is to present the recent advances and applications of new commercially available and in-house extraction phases for the selective SPE of polar EOCs from environmental samples.

2. HYDROPHILIC POLYMERIC SORBENTS FOR SOLID-PHASE EXTRACTION

Over the last few decades, there has been growing interest in the developpment of hydrophilic polymeric sorbents for the SPE of polar compounds. Thus, the functionalisation of porous polymers, mainly based on styrene-divinylbenzene (St-DVB), with polar functionalities was required and achieved using two main strategies: the copolymerisation of monomers with the desired functionalities and the introduction of a functional group to the hydrophobic polymer. Several reviews have reported the analytical applications of these novel polymeric SPE sorbents with enhanced hydrophilicity for extracting a great variety of EOCs with different polarities from environmental samples [3,4].

Nowadays, there is an extensive variety of commercially available SPE sorbents, but the most well-known and widely used commercial hydrophilic sorbent obtained by copolymerisation is Oasis HLB from Waters. This material is a macroporous poly(*N*-vinylpyrrolidone-DVB) (PVP-DVB) copolymer with a specific surface area of ~800 m^2 g^{-1}. Since its commercialisation, Oasis HLB has become more and more popular for sample preparation due to the advantages it offers: extraction of both polar and apolar compounds, high capacity, cleaning complex matrices and effectiveness in terms of removing interferences. As shown in Table 1, this sorbent has been the optimal choice in many studies for the extraction and determination of pharmaceuticals [5,6], personal care products (PCPs) [7], illicit drugs [8] and sweeteners [9], among

others, in environmental samples. The analytical methods developed using Oasis HLB in environmental analysis have enabled high sample volumes to be loaded (from 50 to 1000 mL), providing high enrichment factors. Combining the high capacity of the Oasis HLB sorbent and the sensitivity of MS/MS detection technique, low MDLs have been achieved at levels of ng L^{-1}. For instance, González-Mariño et al. [7] used Oasis HLB as SPE sorbent, followed by LC-MS/MS for extracting PCPs from 200 and 500 mL of waste-waters and surface waters, respect-tively, achieving good recovery values (67-110%). Due the complexity of these samples, a matrix effect was observed for most of the compounds (30-120%). It should be pointed out that matrix effect is expressed as the percentage of the ratio between the peak area of the analyte(s) recorded for the sample spiked with the target compound(s) after extraction and the peak area of the analyte(s) recorded for a standard solution at the same concentration level [10]. In order to compensate this effect, the authors decided to use internal standards (ISs). However, for certain compounds, the matrix effect could not be corrected. Moreover, a good option for avoiding the matrix effect is to percolate a smaller sample volume. For example, Ordóñez et al. [9] developed the extraction of a group of sweeteners from 50 mL of surface and wastewaters using Oasis HLB and LC-MS/MS, obtaining high recovery values (73-112%), low matrix effect (75-100%) and method quantification limits (MQLs) at low levels of ng L^{-1} (0.02-50 ng L^{-1}).

When environmental solid samples are analysed, such as soil and sewage sludge, SPE is usually required to preconcentrate the analytes of interest and clean the extracts obtained after the solid-liquid extraction, reducing the matrix effect. Oasis HLB is one of the most commonly used sorbents for this purpose and is used for the extraction of pharmaceuticals or PCPs from sewage sludge [11]. For instance, Jelić et al. [12] reported the use of pressurised liquid extraction (PLE)/SPE/LC-MS/MS for the extraction of pharmaceuticals from environmental solid samples. Although the Oasis HLB sorbent enabled a higher enrichment factor, the removal of interferences was not complete and the matrix effect was compensated using ISs.

Other hydrophilic polymeric sorbents have been also developed, such as Bond Elut Plexa (Agilent Technologies) or Strata-X (Phenomenex), which are the most commonly used chemically modified sorbents with polar functionalities. In Table 1, a number of studies have been reported in which Strata-X has been applied mainly for the extraction of pharmaceuticals from environmental waters [13,14]. For example, Babić et al. [13] used Strata-X sorbent to extract a group of pharmaceuticals from 100 mL of waste-water samples combined with LC-MS/MS, obtaining successful results in terms of recovery values (50-117% for most of the compounds) and a low matrix effect (70-130%). Moreover, an interesting study, reported by D'Archivo et al. [15], compared five SPE sorbents (Isolute SPE C18, Superclean Envi-Carb, Lichrolut EN, Oasis HLB and Strata-X) for extracting 16 polar pesticides from 1 L of groundwater. Of these, the highest recovery values (54-113%) were provided by both of the

Table 1. Structure and application of some commercial and in-house hydrophilic polymeric sorbents for SPE.

Sorbent (Supplier)	Structure	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Matrix effect (%) [f]	MDLs (ng L^{-1})	Ref.
Oasis HLB (Waters)		Human and veterinary antibiotics	Surface and sewage	Off-line-SPE-LC-MS/MS	100-1000	50-150	42-221 [a,e]	0.2-61.7	[62]
		Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-MS/MS	50	21-116	6-123 [e]	1-500 [b]	[5]
		Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-MS/MS	100-500	35-116	<25 [c,e]	0.5-60	[6]
		PCPs	Surface and sewage	Off-line-SPE-LC-MS/MS	200-500	67-110	30-150 [a,e]	0.02-50	[7]
		Illicit drugs	Surface	Off-line-SPE-LC-MS/MS	250	71-104	80-100 [a,e]	0.01-1.54	[8]
		Sweeteners	Surface and sewage	Off-line-SPE-LC-MS/MS	50	73-112	75-100 [e]	0.01-0.5 [b]	[9]
Strata-X (Phenomenex)		Pharmaceuticals	Sewage	Off-line-SPE-LC-MS/MS	100	26-117	70-130 [e]	0.1-5	[13]
		Trace organic contaminants	Drinking and surface	On-line-SPE-LC-MS/MS	10	60-109	Low [a,e]	0.4-6	[63]
		Pharmaceuticals	Sewage	Off-line-SPE-LC-MS/MS	250-500	71-115	Low [a,e]	1-500 [b]	[14]
Bond Elut Plexa (Agilent Technologies)		PCPs	Surface	Off-line-SPE-LC-MS/MS	500	46-101	45-108 [d,e]	1-4	[16]
PANI (in-house)		Polar pesticides	River and tap	Off-line-SPE-CE-DAD	350	7-111	-	10-500	[64]

Table 1. Continued.

Sorbent (Supplier)	Structure	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Matrix effect (%) [f]	MDLs (ng L^{-1})	Ref.
PPy (in-house)		Phenols and chlorophenols	River and tap	On-line-SPE-LC-UV	25-100	84-96	-, [e]	15-150	[65]
4-VP-DVB (in-house)		Polar contaminants	Surface and sewage	On-line-SPE-LC-UV	100	35-89	-, [e]	100-200	[66]
4-Vim-DVB (in-house)		Polar contaminants	River	On-line-SPE-LC-UV	100	6-96	-	-	[67]
HXLPP polarB (in-house)		Polar contaminants	River and tap	Off-line-SPE-LC-UV	1000	29-137	-, [e]	200-500	[17]

4-VP-DVB: 4-vinylpyridine–divinylbenzene; 4-Vim-DVB: 4-vinylimidazole-divinylbenzene; CE-DAD: capillary electroforesis-diode array detection; HXLPP: hypercrosslinked resin; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-UV: liquid chromatography-UV detection; MDLs: method detection limits; PANI: polyaniline; PPy: polypyrrole; SPE: solid-phase extraction

[a] Matrix effect compensated with the use of ISs.

[b] Method quantification limits (MQLs).

[c] Matrix effect compensated with the use of internal standards or extract dilution.

[d] Matrix effect compensated with sample dilution.

[e] Matrix-matched or standard addition calibrations were applied.

[f] Matrix effect expressed as in reference [10].

-: not reported.

hydrophilic polymeric sorbents, Oasis HLB and Strata-X, due to the pyrrolidone group present in their structure. Although Bond Elut Plexa has been less commonly applied than Oasis HLB or Strata-X, it has been successfully used to preconcentrate PCPs from surface waters [16].

Apart from the commercial polymeric sorbents with polar functionalities, some research groups have focused on the synthesis and the analytical application of in-house hydrophilic polymers as SPE sorbents [3]. Table 1 shows some examples of in-house polymeric SPE sorbents synthesised with polar monomers used in environmental analysis. These in-house sorbents also provided good results in terms of capacity, recovery values and matrix effect when environmental waters were analysed (surface, tap, river and wastewaters). One of these worth highlighting is the in-house hypercrosslinked polymeric sorbent (HXLPP Polar, 850 m^2 g^{-1}) [17], which was able to preconcentrate polar contaminants from 1 L of river and tap waters with similar recovery values to Oasis HLB, even in the presence of matrix interferences.

It is important to remark that in most of the studies cited in the present review, matrix effect has been compensated using matrix-matched or standard addition calibrations, as can be observed in all Tables. Moreover, the use of ISs has been also used.

Although both commercial and in-house hydrophilic polymeric sorbents for SPE have demonstrated their capacity, loading high sample volumes, they are sometimes not able to provide effective washing of the sample, leading to high matrix effect and, as a result, high MDLs. Therefore, more selective materials for SPE are required, which enable the selective extraction of the analytes of interest as well as the removal of matrix interferences, thereby enhancing the sensitivity.

3. SELECTIVE SORBENTS FOR SOLID-PHASE EXTRACTION

As mentioned above, the need for selective SPE sorbents has been a considerable concern, at the same time as the complexity of the matrices increased. The first successful attempts to enhance selectivity were achieved with the development of immunosorbents (IMSs) and restricted-access materials (RAMs) as specific materials to be applied as SPE sorbents [18,19]. These materials have been extensively used in biological and food analysis, and to a lesser extent in environmental analysis, since they are able to retain the analyte of interest selectively, as well as removing high-molecular mass compounds, such as proteins [20,21]. However, these sorbents presented some limitations, such as the high cost and instability of IMSs or the insufficient selectivity of RAMs. In order to solve these shortcomings, molecular recognition was applied in order to synthesise novel SPE sorbents.

3.1. MOLECULARLY IMPRINTED POLYMERS

Molecularly imprinted polymers (MIPs) were designed to establish selective interactions with a target analyte (template), while non-desired compounds are not retained in their structure, removing interferences and, as a result, reducing matrix effect. MIPs

were first synthesised and used as SPE sorbents by Sellergen in 1994 [22]. Since then, molecularly imprinted solid-phase extraction (MISPE) has become the one of the most popular choice for achieving high selectivity during the sample preparation of food, biological or environmental matrices [23,24].

The selectivity of a MIP can be enhanced during the synthesis by selecting a proper functional monomer with complementary functional groups to those present in the template molecule, as well as the type of polymerisation. Several reviews have been reported in which all parameters affecting the synthesis of MIPs are discussed [25-27]. As can be seen in Table 2, 4-vinylpyridine (4-VP), 2-vinylpyridine (2-VP) and methacrylic acid (MAA) are the most commonly used functional monomers in MIP synthesis, due to their ability to establish hydrophobic interactions and hydrogen bonds with the template [27]. For instance, Michailof et al. [28] developed an exhaustive study about the influence of the functional monomer and crosslinker used in the synthesis of two MIPs for caffeic acid to extract polyphenols from olive mill wastewaters. Thus, four functional monomers (4-VP, MAA, allylurea and allylaniline) and three crosslinkers (DVB, ethylene-glycol dimethylacrylate (EGDMA) and pentaerythritol trimethylacrylate (PETRA)) were tested using the same porogenic solvent, tetrahydrofuran. Of the functional monomers used, 4-VP and allylaniline showed better recognition than MAA or allylurea because, as well as electrostatic interactions, hydrogen bonds, π-π interactions between these monomers and the template could be established. Finally, 4-VP allowed a higher affinity with the template due to its stronger basic character and PETRA was the most suitable crosslinker due to its flexibility.

In MISPE, a washing step, using organic solvents with different chemical properties, is usually included, since it is the most crucial step for maximising the interactions between the imprinting cavities and the template, and for simultaneously eliminating interfering compounds. For example, Dai et al. [29] synthesised a MIP to extract diclofenac specifically from water samples. In this study, the authors checked if the interferences present in the matrix (such as organic matter or other pharmaceuticals) could be retained onto the MIP or removed during a washing step. After testing different compositions of an ACN/H_2O solution, 2 mL of ACN/H_2O (40/60, v/v) were enough to release all of the interfering compounds from the MIP during the washing step. In addition, no presence of matrix interferences was detected, since the recovery values for diclofenac were similar when more complex matrices were analysed. Another example of a highly selective MIP was proposed in a study by Beltran et al. [30], in which carbamazepine, an anticonvulsant drug, was selected as template for further MISPE application in wastewaters. In this case, other pharmaceutical (ibuprofen and benzafibrate) were selected to evaluate the selectivity of the MIP and the authors demonstrated that the washing of the sample using 2 mL of ACN was enough to eliminate matrix interferences, as well as other structurally-related compounds.

Template bleeding is considered to be the main drawback of MISPE in trace

analysis, because a small amount of template may remain strongly bound to the polymer, even after exhaustive washings, giving rise to erroneous results. To tackle this problem, the dummy imprinting approach was proposed, consisting of the use of an analogue of the analyte of interest as a template, known as a dummy molecule [31]. For example, López-Nogueroles et al. [31] have recently synthesised a dummy MIP to extract nitro musk compounds from environmental waters, using 2,4-dinitrotoluene as a dummy template. This approach provided successful results for all of the studied compounds with recovery values between 52% and 92%, as well as selective washing being performed with 1.3 mL of MeOH/H_2O (60/40, v/v), when 200 mL of different environmental waters were analysed. Although dummy imprinting is a good option for avoiding the residual leaking of the template, it may result in lower molecular recognition compared to those MIPs synthesised with the analyte of interest [32].

Most MIPs are developed for one target analyte, especially in biological research. However, in some cases, the synthesised MIP can retain the target molecule as well as other structurally-related compounds, resulting in a decrease of selectivity. Although this effect, known as cross-selectivity, may be undesirable, many studies involving environmental analysis have been reported that take advantage of the effect to obtain class-selective MIPs, showing selectivity towards a family of related compounds [23,33,34]. For instance, Chen et al. [34] synthesised a MIP for ciprofloxacin for the determination of fluoroquinolone antibiotics in environmental samples. It was observed that the MIP was class-selective for fluoroquinolones, but not for other antibiotics. Moreover, the authors studied the matrix effect obtained using different washing solvents (water, methanol and acetonitrile) when environmental waters were analysed. After several trials, they concluded that water was not enough to reduce the presence of organic matter, whereas a washing with acetonitrile (2x3 mL) allowed the efficient removal of interferences (>88% of matrix effect) and good recovery values (78-94%). More recently, Herrero-Hernández et al. [35] developed a MIP specific for a group of phenolic compounds and phenoxyacid herbicides using bisphenol-A as a template. In the proposed MISPE-LC-UV method, 250 mL of river and ground waters were percolated through the cartridges, retaining the analytes of interest (recovery values between 91% and 107%), eliminating all interfering compounds (with 5 mL of dichloromethane (DCM)) and achieving MDLs at levels of ng L^{-1} (20-90 ng L^{-1}). As shown in Fig. 1, the feasibility of the MISPE was demonstrated by compareson with the SPE performance obtained with Oasis HLB and C_{18} SPE cartridges. Oasis HLB or C_{18} sorbent were not able to perform a selective washing of the sample and, as a result, many interferences (humic and fulvic acids) coeluted with the target analytes and some of the analytes could not be recovered.

Because of recent concern about environmental pollution, scientists have made great efforts to develop multi-residue methods for extracting and detecting as many contaminants as possible in the same analysis.

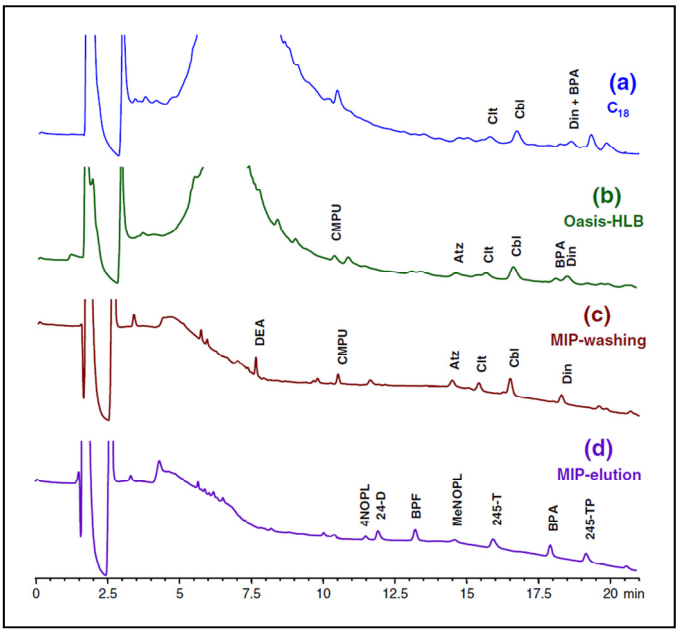


Fig. 1. Chromatograms obtained after the preconcentration of 250 mL of river water samples spiked with 0.3 µg L−1 of each analyte: (a) with C_{18} cartridges as the SPE sorbent; (b) with Oasis HLB cartridges; (c) MISPE, washing step of bisphenol-A-MIP; (d) MISPE, elution step of bisphenol-A-MIP. Reproduced from [35] with permission of Elsevier.

This may sometimes involve the use of several SPE sorbents to cover a wide range of EOCs. With this in mind, and in relation to cross-selectivity, a multi-molecular imprinting approach has recently been proposed by Duan et al. [36], in which a MIP is prepared with more than one compound as a template in order to extract a group of acidic pharmaceuticals simultaneously from environmental waters and sediments. In this study, five pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid) were used as templates, 2-VP as the functional monomer and EGDMA as the crosslinker. Once the MIP was synthesised, its specificity was evaluated using two different pharmaceuticals as interfering compounds. The results showed that the multi-template MIP presented high selectivity for the templates, while the two interferences were completely removed during the washing step using 2 mL of a mixture of DCM/ACN. In addition, the multi-template MISPE was successfully applied to environmental waters with recovery values higher than 80% and an insignificant matrix effect when 1 L of lake and wastewaters were analysed. Another multi-template MIP was synthesised by Song et al. [37] using a group of 16 polycyclic aromatic hydrocarbons (PAHs) as templates, phenyltrimethoxysilane (PTMS) as the func-

tional monomer and tetraethoxysilane (TEOS) as the crosslinker. Despite the lack of functionalities in the structures of PAHs, a promising molecular imprinting was achieved due to the hydrophobic and π-π interactions resulting from the aromatic rings of PAHs. The multi-template MISPE was applied to seawaters (10 mL), obtaining recovery values between 83% and 113%. The authors mentioned that binding capacity of the MIP was influenced by the high presence of inorganic ions in seawaters. However, the mainly aqueous washing step included in the multi-template MISPE (2 mL of MeOH/H_2O (10/90, v/v)) enabled the removal of as many interferences as possible.

The widespread application of in-house MIPs in sample preparation has recently resulted in the commercialisation of a variety of MISPE cartridges. One group of these worth highlighting are the class-selective MISPE cartridges, such as those for non-steroidal anti-inflammatory drugs (NSAIDs), PAHs, triazines, tobacco-specific nitrosamines or estrogens. There are also MIPSE cartridges that are selective for a single target analyte, such as clenbuterol or bisphenol-A. However, few studies have been reported in the field of environmental research, some of which are detailed in Table 2. Gros et al. [38] were the first to use a commercial MISPE cartridge to extract a group of β-blockers from environmental waters by liquid-chromatography-quadrupole-linear ion trap mass spectrometry (LC-MS/MS(QqLIT)). In this study, the MISPE performance was tested and compared to the Oasis HLB sorbent. The results obtained showed that the commercial MIP was completely able to extract all β-blockers selectively from wastewaters (25 mL) with recovery values ranging from 43% to 112% and low MDLs (0.2-6.5 ng L^{-1}). Meanwhile, Oasis HLB provided similar recovery values to the MIP, but slightly higher LODs (1-8 ng L^{-1}), even when percolating a higher sample volume (100 and 200 mL of effluent and influent wastewaters, respectively). Moreover, when the matrix effect was studied using both SPE sorbents, the authors demonstrated that the commercial MIP presented a negligible matrix effect (external calibration curves in influent extracts and solvent were completely parallel), while Oasis HLB was influenced by the matrix interferences, using ISs to compensate this effect. More recently, another interesting study reported the use of a commercial MIP for estrogens (AffiniMIP-Estrogens) for preconcentrating 100 mL of river and tap waters, which was compared to C_{18} SPE cartridges [39]. The MISPE performance was considerably better than using the C_{18} cartridges, since acceptable recovery values (48-99%) were achieved with no significant matrix effect. Other studies, in which a commercial MIP was used, have been reported in Table 2, showing a satisfactory MISPE performance due to their high selectivity, high removal of interferences and low matrix effect, providing good recovery values and low MDLs.

More recently, a new, rapid and selective approach has been reported, consisting of the direct coupling of a MISPE procedure to a detection system. Figueiredo et al. [40,41] were the first to perform a fast and effective MISPE coupled to MS for the extraction of benzodiazepines and phenothiazines

Table 2. In-house and commercial molecularly imprinted solid-phase extraction (MISPE) applications in environmental analysis.

Template	Functional monomer/ Cross-linker	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Washing step	Matrix effect (%) [a]	MDLs (ng L^{-1})	Ref.
Butylparaben	4-VP/EGDMA	Benzylparaben, methylparaben, ethylparaben, butylparaben	River	Off-line MISPE-LC-UV	500	22-67	1 mL of 2-propanol	Low	-	[68]
Diclofenac	2-VP/EGDMA	Diclofenac	River and sewage	Off-line MISPE-LC-UV	1000	90-95	2 mL of ACN/H_2O (40/60)	Low	-	[29]
Bisphenol-A	4-VP/EGDMA	4 phenols and 3 phenoxyacid herbicides	River and ground	Off-line MISPE-LC-UV	250	91-107	5 mL of DCM	Low [b]	20-90	[35]
Monobutyl phthalate	4-VP/EGDMA	Monobutyl phthalate	Bottled	Off-line MISPE-LC-UV	25	80	1mL of ACN/MeOH (50/50)	Low	17	[69]
Cyclobarbital	4-VP/EGDMA	Phenobarbital, amobarbital and phenytoin	River	On-line MISPE-LC-MS/MS	50	91-112	2 mM ammonium acetate	- [b]	0.5-5	[70]
Ciprofloxacin	MAA/EGDMA	ciprofloxacin, enrofloxacin, lomefloxacin, levofloxacin, fleroxacin and sparfloxacin	River and sewage	Off-line MIMSPE-LC-MS/MS	50-500	78-94	2x3 mL of ACN	>88 [b]	3.2-6.2	[34]
2,4-dinitrotoluene	Phenyltrimetho-xysilane/ tetraethoxysilane	Musk tibetene, musk moskene, musk ketone, musk xylene and musk ambrette	River, sea and sewage	Off-line MISPE-GC-MS	200	52-92	1.3 mL of MeOH/H_2O (60/40)	Low [b]	1.5-2.6	[31]
Carbamazepine	MAA/DVB	Carbamazepine	Sewage	Off-line MISPE-LC-MS	100	80	5 mL of H_2O + 2 mL of ACN	Low [b]	-	[30]
Ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid	2-VP/EGDMA	Ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid	Lake	Batch experiment-LC-UV	30	90-100	No	-	-	[71]

Table 2. Continued.

Template	Functional monomer / Cross-linker	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Washing step	Matrix effect (%) [a]	MDLs (ng L^{-1})	Ref.
Ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid	2-VP/EGDMA	Ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid	Lake and sewage and sediments	Off-line MISPE-LC-MS/MS	1000	80-105	2 mL of DCM/ACN (94:6)	≈ 100 [b]	2-12	[36]
Carbamazepine and clofibric acid	2-VP/EGDMA	Carbamazepine and clofibric acid	Tap, lake and river	Batch experiments -LC-UV	3	90-95	No	-	-	[72]
16 PAHs	Phenyltrimethoxysilane/tetraethoxysilane	16 PAHs	Sea	Off-line-MISPE-GC-MS	30	83-113	2 mL of MeOH/H_2O (10/90)	Low [b]	5.2-12.6	[37]
SupelMIP-Amphetamine	Commercial	5 amphetamines	Sewage	Off-line MISPE-LC-MS/MS	50-100	80-100	2× 1mL of basic H_2O + 1mL of ACN/H_2O (60/40) + 1mL of ACN with 1% CH_3COOH	60-100	0.5-2.7	[73]
MIP4SPE-β-blockers	Commercial	8 β-blockers	Sewage	Off-line MISPE-LC-QqLIT MS	25	43-112	2×1mL of H2O + 1mL of ACN + 1mL of DCM	Low [b]	0.2-6.5	[38]
Affinilute MIP-NSAIDs	Commercial	15 pharmaceuticals	Sewage	Off-line MISPE-LC-MS/MS	250	45-85	5 mL of H_2O + 5 mL of ACN/H_2O (40/60)	20-95 [b]	0.5-2	[74]
AffiniMIP-Estrogens	Commercial	7 estrogens	River and tap	Off-line MISPE-LC-MS/MS	100	48-99	4 mL of ACN/H_2O (80/20) + 2 mL of H_2O	Low [b]	4.5-9.8	[39]
Affinilute MIP-NSAIDs	Commercial	6 NSAIDs	Sewage	Off-line MISPE-MS/MS	10-50	62-103	5 mL of ACN/H_2O (40/60)	30-16 [b]	50-100	[42]

2-VP: 2-vinylpyridine; 4-VP: 4-vinylpyridine; ACN: acetonitrile; CH_3COOH: acetic acid; DCM: dichloromethane; DVB: divinylbenzene; EGDMA: ethylene-glycol dimethylacrylate; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-QqLIT MS: liquid chromatography-quadrupole-linear ion trap mass spectrometry; LC-UV: liquid chromatography-UV detection; MAA: mehtacrylic acid; MDLs: method detection limits; MeOH: methanol; MISPE: molecularly imprinted solid-phase extraction; MIMSPE: molecularly imprinted magnetic solid-phase extraction; PAHs: polycyclic aromatic hydrocarbons.

[a] Matrix effect expressed as in reference [10]. [b] Matrix-matched or standard addition calibrations were applied.

-: not reported.

from biological samples. However, the first study to apply this new approach in environmental analysis was developed by Gilart et al. [42], in which a group of NSAIDs were extracted using MISPE-MS/MS methodology. Although MS/MS detection is highly susceptible to interfering compounds from complex matrices, the clean and selective extraction provided by the MISPE procedure and a proper validation method using matrix-matched calibration enabled suitable and rapid detection and quantification of these contaminants in wastewaters (10-50 mL) without chromatographic separation.

As can be seen in Table 2, the sample volumes percolating through a MIP are usually lower than those loaded onto a polymeric sorbent, such as Oasis HLB or Strata-X (Table 1). The reason remains the fact that a lower amount of polymeric material is present in the MIP structure and less imprinted sites are available to retain the analytes of interest [23,24]. However, a low sample volume can be an advantage in comparison to the polymeric sorbents, since less time is required during the extraction process and, in particular, less matrix effect is obtained.

3.2. MIXED-MODE ION-EXCHANGE SORBENTS

In order to achieve selectivity and capacity in a single material, dual-phase or mixed-mode sorbents emerged, allowing selective extractions through ion-exchange mechanisms with the target analytes, as well as percolating higher sample volumes due to an increase in the surface area of the polymer, in comparison to a MIP. Ion-exchange silica-based sorbents were the first to be commercialised. However, mixed-mode polymeric sorbents were then designed to overcome the low stability at extreme pH and the low retention of polar compounds of ion-exchange silica-based sorbents [43].

Depending on the ion-exchange interactions established between the polymer and the analytes, mixed-mode sorbents can be divided in four main groups: strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX) and weak anion-exchange (WAX). These ionic interactions can be achieved by modifying the polymer structure with specific functional groups, such as sulphonic acid for SCX, a carboxylic acid for WCX, a quaternary amine for SAX and a secondary amine for WAX. Due to their mixed-mode behaviour, these sorbents are able to extract both charged and uncharged analytes by ion-exchange and reversed-phase interactions, respectively. Therefore, it is important to select the correct sorbent and follow a suitable SPE protocol in order to enhance the selectivity and the retention of the analytes of interest [4,43]. In mixed-mode SPE, loading, washing and elution steps are essential in order to enhance selectivity, and these steps need to be evaluated. In the loading step, a selective retention of the analytes can be achieved depending on the sample pH promoting ion-exchange interactions. However, in the washing step, a proper washing solvent (usually an organic solvent) is required to disrupt reversed-phase interactions and remove interferences. And finally, the target analytes are eluted from the sorbent, usually using a basic or acidic organic solution to break the ion-exchange interactions. It has been

demonstrated that the whole mixed-mode SPE procedure allows cleaner extracts, lower matrix effects and MDLs [43].

The first commercially available mixed-mode polymeric sorbents were pioneered by Waters Corporation (Milford, MA, USA), which developed four novel sorbents for SPE: Oasis MCX (SCX), Oasis WCX (WCX), Oasis MAX (SAX) and Oasis WAX (WAX). All of these mixed-mode sorbents were based on the Oasis HLB skeleton and further chemically modified with sulphonic groups for SCX, carboxylic acids for WCX, dimethylbutylamine for SAX and piperazine for WAX. As can be seen in Table 3, all four sorbents have been applied in environmental analysis, especially, for the extraction of pharmaceuticals and illicit drugs from environmental waters [44-47]. Moreover, other companies have commercialised similar mixed-mode polymeric sorbents, such as Phenomenex (Torrence, CA, USA) with the Strata-X family sorbents, Agilent Technologies (Santa Clara, CA, USA) with Bond Elut Plexa family sorbents and Biotage (Uppsala, Sweden) with the Evolute family sorbents. Table 3 provides some examples of the application of these commercial mixed-mode sorbents. More recently, new mixed-mode polymeric sorbents have been commercialised, known as SiliaPrepX from SiliCycle (Quebec, Canada), including all four ion-exchange sorbents. However, as yet, there have been no publications related to these SPE sorbents. Current commercialised mixed-mode polymeric sorbents present a macroporous structure with a specific surface area over 800 $m^2 g^{-1}$.

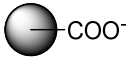
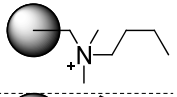
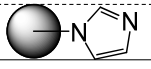
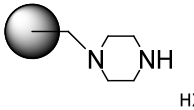
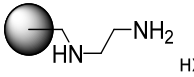
Of the studies cited in Table 3, it is worth highlighting the analytical method developed by Bijlsma et al. [44], in which a group of illicit drugs were extracted from surface and wastewaters. Since the studied compounds presented basic and acidic functionalities, Oasis MCX and Oasis HLB were tested as SPE sorbents, with Oasis MCX being selected due to its improved selectivity towards basic compounds. However, in this study, the washing step was performed using an aqueous basic solution instead of a pure organic solvent, such as MeOH, in order to keep the acidic analytes retained, as well as removing inorganic salts from the matrix. This fact caused a high matrix effect for all of the compounds (40-120%), which was compensated with the use of ISs. In some cases, the use of organic solvents in the washing step could be crucial for extracting and determining the target analytes. For instance, Barclay et al. [48] proposed the enantiomeric separation of fluoxetine and norfluoxetine and their extraction from wastewaters using a commercial SCX SPE sorbent (Evolute-CW). After the SPE optimisation, the authors concluded that including a washing step with 4 mL of MeOH resulted in cleaner extracts, less ion suppression and, in particular, an improvement in the detection of norfluoxetine, which was undetectable when the washing step was not performed.

With respect to WCX and WAX mixed-mode sorbents, Fontanals et al. [45] have recently developed an on-line SPE procedure using the Oasis WCX sorbent to extract basic illicit drugs from environmental waters. The coupling between a mixed-mode sorbent with LC was a challenge since the elution solvent had to be compatible with the

Table 3. In-house and commercial ion-exchange polymeric sorbents for SPE applications in environmental analysis.

Ion-exch. behaviour	Sorbent	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Washing step	Matrix effect (%) [g]	MDLs (ng L^{-1})	Ref.
SCX	Oasis MCX [a]	Illicit drugs	Surface and sewage	Off-line-SPE-LC-MS/MS	10-50	48-120	5 mL of 2% NH4OH in H_2O	80-200 [c, i]	0.2-2500	[44]
		Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-MS/MS	50	40-110	6 mL of H_2O	9-140 [c, i]	0.5-25 [d]	[75]
		PPCPs and illicit drugs	Surface and sewage	Off-line-SPE-LC-MS/MS	250-1000	5-144	2 mL of 2% HCOOH in H_2O	14-200 [i]	0.15-538 [d]	[76]
	Strata-X-C [a]	Illicit drugs	Surface and sewage	Off-line-SPE-LC-MS/MS	500	4-65	50 mL of 10% MeOH in 100 mM HCOOH	- [i]	1-257	[77]
	Evolute-CW [a]	Antidepressant drugs	Sewage	Off-line-SPE-LC-MS/MS	200-500	65-82	6 mL of 2% HCOOH in H_2O + 4 mL of MeOH	- [i]	<1	[48]
	AMPSA/HEMA /PETRA [b] Sulfonated HEMA/DVB [b]	Pharmaceuticals and illicit drugs	Sewage	Off-line-SPE-LC-MS/MS	25-50	5-107	5 mL of MeOH	80-110 [i]	2-40	[53]
WCX	Oasis WCX [a]	Illicit drugs	Surface and sewage	On-line-SPE-LC-MS	10	51-115	0.5 mL of MeOH	80-110 [i]	0.5-2	[45]
		Fluoroquinolones	Sewage	Off-line-SPE-LC-MS/MS	250	87-94	5 mL of MeOH	High [e]	2-10 [d]	[78]
	Strata-X-CW [a]	Antidiabetic drug	Surface and sewage	Off-line-SPE-LC-MS/MS	10	91	-	Low [i]	3-10	[49]

Table 3. Continued.

Ion-exch. behaviour		Sorbent	Analytes	Water matrix	Technique	Sample volume (mL)	Recovery values (%)	Washing step	Matrix effect (%) [g]	MDLs (ng L^{-1})	Ref.
WCX		HXLPP-WCX [b]	Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-UV	250-500	54-92	2 mL of 5% NH_4OH in MeOH	Low [i]	100-1000	[51]
SAX		Oasis MAX [a]	Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-MS/MS	50-100	6-145	-	High [c, i]	1-65	[46]
		HXLPP-SAXa [b]	Pharmaceuticals	Surface and sewage	Off-line-SPE-LC-UV	100-500	60-90	10 mL of MeOH	Low [i]	50-100	[52]
		NVIm-DVB [b]	Pharmaceuticals	Aqueous samples	Off-line-SPE-LC-UV	1	54-96	1 mL of MeOH	-	-	[79]
WAX		Oasis WAX [a]	Whitening agents	Surface and sewage	Off-line-SPE-LC-MS/MS	50	74-92	2 mL of 25 mM acetate buffer (pH 5) + 2 mL of MeOH	- [i]	1-6	[50]
			Pharmaceuticals	River	Off-line-SPE-LC-UV	50	60-110	-	Low [i]	40-67	[47]
		HXLPP-WAX-piperazine [b]	Acidic pharmaceuticals	Surface	Off-line-SPE-LC-UV	500	56-94	4 mL of MeOH	-	-	[80]
		Strata-X-AW [a]	Estrogens	River sediments	MASE-Off-line-SPE-LC-MS/MS	1 (gr)	82-98	4 mL of MeOH/pH 7acetate buffer (4:6)	<82 [f, i]	0.15-0.04 [h]	[81]
		HXLPP-WAX-EDA [b]	Pharmaceuticals	Surface and sewage	On-line-SPE-LC-UV	100-250	82-97	1 mL of MeOH	- [i]	50-100	[82]

HCOOH: formic acid; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-UV: liquid chromatography-UV detection; MASE: microwave-assisted solvent extraction; MDLs: method detection limits; MeOH: methanol; NH4OH: ammonium hydroxide; SAX: strong anion-exchange; SCX: strong cation-exchange; SPE: solid-phase extraction; WAX: weak anion-exchange; WCX: weak cation-exchange

[a] Commercial mixed-mode sorbents; [b] In-house mixed-mode sorbents; [c] Matrix effect compensated with the use of internal standards; [d] Method quantification limits (MQLs); [e] Matrix effect compensated with the extract dilution; [f] Matrix effect compensated with a different chromatographic separation.; [g] Matrix effect expressed as in reference [10]; [h] ng g^{-1}; [i] Matrix-matched or standard addition calibrations were applied.

-: not reported.

mobile phase of the chromatographic system. In this study, an acidic elution solvent was required to disrupt the ion-exchange interactions between the analytes and the sorbent while, at the same time, being suitable for chromatographic separation. Therefore, the proposed analytical method provided satisfactory results in terms of recovery values (51-115%), matrix effect (80-110%) and sensitivity (MDLs = 0.5-2 ng L^{-1}) taking into account the low sample volume used (10 mL) and the effectiveness of the Oasis WCX in terms of removing interferences with just 0.5 mL MeOH. With respect to Strata-X family sorbents, a few applications have been reported, especially in environmental analysis. For example, Strata-X-CW has been used to extract an anti-diabetic drug from environmental waters by LC-MS/MS [49]. The authors emphasised that the excellent recovery values (91%) and the absence of matrix effect was attributed to the selectivity of the mixed-mode sorbent as well as the small sample volume used. As shown in Table 3, WAX mixed-mode sorbents have been also used in environmental analysis, such as for extracting whitening agents from surface and wastewaters [50]. Oasis WAX was selected because it can establish weak ionic-exchange and reversed-phase interactions with the sulphonate groups and aromatic rings of the target analytes, respectively.

Despite the large number of publications in the literature reporting the use of mixed-mode polymeric sorbents, there has been constant interest in improving the current commercial sorbents, developing novel extracting phases for SPE with mixed-mode behaviour. Our research group has become a pioneer in the research area of the synthesis of hypercrosslinked mixed-mode polymers as SPE sorbents. These materials have a hypercrosslinked and microporous structure with a higher specific surface area (~1000 m^2 g^{-1}) than the commercial macroporous sorbents (~800 m^2 g^{-1}), leading to higher extraction efficiencies. Table 3 also details the in-house mixed-mode polymers synthesised as SPE sorbents for environmental analysis. For instance, Bratkowska et al. [51] obtained a hypercrosslinked WCX polymeric sorbent (HXLPP-WCX) for the SPE of basic pharmaceuticals from environmental waters. Unlike commercial WCX sorbents, the in-house HXLPP-WCX sorbent allowed a washing step to be performed using a basic organic solvent (2 mL of 5% NH_4OH in MeOH) instead of pure organic solvent, eluting the acidic compounds while the basic ones were still retained onto the sorbent. In contrast, under the same SPE conditions, the commercial mixed-mode Strata-X-CW and Oasis WCX were unable to keep the basic pharmaceuticals retained onto the sorbent during the washing step, since the ionic interactions were entirely disrupted. Fig. 2 shows the effect of including a washing step when 250 mL of effluent wastewaters were loaded. It was observed that acidic and interfering compounds were totally removed during the washing step, while only the basic compounds were released from the sorbent during the elution step with high recovery values (54-92%). In addition, the same authors applied an in-house hypercrosslinked SAX polymer (HXLPP-SAXa) as SPE sorbent for extracting a group of acidic pharmaceuticals from surface and wastewaters [52].

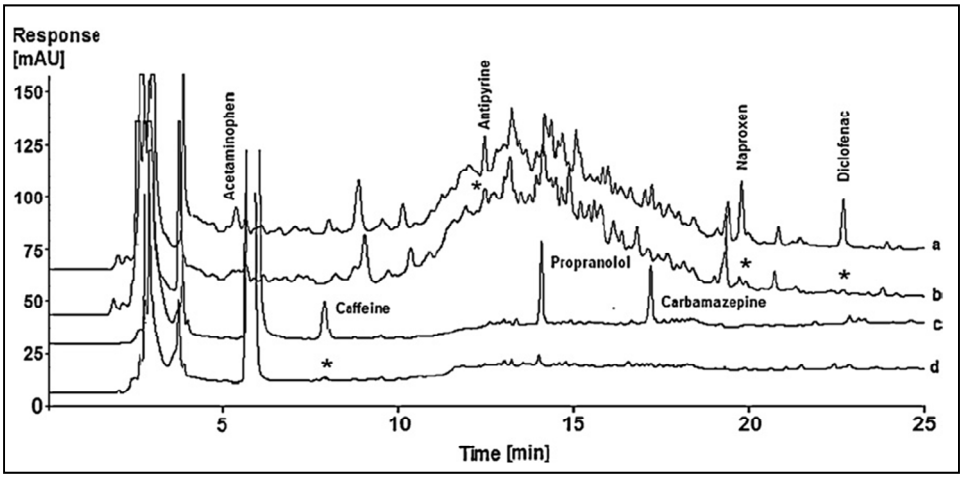


Fig. 2. Chromatograms obtained after off-line SPE with HXLPP-WCX of 250 ml of effluent WWTP sample with (a and c) and without (b and d) the addition of a 5 µg L-1 level of analyte mixture: washing step (a and b) and elution step (c and d).*Peaks at the same time of studied analytes. Reproduced from [51] with permission of Elsevier.

It should be noted that the proposed SPE procedure included a washing step using 10 mL of MeOH, a higher washing volume than usual, which helped to remove interferences effectively while maintaining the target analytes attached to the sorbent without losses. After comparing it to two commercial SAX sorbents (Oasis MAX and SampliQ SAX), the in-house HXLPP-SAXa sorbent provided promising recovery values (60-90%) without the presence of matrix interferences. More recently, two in-house SCX copolymers have been synthesised and applied in SPE of a group of illicit drugs and pharmaceuticals from wastewaters [53]. Both in-house SCX sorbents were evaluated and compared to Oasis MCX. The results demonstrated the feasibility of one of the sorbents in complex environmental water samples, with similar results to the commercial SCX sorbent. Moreover, acceptable recovery values (39-98%), low matrix effect (80-110%) and low MDLs (1-40 ng L^{-1}) were obtained after loading 25 and 50 mL of influent and effluent wastewaters.

4. OTHER SORPTIVE MATERIALS FOR SOLID-PHASE EXTRACTION

As mentioned before, selectivity can mainly be achieved using a MIP or mixed-mode sorbents when SPE is required in environmental analysis. As well as these, since 2009, ionic liquids (ILs - inorganic and organic salts with melting points below 100ºC) immobilised onto a silica or polymeric surface have attracted greater interest as materials for SPE [54,55]. In environmental analysis, a few publications have reported the use of ionic liquids as SPE sorbents, in which selectivity has been also enhanced thanks to the ion-exchange mechanisms. For example,

Bratkowska et al. [56] and Fontanals et al. [57] evaluated three polymeric ionic liquid phases containing *N*-methylimidazolium with different anions, trifluoromethanesulphonate [$CF_3SO_3^-$] [56], tetrafluoroborate [BF_4^-][56] and trifluoroacetate [CF_3COO^-][57], to be used as sorbents for the SPE of acidic pharmaceuticals from wastewaters. These polymeric IL sorbents were evaluated under reversed-phase, WAX, SAX and SCX SPE protocols, providing successful results under SAX behaviour, removing all basic compounds in the washing step while retaining the acidic analytes onto the sorbent. When all ILs were compared, [MI^+][CF_3COO^-] provided the best SPE performance with recovery values for acidic pharmaceuticals between 55% and 101%, being similar to the results obtained using the commercial Oasis MAX. Another interesting approach was developed by Yang et al. [58], in which magnetic microspheres modified with an IL were synthesised and then applied as a sorbent for magnetic solid-phase extraction (MSPE) of chlorophenols from environmental waters. The authors also highlighted that the anion-exchange mechanisms were responsible for the selective retention of the analytes due to the silica IL phase based on *N*-methylimidazolium chloride, with recovery values ranging between 70% and 89%.

Over the last few years, magnetic beads applied in MSPE have gained popularity for sample preparation due to their magnetic, mechanical and surface properties, easy surface modification, easy operation and high extraction efficiency [59]. With this in mind, the functionalisation of magnetic particles (mainly magnetite (Fe_3O_4)) with MIPs has been successful in environmental analysis, such as for the extraction of fluoroquinolone antibiotics [34], sulphonylurea herbicides [60], and estrogens [61] from environmental waters, among others. The mentioned studies highlight the fast and simple sample extraction (7-30 min) displayed by the magnetic MIP particles, as well as their high capacity (100-500 mL) and recovery values (72-102%). Moreover, most of the studies involving molecularly imprinted MSPE (MI-MSPE) procedures do not usually include a washing step, which could result in the presence of interferences. However, Chen et al. [34] performed a washing of the magnetic MIP particles (2x3 mL of ACN) when analysing environmental waters. Therefore, the interfering compounds were discarded from the particles, whereas the target analytes (fluoroquinolone antibiotics) were still retained onto the imprinting surface, showing good recovery values (78-94%). Although there has been efforts to enhance the selective retention of EOCs; further improvements in new extracting phases and formats for SPE to simplify sample preparation are still expected.

5. CONCLUSIONS

The presence of EOCs at low levels of concentration (ng L^{-1}) and the analysis of high complex matrices have consistently involved the commercialisation or synthesis of polar and selective materials for SPE, including hydrophilic polymers, MIPs, mixed-mode sorbents or other materials.

The main requirement in sample preparation is to provide clean extracts in order to avoid the matrix effect,

which is highly undesirable when specific and sensitive detection techniques are used, such as MS or MS/MS. In this review, it has been demonstrated that matrix effect has been almost eliminated due to the introduction of a selective washing of complex matrices when MIPs or mixed-mode sorbents are used, leading to a significant decrease in this effect.

Despite the huge number of applications of commercial hydrophilic and selective sorbents for SPE, research into the development of improved sorbents materials should continue in order to contribute in fast, simple, selective and automated sample extraction techniques.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] M. Farré, L. Kantiani, M. Petrovic, S. Pérez, D. Barceló, J. Chromatogr. A 1259 (2012) 86.
[2] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, TrAC, Trends Anal. Chem. 30 (2011) 749.
[3] N. Fontanals, R.M. Marcé, F. Borrull, TrAC, Trends Anal. Chem. 24 (2005) 394.
[4] N. Fontanals, R.M. Marcé, F. Borrull, J. Chromatogr. A 1152 (2007) 14.
[5] A.L.N. van Nuijs, I. Tarcomnicu, W. Simons, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Anal. Bioanal. Chem. 398 (2010) 2211.
[6] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678.
[7] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Rapid Commun. Mass Spectrom. 23 (2009) 1756.
[8] P. Vázquez-Roig, V. Andreu, C. Blasco, Y. Picó, Anal. Bioanal. Chem. 397 (2010) 2851.
[9] E.Y. Ordóñez, J.B. Quintana, R. Rodil, R. Cela, J. Chromatogr. A 1256 (2012) 197.
[10] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[11] O. Zuloaga, P. Navarro, E. Bizkarguenaga, A. Iparraguirre, A. Vallejo, M. Olivares, A. Prieto, Anal. Chim. Acta 736 (2012) 7.
[12] A. Jelić, M. Petrović, D. Barceló, Talanta 80 (2009) 363.
[13] S. Babić, D.M. Pavlović, D. Ašperger, M. Periša, M. Zrnčić, A.J.M. Horvat, M. Kaštelan-Macan, Anal. Bioanal. Chem. 398 (2010) 1185.
[14] F. Busetti, K.L. Linge, A. Heitz, J. Chromatogr. A 1216 (2009) 5807.
[15] A.A. D'Archivio, M. Fanelli, P. Mazzeo, F. Ruggieri, Talanta 71 (2007) 25.
[16] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1216 (2009) 6994.
[17] D. Bratkowska, N. Fontanals, F. Borrull, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, J. Chromatogr. A 1217 (2010) 3238.

[18] C.F. Poole, TrAC, Trends Anal. Chem. 22 (2003) 362.
[19] F. Augusto, L.W. Hantao, N.G.S. Mogollón, S.C.G.N. Braga, TrAC, Trends Anal. Chem. 43 (2013) 14.
[20] N. Delaunay-Bertoncini, M.C. Hennion, J. Pharm. Biomed. Anal. 34 (2004) 717.
[21] L. Chen, H. Wang, Q. Zeng, Y. Xu, L. Sun, H. Xu, L. Ding, J. Chromatogr. Sci. 47 (2009) 614.
[22] B. Sellergren, Anal. Chem. 66 (1994) 1578.
[23] A. Beltran, F. Borrull, P.A.G. Cormack, R.M. Marcé, TrAC, Trends Anal. Chem. 29 (2010) 1363.
[24] M. Lasáková, P. Jandera, J. Sep. Sci. 32 (2009) 799.
[25] P.A.G. Cormack, A.Z. Elorza, J. Chromatogr. B 804 (2004) 173.
[26] H.Y. Yan, K.H. Row, Int. J. Mol. Sci. 7 (2006) 155.
[27] L.X. Chen, S.F. Xu, J.H. Li, Chem. Soc. Rev. 40 (2011) 2922.
[28] C. Michailof, P. Manesiotis, C. Panayiotou, J. Chromatogr. A 1182 (2008) 25.
[29] C.M. Dai, X.F. Zhou, Y.L. Zhang, S.G. Liu, J. Zhang, J. Hazard. Mater. 198 (2011) 175.
[30] A. Beltran, E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 597 (2007) 6.
[31] M. López-Nogueroles, S. Lordel-Madeleine, A. Chisvert, A. Salvador, V. Pichon, Talanta 110 (2013) 128.
[32] F.G. Tamayo, E. Turiel, A. Martín-Esteban, J. Chromatogr. A 1152 (2007) 32.
[33] V. Pichon, F. Chapuis-Hugon, Anal. Chim. Acta 622 (2008) 48.
[34] L.G. Chen, X.P. Zhang, Y. Xu, X.B. Du, X. Sun, L. Sun, H. Wang, Q. Zhao, A.M. Yu, H.Q. Zhang, L. Ding, Anal. Chim. Acta 662 (2010) 31.
[35] E. Herrero-Hernández, E. Rodríguez-Gonzalo, M.S. Andrades, S. Sánchez-González, R. Carabias-Martínez, Sci. Total Environ. 454 (2013) 299.
[36] Y.-P. Duan, C.-M. Dai, Y.-L. Zhang, C. Ling, Anal. Chim. Acta 758 (2013) 93.
[37] X. Song, J. Li, S. Xu, R. Ying, J. Ma, C. Liao, D. Liu, J. Yu, L. Chen, Talanta 99 (2012) 75.
[38] M. Gros, T.M. Pizzolato, M. Petrović, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374.
[39] P. Lucci, O. Núñez, M.T. Galceran, J. Chromatogr. A 1218 (2011) 4828.
[40] E.C. Figueiredo, R. Sparrapan, G.B. Sanvido, M.G. Santos, M.A.Z. Arruda, M.N. Eberlin, Analyst 136 (2011) 3753.
[41] E.C. Figueiredo, G.B. Sanvido, M.A.Z. Arruda, M.N. Eberlin, Analyst 135 (2010) 726.
[42] N. Gilart, R.M. Marcé, N. Fontanals, F. Borrull, Talanta 110 (2013) 196.
[43] N. Fontanals, P.A.G. Cormack, R.M. Marcé, F. Borrull, TrAC, Trends Anal. Chem. 29 (2010) 765.
[44] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, J. Chromatogr. A 1216 (2009) 3078.
[45] N. Fontanals, F. Borrull, R.M. Marcé, J. Chromatogr. A 1286 (2013) 16.
[46] M.A. Sousa, C. Gonçalves, E. Cunha, J. Hajslová, M.F. Alpendurada, Anal. Bioanal. Chem. 399 (2011) 807.

[47] L.H.M.L.M. Santos, P. Paíga, A.N. Araújo, A. Pena, C. Delerue-Matos, M.C.B.S.M. Montenegro, J. Chromatogr. B 930 (2013) 75.
[48] V.K.H. Barclay, N.L. Tyrefors, I.M. Johansson, C.E. Pettersson, J. Chromatogr. A 1218 (2011) 5587.
[49] M. Scheurer, F. Sacher, H.-J. Brauch, J. Environ. Monit. 11 (2009) 1608.
[50] H.C. Chen, S.P. Wang, W.H. Ding, J. Chromatogr. A 1102 (2006) 135.
[51] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N. Fontanals, J. Chromatogr. A 1217 (2010) 1575.
[52] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C. Sherrington, R.M. Marcé, J. Sep. Sci. 35 (2012) 2621.
[53] N. Gilart, P.A.G. Cormack, R.M. Marcé, N. Fontanals, F. Borrull, J. Chromatogr. A (*In press*) (2014).
[54] N. Fontanals, F. Borrull, R.M. Marcé, TrAC, Trends Anal. Chem. 41 (2012) 15.
[55] L. Vidal, M.L. Riekkola, A. Canals, Anal. Chim. Acta 715 (2012) 19.
[56] D. Bratkowska, N. Fontanals, S. Ronka, A.W. Trochimczuk, F. Borrull, R.M. Marcé, J. Sep. Sci. 35 (2012) 1953.
[57] N. Fontanals, S. Ronka, F. Borrull, A.W. Trochimczuk, R.M. Marcé, Talanta 80 (2009) 250.
[58] F. Yang, R. Shen, Y. Long, X. Sun, F. Tang, Q. Cai, S. Yao, J. Environ. Monitor. 13 (2011) 440.
[59] X.S. Li, G.T. Zhu, Y.B. Luo, B.F. Yuan, Y.Q. Feng, TrAC, Trends Anal. Chem. 45 (2013) 233.
[60] M. Lerma-García, M. Zougagh, A. Ríos, Microchim. Acta 180 (2013) 363.
[61] Z. Lin, Q. He, L. Wang, X. Wang, Q. Dong, C. Huang, J. Hazard. Mater. 252-253 (2013) 57.
[62] L.-J. Zhou, G.-G. Ying, S. Liu, J.-L. Zhao, F. Chen, R.-Q. Zhang, F.-Q. Peng, Q.-Q. Zhang, J. Chromatogr. A 1244 (2012) 123.
[63] A. García-Ac, P.A. Segura, L. Viglino, A. Fürtös, C. Gagnon, M. Prévost, S. Sauvé, J. Chromatogr. A 1216 (2009) 8518.
[64] H. Bagheri, M. Saraji, D. Barceló, Chromatographia 59 (2004) 283.
[65] H. Bagheri, A. Mohammadi, A. Salemi, Anal. Chim. Acta 513 (2004) 445.
[66] N. Fontanals, P. Puig, M. Galià, R.M. Marcé, F. Borrull, J. Chromatogr. A 1035 (2004) 281.
[67] N. Fontanals, M. Galià, R.M. Marcé, F. Borrull, Chromatographia 60 (2004) 511.
[68] A. Beltran, R.M. Marcé, P.A.G. Cormack, F. Borrull, Anal. Chim. Acta 677 (2010) 72.
[69] P. Qi, J. Wang, Y. Li, F. Su, J. Jin, J. Chen, J. Sep. Sci. 34 (2011) 2712.
[70] K. Hoshina, S. Horiyama, H. Matsunaga, J. Haginaka, J. Chromatogr. A 1216 (2009) 4957.
[71] C.M. Dai, J. Zhang, Y.L. Zhang, X.F. Zhou, Y.P. Duan, S.G. Liu, Chem. Eng. J. 211 (2012) 302.
[72] C.M. Dai, J. Zhang, Y.L. Zhang, X.F. Zhou, Y.P. Duan, S.G. Liu, Environ. Sci. Pollut. Res. Int. 20 (2013) 5492.
[73] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Rodil, J. González-Penas, R. Cela, J. Chromatogr. A 1216 (2009) 8435.
[74] N. Gilart, R.M. Marcé, F. Borrull, N. Fontanals, J. Sep. Sci. 35 (2012) 875.

[75] I. Tarcomnicu, A.L.N. van Nuijs, W. Simons, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Talanta 83 (2010) 795.
[76] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Anal. Bioanal. Chem. 391 (2008) 1293.
[77] J. Bones, K.V. Thomas, B. Paull, J. Environ. Monit. 9 (2007) 701.
[78] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr. A 1139 (2007) 45.
[79] N. Fontanals, B.C. Trammell, M. Galià, R.M. Marcé, P.C. Iraneta, F. Borrull, U.D. Neue, J. Sep. Sci. 29 (2006) 1622.
[80] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. A 1215 (2008) 21.
[81] P. Labadie, E.M. Hill, J. Chromatogr. A 1141 (2007) 174.
[82] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, F. Borrull, J. Chromatogr. A 1217 (2010) 2855.

1.2. Stir bar sorptive extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

As mentioned earlier, another sorptive extraction technique is stir bar sorptive extraction (SBSE), which is an equilibrium technique, similar to solid-phase microextraction (SPME). In SBSE, the extraction of the solute from an aqueous phase into an extraction phase is controlled by the partitioning coefficient of the solute between the extraction phase and the aqueous phase. As its name indicates, the extracting device is a glass stirring bar covered with the extraction phase. The principal parameter to take into account in sorptive extraction is the nature and amount of the extracting phase. In SPME and SBSE, the most commonly-used extracting material is a polymeric coat based on polydimethylsiloxane (PDMS) because, for many years, it was the only extracting phase that was commercially available [88,231]. Unlike SPME, the amount of sorptive phase in SBSE is much higher (50-250 times), resulting in higher capacity, a fact which is the main advantage of SBSE over SPME. Some studies into SBSE principles correlate the partition coefficient between the PDMS phase and the aqueous phase ($K_{PDMS/W}$) with the octanol-water partition coefficient ($K_{O/W}$), providing a suitable indication of how well the analyte can be extracted in a PDMS phase, either as a SPME fibre or SBSE coating. Figure 8 shows the existing relation between the theoretical recoveries of analytes using SPME and SBSE as a function of the log $K_{O/W}$ partition coefficient [88].

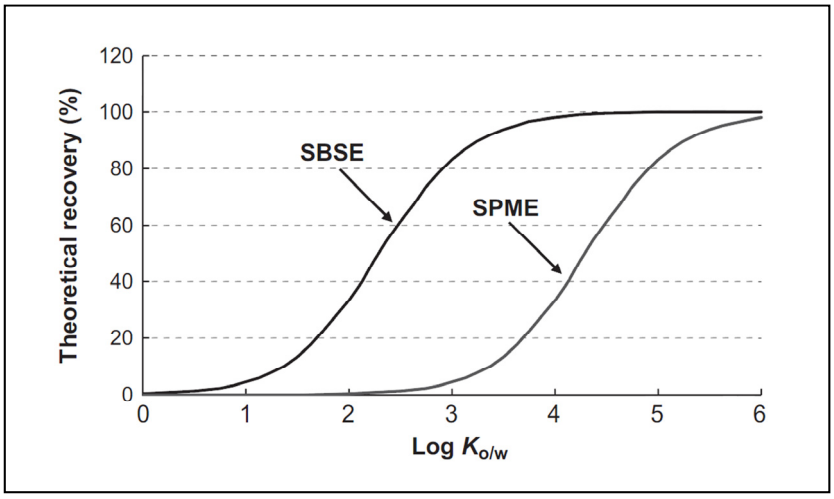


Figure 8. Theoretical recovery values for solutes as function of the log $K_{O/W}$ partition coefficient for SBSE (10 mL, 50 µL of PDMS phase) and SPME (10 mL, 0.5 µL of PDMS phase) [88].

From Figure 8, it can be concluded that the higher amount of PDMS phase in SBSE allows higher recovery values than using a SPME fibre. However, the apolar nature of the PDMS phase means that SBSE provides satisfactory recovery values for analytes with

log $K_{O/W}$ > 3, being particularly suitable for extracting compounds with low and moderate polarity, such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) or pesticides, among others [232,233]. The PDMS extracting phase as a coating for SBSE has only been commercialised by Gerstel under the trademark Twister.

Normally, SBSE is applied for extracting organic compounds from liquid samples. The sample extraction is performed by immersing the stir bar in the liquid phase and being stirred for a certain period of time. The immersion sampling mode allows direct contact between the analytes and the sorptive material, and the stirring of the sample is included within the same extraction device, enabling faster equilibration. However, stirring at a high agitating speed can result in damage of the extracting phase. Apart from immersion mode, SBSE can also be performed under headspace mode, consisting of the extraction of organic compounds in a vapour phase from liquid or solid samples. Nevertheless, this sampling mode is much less used than immersion, due to the specific material required for conducting it and the limited extraction towards volatile and semi-volatile compounds [88,90]. For these reasons, few publications can be found in literature.

Once equilibrium is reached, the stir bar is removed, rinsed with water to remove interfering compounds (salts or sample components) and cleaned with a paper tissue, before proceeding to the analyte desorption. The aim of this step is to ensure the complete transfer of the analytes from the extracting phase directly to the chromatographic system (thermal desorption (TD)) or towards an organic solvent (liquid desorption (LD)). TD has become the desorption method of choice and is widely applied, as the total amount of the analytes adsorbed onto the coating is entirely introduced into the gas chromatography (GC) system, resulting in an enhancement of the method sensitivity. TD is performed at elevated temperatures, only being suitable for thermally stable volatile and semi-volatile analytes. The main disadvantage of TD is the need for an expensive thermal desorption unit (TDU) coupled to the GC system. Despite the great method sensitivity obtained using TD, it is limited to the coupling with GC and for the extraction of mainly volatile compounds [88,90,232]. Hence, LD emerged as an alternative to TD for desorbing non-volatile and thermally labile compounds, with the stir bar being dipped in a small amount of a suitable organic solvent or solvent mixture and stirring it, or in an ultrasonic bath for a certain period of time. LD is usually followed by liquid chromatography (LC) or capillary electrophoresis (CE). Proper selection of the solvent is essential because it must be compatible with the sample as well as the extracting phase. LD offers an easy and cost-effective desorption step and the possibility of re-analysing. Figure 9 depicts the different applicability of both LD and TD in SBSE.

Despite the ease of the LD procedure, this type of desorption has been less applied than TD, because only a fraction of the desorption solvent is analysed and the sensitivity of the whole analytical method decreases. To a lesser extent, LD has also been followed by large-volume injection (LVI)-GC to increase the method sensitivity, because a larger amount of analyte can be injected to the system [234]. It should be noted that some authors mention that SBSE is considered to be a solventless technique, like SPME. However, in our point of view, SBSE is partially a solvent-free sorptive extraction technique, since TD and also LD can be used to desorb the analytes from the coating. In this Doctoral Thesis, special attention has been paid to SBSE(LD), as the studies included in it involve LC and, therefore, SBSE(LD) was required.

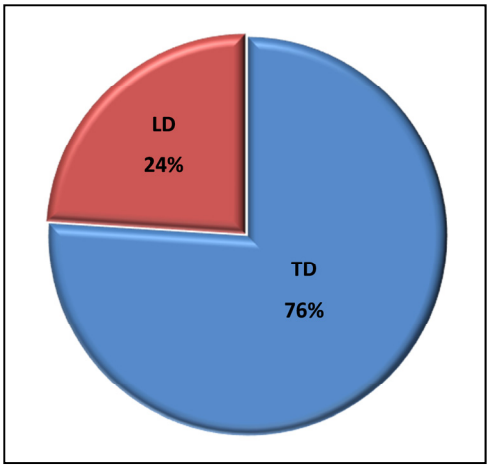


Figure 9. Percentage of the number of SBSE applications using TD and LD found in Isi Web of Knowledge until 2014.

Over the last few years, several reviews dealing with SBSE and its applications have been published [88,90,91,231-233,235-237]. It should be pointed out that most of the SBSE applications are found in environmental analysis, followed by food analysis and, finally, biomedical analysis. Table 7 presents a list of some examples of SBSE applications using the commercial PDMS stir bar in different research fields. The successful combination of SBSE(TD) and GC has provided numerous applications in the literature, some of which are reported in Table 7, with far fewer applications being found related to SBSE(LD) followed by LC or CE.

Moreover, it can be observed that SBSE has been widely and directly applied in liquid samples. Nevertheless, there are some studies in which organic contaminants in solid samples (sediments, soils and fruits) are firstly extracted using other sample

Table 7. Main applications of SBSE of organic compounds from environmental, food and biological analysis, using the commercial PDMS stir bar (Twister).

Analyte	Matrix	Sample amount	Dimension (mm)	Extraction time	Desorption	Analysis	MDLs (ng L^{-1})	Ref.
PCPs (UV filters and antimicrobial agents)	Wastewaters and river waters	50 mL	10 x 0.5	3 h	LD (1 mL of ACN, stirring)	LC-MS/MS [a]	2.5-10	[238]
PCPs (parabens and synthetic musks)	Wastewaters and river waters	100 mL	20 x 0.5	4 h	TD (300ºC)	GC-MS [a]	0.02-0.3	[239]
Triclocarban	Wastewaters	10 mL	10 x 0.5	22 h	LD (1.5 mL of MeOH, sonication)	LC-MS/MS [a]	1	[240]
15 polar pesticides	Surface waters	20 mL	20 x 1	3h	LD (0.2 mL ACN/MeOH (1/1), sonication)	LC-MS/MS [a]	20-1000 (MQLs)	[241]
PCBs and PBDEs	Sediments	0.2 g	10 x 0.5	1 h	TD (280ºC)	GC-MS [a]	0.10-0.55 (ng g^{-1})	[242]
51 POPs (PAHs, OCPs, PCBs, PBDEs)	Soil	1 g	20 x 0.5	14 h	TD (280ºC)	GC-MS [a]	0.01 to 2.0 µg kg^{-1}	[243]
35 micropollutants, pesticides and PAHs	Surface, ground and tap waters.	100 mL	20 x 0.5	14 h	TD (280ºC)	GC-MS [a]	0.1-10.7	[244]
PAHs	Environmental waters	500 mL	20 x 1	50 min/1h	LD (0.15 mL MeOH, sonication)	LC-FD [a,b]	0.03-3.41	[245]
PPCPs	Wastewaters, seawaters and sediments	10-100 mL/2 g	10 x 0.5	8 h	LD (0.2 mL ethyl acetate, sonication)	GC-MS [a]	1-853 ng L^{-1}/0.1-5 ng g^{-1}	[246]
86 POPs (PAHs, PCBs, PBDEs, OPPs, OCPs)	Marine sediments	10 g	20 x 0.5	24 h	TD (280ºC)	GC-MS/MS [a]	0.001-0.3 ng g^{-1}	[247]
Steroid sex hormones	Environmental waters/Urine	30 mL/5 or 20 mL	20 x 1	2 or 4 h	LD (1.5 mL ACN/MeOH (1/1), sonication)	LC-DAD [a]	300-1000	[248]
19 EDCs (bisphenol-A, hormones and sterols)	Wastewaters	100 mL	10 x 0.5	15 h	TD (300ºC)	GC-MS [a]	0.8-84	[249]
Organochlorine pesticides	River waters	50 mL	20 x 0.5	3 h	TD (280ºC)	GCxGC-HRTOF-MS [a]	10-44 pg L^{-1}	[250]

Table 7. Continued.

Analyte	Matrix	Sample amount	Dimension (mm)	Extraction time	Desorption	Analysis	MDLs (ng L^{-1})	Ref.
PBDEs and PBBs	Seawaters	20 mL	10 x 0.5	5 h	TD (300ºC)	GC-MS[a]	0.2-1.9	[251]
Triazines	Underground waters	20 mL	10 x 0.5	1 h	TD (275ºC)	GC-MS[a]	0.2-3.4	[252]
PAHs	Tap and surface waters/Sediments/ Fish bile	30 mL/2 g/0.3 g	10 x 0.5	1.5 h	LD (0.15 mL ACN, sonication)	MECK-DAD[a]	2000-11000	[253]
11 monohydroxylated PAHs	Human urine	5 mL + 10 mL buffer solution	20 x 1	3 h	LD (5 mL MeOH, stirring)	LC-MS/MS[a]	1-22	[254]
Fluoxetine	Plasma	1 mL + 4 mL aqueous solution	10 x 0.5	30 min	LD (0.150 mL ethyl acetate, 30 min)/TD (300ºC)	GC-MS[a]	10 (LD) / 0.46 (TD)	[255]
Testosterone and epitestosterone	Human urine	1 mL	10 x 0.5	1 h	TD (300ºC)	GC-MS[a]	300-900	[256]
Diclofenac	Human urine	0.5 mL + 4.5 mL aqueous solution	20 x 0.5	2 h	LD (3 mL ACN, stirring)	LC-UV[a]	12030	[257]
Antidepressants	Plasma	1 mL	10 x 0.5	45 min	LD (1 mL ACN, stirring)	LC-UV[a]	10000-40000 (MQLs)	[258]
VOCs	Wine	10 mL	10 x 0.5	12 h	TD (300ºC)	GC-MS[a]	0.1-174 ($\mu g\ L^{-1}$, MQLs)	[259]
Fungicides	Grapes	5 g	10 x 1	2 h	LD (1 mL of MeOH, sonication)	LC-MS/MS[a]	10 ng g^{-1}	[260]
VOCs	Wines	0.5 mL	10 x 0.5	1 h	TD (260ºC)	GC-MS[b]	0.05 - 281	[261]
Pesticides and benzo[a]pyrene	Sugarcane juice	10 mL	10 x 0.5	3 h	TD (250ºC)	GC-MS[a]	2-400	[262]
Resveratrol, piceatannol and oxyresveratrol	Wines	10 mL	10 x 0.5	3 h	TD (260ºC)	GC-MS[a]	4-14.8	[263]

[a] Immersion as SBSE sampling mode.
[b] Headsapce as SBSE sampling mode.

pretreatments, such as ultrasonic extraction (USE) or microwave-assisted extraction (MAE), before subsequently being preconcentrated with SBSE. As can be seen in Table 7, the preferred sampling mode in SBSE is immersion because of its easy performance and direct contact with the sample containing the analytes. With respect to the analytes of interest, many studies have reported the extraction of PAHs, VOCs, pesticides and pharmaceuticals and personal care products (PPCPs) from different complex matrices. The majority of the compounds analysed by SBSE show an apolar or low polar nature (high log $K_{O/W}$ values), showing high or certain affinity, respectively, towards the commercial coating based on PDMS. An important fact to mention is that, in some cases, several compounds with different chemistries and polarities are included in the same study and, therefore, the PDMS coating might fail to interact with analytes with hydrophilic properties, resulting in lower extraction efficiencies. For this reason, over the last few years, the lack of commercially available coatings for SBSE has led to increasing interest in the development of novel coatings in order to overcome the limitations of PDMS and obtain improved extraction efficiencies for a wider range of analytes. In this Doctoral Thesis, a special section (Section 1.2.2.1) has been included in which a review outlines different strategies for developing new SBSE coatings and their main applications.

Lastly, Table 7 also includes some parameters that affect SBSE performance, such as sample amount, dimensions of the stir bar or extraction time. These parameters and others related to SBSE will be discussed in the following section, showing their effect on the extraction efficiencies of the analytes of interest.

1.2.1. Parameters affecting stir bar sorptive extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

In stir bar sorptive extraction (SBSE), all of the parameters involving the extraction and desorption procedures should be taken into account for enhancing the transfer of the analytes to the extracting phase of the stir bar. With regard to the extraction step, the most commonly studied factors are sample pH, addition of an inert salt or an organic modifier, stirring speed and extraction time, followed by sample volume, extraction temperature and volume of the extracting phase. It is worth mentioning that the cited extraction parameters are directly related to the immersion sampling mode, since it is the most widely used along with the SBSE applications and is also applied in the studies reported in this Doctoral Thesis. In contrast, fewer variables need to be evaluated during the desorption step, with desorption solvent, volume, time and mode being the most relevant for liquid desorption (LD), and desorption temperature and time for thermal desorption (TD). In this section, the main variables affecting the whole SBSE procedure are studied in detail in order to observe their influence on the extraction efficiencies of the analytes of interest. Most of the examples described below mainly focus on improving the performance of the polydimethylsiloxane (PDMS) coating, but other studies have also been included in which the effect of certain parameters on the SBSE performance of in-house coatings is shown.

1.2.1.1. Extraction procedure

During extraction by immersion of the stir bar, numerous parameters have to be borne in mind to obtain the highest extraction efficiencies, as can be observed in Figure 10. Among these variables, sample pH and addition of an inert salt are crucial for modifying the analytes or sample conditions and affecting the equilibrium, while stirring speed, for example, accelerates the process, reaching the equilibrium faster.

Sample pH

Starting with the effect of the sample pH on the extraction efficiencies, the control of this parameter is necessary to promote the non-ionic form of the analytes with acidic or basic functionalities and to enhance the sorption towards the PDMS phase. However, samples should not be adjusted to pH values that are too basic or acidic because this may damage the PDMS phase and shorten its lifetime [88,90]. As an example, Chaves et al. [258] evaluated the effect of the pH (from 7 to 11) in order to extract a group of basic antidepressants drugs with pK_a values ranging between 8.7 and 10.2. The authors observed that the highest extraction efficiencies were achieved by adjusting the sample to pH 9, at which the analytes of interest were partially or totally in their non-ionic form. Another study reported the effect of the sample pH (2, 4 and 7) when ionisable compounds are extracted, such as a group of pharmaceuticals and personal care

products (PPCPs) with acidic and basic behaviour [246]. It was observed that the extraction efficiencies of the acidic analytes (pK_a~4) increased when working at pH 2 because the non-ionic form of those compounds prevails in acidic aqueous solutions. The rest of the compounds were not significantly affected when working at different pH values. Therefore, in this study, pH 2 was selected as the optimal value. However, in other studies, no significant influence of the sample pH over the signal response of the compounds was observed, especially for extracting non-ionisable compounds, such as PAHs. For these compounds, most of the SBSE procedures were performed without a sample pH evaluation [243-245].

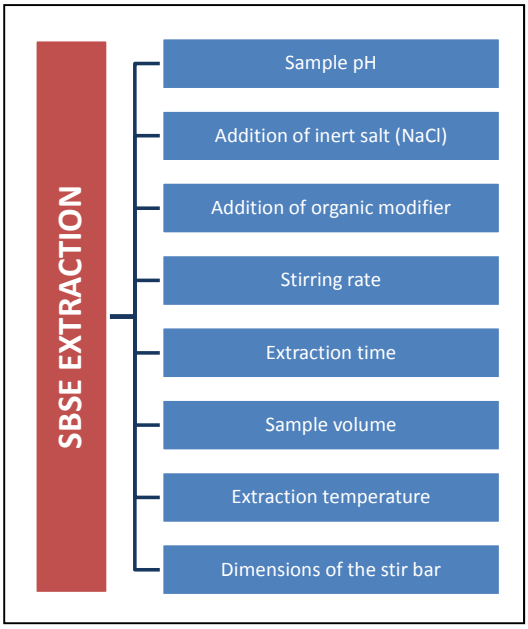


Figure 10. Main parameters studied in SBSE extraction.

Sample pH becomes a more important factor when an in-house coating for SBSE is used, which usually contains polar functional groups, ionisable or otherwise, in order to enhance the sorption of more polar compounds. For instance, Melo et al. [264] synthesised an in-house coating based on PDMS and polypyrrole (PPy) for the SBSE of six basic antidepressants (pK_a values from 8.7 to 10.2) from plasma samples. After testing different sample pH values (5, 7 and 9), the results showed that, at pH 9, the highest extraction efficiencies were obtained for all the compounds because, under these conditions, the compounds were partially or totally in the non-ionic form, interacting with PPY functionalities through hydrogen bondings. A more interesting study, reported by Huang et al. [265], showed the significant effect of varying the pH of

the sample on the sorption of the analytes since both the in-house coating and the analytes (quinolones) presented ionisable functional groups. The in-house coating for SBSE was based on methacrylic acid-3-sulphopropyl ester potassium salt (MASPE) and divinylbenzene (DVB). From low pH values, the extracting phase was totally deprotonated and, at the same time, the basic compounds possessed positive charges, so cation-exchange and hydrophobic interactions were established thanks to MASPE and DVB monomers, respectively. At pH values higher than 5, the extraction efficiencies decreased because the cation-exchange interactions were weakened. Eventually, a sample pH of 5 was the optimal value.

Salt addition

Another parameter to take into account is the addition of an inert salt, which modifies the ionic strength of the sample solution, which may or may not improve the extraction efficiencies of the analytes. Throughout the studies published about SBSE applications, it is observed that the presence of an inert salt, such as sodium chloride (NaCl), in the solution leads to an increase in the extraction efficiencies for polar compounds, because the ionic strength of the solution is modified and the solubility of the analytes in water is reduced, presenting more affinity towards the PDMS phase [88,90,241,248,253]. In contrast, it has been observed in some cases that the addition of salt produces the opposite effect for apolar compounds (usually with log $K_{O/W}$ >3). According to some authors [241,248,253,266], salt addition causes an “oil effect”, in which NaCl helps to move the apolar analytes to the water surface, minimising their interaction with the PDMS phase.

For instance, León et al. [266] developed an analytical method based on SBSE(TD) followed by gas chromatography-mass spectrometry (GC-MS) for extracting 35 semi-volatile organic compounds (pesticides and polycyclic aromatic hydrocarbons (PAHs)) from water samples using a PDMS-coated stir bar. For the compounds investigated, with log $K_{O/W}$ values ranging from 2 to 7.66, the effect of adding NaCl to the sample solution was studied. Firstly, it was observed that the compounds with the lowest log $K_{O/W}$ (atrazine, simazine and propazine) displayed a significant increase in their signal responses when increasing amounts of NaCl (5, 10, 20 and 30%) were added. Unfortunately, the addition of 30% of NaCl led to a marked decrease in the retention of the most apolar compounds, such as PAHs and some pesticides (DDD, DDT, DDE, aldrin and heptachlor), with a global signal reduction of approximately 75% being observed for these compounds. As a compromise, 20% of NaCl was eventually added. Another study describes the effect of adding several concentrations of NaCl (5, 10, 20 and 30%) to the sample solution for improving the extraction of steroid sex hormones from water and

urine samples using a PDMS-coated stir bar [248]. The studied analytes could basically be considered to be apolar, due to their high log $K_{O/W}$ values (3.94-4.68). Despite their hydrophobicity, the results showed that the higher the ionic strength, the higher the extraction efficiencies obtained for most of the compounds. However, it should pointed out that the most apolar compound (mestranol, log $K_{O/W}$ = 4.68) displayed an opposite effect, with its extraction efficiency decreasing considerably. In the end, the addition of 20% of NaCl was selected as a compromise. Moreover, adding a high concentration of NaCl is not recommended, because it may seriously damage the PDMS phase [241,267].

Salt addition could help to increase the extraction efficiencies of the analytes even when a polar in-house coating is used. As an example, an in-house coating for SBSE based on vinylimidazole (VI) and DVB was used for extracting a group of sulphonamides, which are strongly polar compounds (log $K_{O/W}$ between -0.09 and 1.68), from milk samples [268]. Although the extracting phase of the stir bar presented polar functionalities to interact with these compounds, the presence of 15% of NaCl enhanced their affinity towards the in-house coating. The authors mentioned that the high polarity and water solubility of these compounds was slightly reduced with the salt addition. Therefore, it may be concluded that the addition of an inert salt must be evaluated carefully in order to improve the extraction efficiencies of the analytes of interest as much as possible.

Addition of an organic modifier

As well as salt addition, it is also possible to include an organic modifier, such as methanol (MeOH) or acetonitrile (ACN), during the SBSE procedure. The aim of the organic modifier is to minimise the adsorption of the most hydrophobic compounds to the glass wall. Nevertheless, the addition of an organic modifier can also have a negative effect towards polar analytes (log $K_{O/W}$ < 2.5), since their solubility in the water phase can increase, reducing the extraction efficiencies [90]. To prove this, Chary et al. [269] studied the influence of adding 5% and 10% of MeOH to the sample solution for extracting 25 endocrine disrupting compounds (EDCs), mainly pesticides (log $K_{O/W}$ = 2.1-6.9), from environmental water samples. The results confirmed that the presence of 5% of MeOH in the solution enhanced the retention of all of the studied compounds onto the PDMS phase. However, at the highest MeOH content (10%), there was a significant decrease in their extraction efficiencies, because the sample solution became less polar, helping to solubilise the hydrophobic compounds and reduce their affinity towards the PDMS phase. When MeOH was added, satisfactory results in terms of extraction efficiencies were observed in the determination of PPCPs [246], pyrethroid pesticides [270] and personal care products (PCPs) [238], among others. However, other studies

reported that the effect of adding MeOH as an organic modifier was negative [241,248,253,266,271]. In particular, Margoum et al. [241] agreed that a certain amount of MeOH could help to increase the extraction efficiencies of 15 polar pesticides, but it was observed that, when the sample also contained NaCl, the presence of MeOH provided worse results. As the effect of salt addition was more significant, the use of MeOH was discarded.

It is important to point out that the effect of adding an organic modifier is more relevant when low or moderate polar compounds are analysed using the PDMS coating, as observed in the examples cited above. In contrast, when high polarity compounds were extracted using an in-house coating with a polar structure, no addition of an organic modifier is required since polar compounds do not display adsorption onto the glass wall and, as a result, a decrease in the extraction efficiencies of the analytes would be detected [272,273].

Stirring rate

The stirring rate is also evaluated because it can accelerate the extraction and higher extraction efficiencies can be achieved within a fixed extraction time. This fact can be attributed to a decrease in the thickness of the boundary layer between the PDMS phase and the sample solution. Nevertheless, the use of high stirring speeds can lead to damage of the PDMS phase, because of the direct contact between the coating and the bottom of the sample vessel [88,90]. In the literature, the most commonly-applied stirring rates range from 500 to 1500 rpm. However, discrepancies with this statement can be observed. On the one hand, some authors have demonstrated that the higher the stirring rate applied, the higher the extraction efficiencies obtained [238,241,253]. An example of the effect of the stirring rate is provided by Do Rosário et al. [253], who tested different stirring rates (750, 1250 and 1500 rpm) to increase the extraction efficiencies of a group of PAHs using the PDMS-coated stir bar. The results showed that the highest stirring rate presented better recovery values without any physical deterioration, with 1500 rpm being selected as the optimal value for further experiments. On the other hand, other studies state that this parameter may have a negative effect on the extraction efficiencies or that no significant differences were observed at higher stirring rates [248,270,274]. For instance, Quintana et al. [274] explained that increasing the stirring rate caused a negative effect on SBSE performance, showing lower extraction efficiencies at higher stirring rates. One possible explanation given by the authors is the lack of homogeneity in agitation and bubble formation.

Despite the high mechanical stability of the commercial PDMS-coated stir bar and the possibility of applying a high stirring rate, it may be concluded that this parameter can help to improve SBSE results, but it is not as essential a parameter to control as extraction time or salt addition. In contrast, the stirring rate becomes a crucial factor to take into account when using in-house SBSE coatings, which usually present lower mechanical stability than the commercial one. The evaluation of this parameter is also performed, but the stirring rates tested are usually lower than those studied using the PDMS-coated stir bar. Hence, the most commonly-applied stirring rate using an in-house coating is between 250 and 900 rpm. Most of the studies relating to an in-house coating for SBSE usually evaluate different stirring rates, but in order to increase the lifetime of the synthesised coating, the lowest stirring rate is usually selected [275,276].

Extraction time

One of the most widely-studied SBSE parameters is the extraction time, since it is important to control precisely when the equilibrium is reached to obtain the best SBSE performance. The extraction time basically depends on the sample volume, stirring speed, dimension of the stir bar and extraction temperature, and it can range from 10 min to 25 hours [90,232,277]. In some cases, some authors preferred not to work under equilibrium conditions because it took longer, resulting in a decrease in the enrichment factor. For instance, Vercauteren et al. [278] proposed the determination of tributyltin and triphenyltin (organotin compounds) in environmental samples by SBSE(TD) and gas chromatography-inductively coupled plasma mass spectrometry (GC-ICPMS). In this study, although the authors stated that several hours would be required to achieve the equilibrium, they eventually decided to sacrifice the accomplishment of equilibrium, due to the high sensitivity provided by GC-ICPMS, with 15 min being selected as the optimal extraction time.

The sample volume also has an effect on the extraction time, as can be observed in different studies, in which polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are determined in environmental solid samples by ultrasonic extraction (USE) or pressurised liquid extraction (PLE) followed by SBSE(TD) coupled to GC-MS or GC-MS/MS [242,243,247]. From the results of these studies, it can be deduced that the sample volume really affects the extraction time, because samples volumes of 5, 100 and 210 mL led to extraction times of 1, 14 and 24 h, respectively.

Proof that the extraction time is also related to the dimensions of the stir bar of the PDMS phase and its ability to stir the sample is reported by Ochai et al. [250], who investigated the extraction time profile using two PDMS-coated stir bars with different

dimensions (10 x 0.5 mm and 20 x 0.5 mm) for extracting organochloride pesticides from 50 mL water samples, under the same stirring rate conditions. The results demonstrated that the 20 mm-length stir bar with larger dimensions and higher amount of extracting phase could stir the sample more effectively and reach equilibrium in just 3 h. In contrast, the 10 mm-length stir bar managed to achieve equilibrium in 6 h. In addition, the thickness of the extracting phase also influences the extraction time. It is observed that the mass transfer rate of the target analytes is very slow in thick coatings, which results in longer extraction times [279,280]. Huang et al. [279] synthesised three in-house SBSE coatings with different thicknesses (1.0, 1.5 and 2.0 mm) and they observed that the increase in thickness led to more time being required to achieve equilibrium and, at the same time, the same effect was observed in the desorption step, with longer times being needed when the thicker coating was used.

Extraction temperature

At elevated temperatures, equilibrium can be reached more quickly due to an increase in the diffusion of the analytes by lowering the viscosity [235]. With respect to extraction efficiencies, the application of high temperatures can lead to a decrease in log $K_{O/W}$ value, resulting in lower affinity of the analytes towards the PDMS-coated stir bar, as well as the reduction of the lifetime of the PDMS phase [90,281]. However, there are some studies that have reported that the higher the extraction temperature applied, the higher the extraction efficiencies obtained [282,283]. Other authors reported that there is no significant or positive effect of increasing the extraction temperature. For instance, Pedrouzo et al. [238] studied this parameter in order to improve the extraction efficiencies of UV filters and antimicrobial agents from water samples. Thus, a range of temperatures (from 30 to 70ºC) were tested and the results did not show a significant increase in the signal responses of the target analytes, with 30 ºC being selected as the best extraction temperature. Other studies have also presented the same results under the optimisation of the extraction temperature, without any improvement in the extraction efficiencies [239,249]. It is important to add that, in most of the studies in which an in-house SBSE coating was synthesised, SBSE was carried out at room temperature. A possible explanation could be that these in-house coatings would lack thermal stability.

Sample volume

Sample volume is a less studied parameter in SBSE. As mentioned previously, sample volume usually affects the extraction time and, at the same time, it can also influence the extraction efficiencies of the analytes. The total analyte amount extracted depends

on the phase ratio (β), which is the quotient of the volume of the water sample and the volume of the coating, with an inverse relationship between them [87]. Therefore, the higher the sample volumes applied, the lower the extraction efficiencies obtained within a fixed period of time [87,90,284,285]. However, the chromatographic response can increase due to an increase in the amount of analyte in the sample solution [247,284]. As an example, Giordano et al. [284] stirred different water sample volumes (20, 40, 50 and 100 mL) containing the same amount of pesticides for 1 h. The results confirmed the extraction efficiencies were lower when the sample volume increased, particularly due to the fact that higher extraction times were required. Therefore, as a compromise between the extraction efficiencies and the preconcentration factor, 50 mL was selected as the sample volume for further experiments.

Dimension of the stir bar

The dimensions of the stir bar can influence SBSE performance, not only in terms of the size of the stir bar, but also the volume of extracting phase. As discussed earlier, the extraction time can be reduced if stir bars with high dimensions are used, since it favours the movement of the analytes towards the extracting phase. With respect to the volume of extracting phases, this parameter usually has a positive effect on the extraction efficiencies. Higher amounts of analytes are expected to be extracted if higher volumes of extracting phases are used [90,250,266].

For instance, León et al. [266] observed that increasing the volume of PDMS phase (from 20 x 0.5 mm, 50 µL to 10 x 0.5 mm, 25 µL), under non-equilibrium conditions, provided higher extraction efficiencies for a group of pesticides and PAHs from 100 mL of water samples. In contrast, more recently, Camino-Sánchez et al. [247] reported the opposite effect for extracting a group of 86 persistent organic pollutants (POPs). They observed that higher recovery values were obtained for 50 mL of water samples using the PDMS stir bar with dimensions of 20 x 0.5 mm, instead of using the thicker stir bar (20 x 1 mm). In this study, the extracting phase volumes of the stir bars were different, but also the thickness of their coatings. Therefore, a possible justification of the decrease in the extraction efficiencies could be due to the thickness. It is known that higher thickness leads to higher extraction times and so it is possible that the evaluation of this parameter was performed under non-equilibrium conditions, showing lower extraction efficiencies using the thicker coating. Moreover, other authors did not find any significant differences when the amount of PDMS phase was increased, such as for the extraction of PBDEs and polybrominated biphenyls (PBBs) [251] or for a group of PPCPs [246], with the smallest stir bar (10 x 0.5 mm) being selected in most cases, because lower desorption volumes were required.

To sum up, many extraction parameters should be taken into account to achieve the highest enrichment factor possible. Based on the discussion above, special attention should be paid to parameters including extraction time, addition of modifiers (NaCl or MeOH), sample pH and log $K_{O/W}$ value of the analytes, with the last of these being essential for predicting their behaviour towards the PDMS-coated stir bar. In addition it has been demonstrated that PDMS phase shows low affinity towards moderate and high polar compounds, which could be improved with the addition of NaCl. Another option for promoting the sorption of more polar compounds is the use of a different SBSE coating that contains polar functional groups to interact with the analytes. Therefore, the type of coating is an essential parameter to take into account if more polar and improved SBSE performances are desired. This will be extensively discussed in Section 1.2.2.1. This parameter has been the focus of different research studies and the present Doctoral Thesis.

1.2.1.2. Desorption procedure

The desorption step is also a crucial procedure in SBSE. As mentioned in the previous section, analytes can be released from the SBSE coating through LD or TD, depending on the separation technique that follows. In Figure 11, the most commonly studied variables in both LD and TD are shown. These must ensure the complete desorption of the analytes from the coating and avoid memory effects. Throughout this section, all parameters involving LD and TD are discussed.

Liquid desorption

The LD of an SBSE coating consists of immersing the stir bar in a suitable organic solvent to release the analytes from the extracting phase, by stirring or sonication for a certain time. Then, an aliquot of the LD extract can be directly injected into the chromatographic system or the LD extract can be evaporated to dryness (to switch the solvent or achieve higher enrichment factors), redissolved and injected into liquid chromatography (LC) or capillary electrophoresis (CE). In the research for this Doctoral Thesis, LD was the chosen desorption method because it focuses on improving the extraction of polar organic compounds using SBSE followed by LC. As shown in Figure 11, the most frequently studied variables are the nature of the desorption solvent, desorption time, desorption volume and the desorption mode (agitation or sonication).

The **nature of the desorption solvent** is the principal factor to be evaluated during LD, since the analytes must have higher affinity towards the solvent than the extracting phase to assure total desorption. It is well known that LD is suitable for non-volatile and

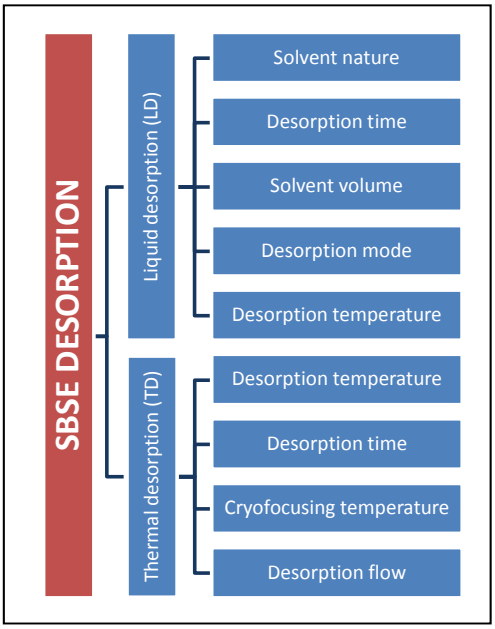


Figure 11. Main parameters studied in SBSE desorption.

thermolabile compounds with high and moderate polarity (log $K_{O/W}$ < 3). Therefore, a polar organic solvent is usually required to desorb the analytes, with ACN, MeOH or a mixture of these two solvents being most commonly used in LD [238,240,241,245,248,253,286,287]. All these studies agree that, in some cases, the differences observed in terms of recovery values using ACN, MeOH or a mixture were not significant. However, it is important to mention that ACN usually displays a slightly higher stripping capacity than MeOH or a mixture of the two [238,253,286,287]. This can be attributed to the fact that ACN is slightly less polar than MeOH and, as a result, it is able to desorb analytes with low polarities and moderate polarities, such as PAHs or some PCPs. Although the use of LD is mainly followed by LC or CE, there are some examples in which LD is followed by GC-MS [246,269] or large volume injection (LVI)-GC-MS [270,274,288], with the latter technique showing an increase in sensitivity due to the injection of larger volumes. In some of these studies, as well as testing the common organic solvents (MeOH and ACN), ethyl acetate or hexane were evaluated to desorb the analytes from the PDMS phase, before being injected directly into the GC system, thereby avoiding solvent switch (evaporation).

Some differences in the nature of the desorption solvent can be found when in-house coatings are used. The in-house SBSE extracting phase usually interacts with the analytes by hydrophilic interactions, such as hydrogen bondings or ion-exchange

interactions. Sometimes, these interactions are not completely disrupted by an organic solvent. Therefore, solvent mixtures of MeOH or ACN with acids or bases are commonly used [265,289,290]. For instance, an in-house coating based on methacrylic acid-3-sulphopropyl ester potassium salt (MASPE) and divinylbenzene (DVB) was able to extract nitroimidazole compounds from honey samples through ion-exchange interactions (sulphonic group in MASPE structure) [290]. The authors observed that better extraction efficiencies were obtained when a mixture of MeOH and an acidic solution at pH 2 was used as the desorption solvent rather than a pure organic solvent. Moreover, the optimal composition of the mixture was also evaluated and MeOH:water at pH 2 (9:1) was chosen as the optimal mixture.

Another parameter to control during LD is the **desorption time**, which usually ranges from 10 min to 30 min [90,245,246,248,254]. Generally, desorption times are shorter than extraction times, since the analytes present higher affinity to the stripping solvent and they are quickly released from the PDMS phase. However, there are some studies in which the desorption step takes longer, up to 60 min [240,254]. With reference to in-house coatings, it has been observed that long desorption times are usually required to desorb the analytes from the in-house coatings (0.5-2.5 h), especially for those stir bars covered by a monolithic extracting phase [275,279,291]. The reason for this is the thickness of this type of in-house coating, which depends on the mould used to obtain the desired dimension of the final stir bar and the usual values are between 1 and 1.5 mm. In contrast, the thickness of in-house coatings obtained by the sol-gel process is controlled during their synthesis, with thickness values in the range of μm, leading to shorter desorption times.

Moreover, the **volume of the organic solvent** used during the LD should be evaluated because it must ensure the complete immersion using the minimal volume possible (0.15-5 mL) and, as such, it depends on the stir bar dimensions. In order to achieve the highest preconcentration factor, stir bars are desorbed using small volumes of organic solvents (0.15-1 mL) inside a glass insert [238,253,274]. Some studies report the evaluation of the desorption volume and all of them agreed that the use of large volumes of solvent is not necessary to achieve the complete desorption of the analytes. For example, several volumes of ethyl acetate (0.1, 0.2 and 0.5 mL) were tested to desorb a group of phenols, herbicides and pharmaceuticals from the PDMS-coated stir bar. The authors observed that there was an increase in recovery values between using 0.1 mL and 0.2 mL. However, when 0.5 mL was tested, the recovery values were similar to those using 0.2 mL. Therefore, in order to obtain the highest enrichment factor, 0.2 mL was selected as the desorption volume.

The **mode** in which the analytes are desorbed from the stir bar, such as magnetic stirring or sonication, can also have an effect on extraction efficiencies. Firstly, desorption using sonication usually enables the use of smaller volumes of stripping solvent, since desorption takes place in a small vial. However, if magnetic stirring is applied, higher desorption volumes are usually required to cover the stir bar completely and allow suitable agitation. Apart from this feature, it has been observed that sonication can accelerate LD [270,284] in comparison to magnetic stirring. Due to the possibility of using smaller volumes as well as achieving quicker analyte desorption, in most of the cases, sonication is preferred to magnetic stirring [240,241,248,253,269,270,284,286,288]. The commercial PDMS stir bar can withstand sonication treatment. However, the novel in-house SBSE coatings based on monolithic phases are more susceptible to damage when sonication liquid desorption is used, since they usually present lower physical stability than the commercial ones. Hence, most of the studies choose magnetic stirring to release the analytes from the coating [279,292-296].

The **desorption temperature** is another parameter that can be optimised, but it is less widely studied than the other parameters discussed above. As with sonication, applying temperature during the desorption step can also accelerate the stripping of the analytes from the stir bar. However, the volatilisation of the organic solvent should be taken into account, as well as the lifetime of the extracting phase stir bar. For these reasons, most of the studies involving commercial or in-house SBSE coatings performed the liquid desorption at room temperature [241,287,297,298].

Thermal desorption

Although TD was not used during the development of the studies included in this Doctoral Thesis, a brief description and discussion of most commonly evaluated parameters in TD is included due to its widespread use and numerous SBSE applications in the literature. In TD, the stir bar is placed into a glass or stainless steel thermal desorption tube and introduced into the thermal desorption unit (TDU). A TDU consists of two programmable temperature vaporizers (PTVs). The first PTV is heated to desorb the analytes from the coating for a certain period of time and, subsequently, the analytes are transported through a carrier gas (usually helium) to the second PTV (cryogenic trap), which is maintained at lower temperatures to cryofocus the desorbed analytes before GC injection. It should be noted that TD enables a total automated desorption step without any sample or organic solvent manipulation, resulting in an environmentally friendly procedure [88,90,237]. Therefore, the optimisation of TD

parameters, as shown in Figure 11, is essential for ensuring the total transfer of the desorbed analytes into the chromatographic system, thereby enhancing sensitivity.

With respect to the **desorption temperature**, the higher temperature applied, the better the volatilisation and desorption of the analytes from the coating that is usually obtained, resulting in an increase in sensitivity [251,252,263,299]. However, it can result in a reduction of the lifetime of the stir bar [300]. The analytes are usually desorbed at elevated temperatures, ranging from 150 to 300ºC [231,239,285,299]. For instance, Kawaguchi et al. [299] developed an analytical method based on SBSE(TD)-GC-MS for extracting benzophenone and two derivatives from water samples using PDMS phase. The authors evaluated the effect of the desorption temperature (from 150 to 275ºC) for a fixed period of time (5 min). The results showed that benzophenone could be easily desorbed from the PDMS phase even at the lowest temperature (150ºC) with recovery values of about 90%. In contrast, its two derivatives required high temperatures (above 250ºC) to achieve complete desorption, due to their higher affinity towards PDMS, since they presented high log $K_{O/W}$ values. Eventually, a temperature of 250ºC was selected to desorb the analytes. It is worth mentioning that few studies report the use of TD to release analytes from an in-house coating [280,282]. The first reason is that most of the analytes extracted using an in-house coating usually have a high polarity and low volatility, and are unsuitable for TD. However, in addition, these in-house coatings are sometimes characterised not to be fully thermally stable and so LD is the most appropriate option for desorbing the analytes and maintaining the integrity of the in-house coating.

Another parameter to take into account is the **desorption time**. This variable also plays an important role in ensuring the total desorption of the analytes. Therefore, high desorption times usually have a positive effect on sensitivity. Moreover, higher desorption times are also used to minimise the carryover effect [90,252,263,301]. For instance, Rodil et al. [301] showed that lower temperatures (220ºC) required longer desorption time to obtain a low carryover effect. However, at higher temperatures (280ºC), the same carryover effect was observed but in a shorter desorption time. In the end, the authors decided to work at 250ºC for 15 min, with the lowest carryover effect being observed.

Cryogenic focusing is another important step to control prior to chromatographic separation, focusing the analytes in a trap at low temperatures to ensure their entrance into the GC column. Therefore, the main parameter to evaluate is the **cryofocusing temperature**, which depends on the volatility of the analytes of interest. The range of

study is between -150ºC and 20ºC. With this in mind, the most volatile compounds require low cryofocusing temperatures [242,266,285,299,302].

Desorption flow is another parameter studied if TD is applied, but to a lesser extent. The carrier gas, usually helium, facilitates the transfer of the analytes towards the cryogenic trap and so can result in an enhancement of the analyte signal. The most common values of desorption flows range from 50 to 100 mL min^{-1}. Generally, an increase in the signal is observed at higher desorption flows. An insufficient carrier gas can lead to poor transfer of the less volatile compounds, as well as carryover effects [266,302]. Other authors did not observe significant differences when increasing values of desorption flows were applied [252,263].

To conclude, some studies have been reported, in which they compare the SBSE performance obtained using TD or LD followed by GC-MS for determining pyrethroids (insecticides) in water samples [303] and fluoxetine (antidepressant drugs) in human plasma [255]. In both studies, the authors agreed that TD provided higher sensitivity and lower method detection limits (MDLs) than using LD. For instance, in one study [303], the pyrethroid compounds had high log $K_{O/W}$ values (5.62-8.15), being suitable for proper retention onto the PDMS coating. For LD, when ethyl acetate was used as the optimal desorption solvent, recovery values ranging from 29 to 62% were obtained for 10 mL of water samples. However, under the same extraction conditions, but using TD, higher recovery values were achieved, between 41 and 92%. Moreover, MDLs obtained using TD and LD considerably differed, with values between 0.02 and 1.4 ng L^{-1} for TD and 8 and 320 ng L^{-1} for LD when 10 mL of water sample were analysed.

1.2.2. SBSE limitations and promising solutions

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

Throughout the previous section, the wide applicability of stir bar sorptive extraction (SBSE) has been demonstrated in environmental, food and biological research fields. However, this technique also presents certain limitations, such as physical damage of the coating, carryover effect or the lack of more commercial and polar coatings, with the last of these drawbacks being the most important limitation for SBSE. In recent years, advances in novel extraction devices, modes and phases related to SBSE have been made and have gradually resolved the main limitation of this extraction technique.

Sometimes, the carryover effect can be observed in SBSE stir bars and it is extremely undesirable, especially in quantitative analysis. It is possible that certain compounds or interferences cannot be completely desorbed from the polydimethylsiloxane (PDMS) extracting phase, due to high hydrophobicity of the studied compounds as well as of the PDMS extracting phase. Therefore, it is really important to ensure that the reconditioning procedure is reliable and efficient for removing any undesorbed impurities/target analytes. When SBSE with thermal desorption (TD) is applied, the most common way to avoid carryover effects is to perform the conditioning of each stir, keeping it at high temperatures (250-300ºC) for several hours (2-4 h) in the thermal desorption unit (TDU) [239,243,251,302,304]. In contrast, the stir bars desorbed by liquid desorption (LD) are conditioned by agitation/sonication for a certain period of time (30 min - 2 h) with a suitable solvent (acetonitrile (ACN) or methanol (MeOH)/dichloromethane (DCM)) [240,241,253,257,305]. In particular, Kole et al. [257] have studied the carryover effect when diclofenac was extracted from human urine using SBSE(LD) followed by liquid chromatography-ultraviolet-visible detection (LC-UV). In this study, after the reconditioning step (MeOH/DCM solution, 10 min sonication before drying at 220ºC for 15 min), the stir bars were desorbed again using the optimal desorption solvent (3 mL ACN) and no peak appeared corresponding to diclofenac. This shows the effectiveness of this reconditioning step. Therefore, it is important to control the possibility of remaining compounds retained onto the extracting phase.

In previous sections, a number of studies have reported the development of multi-residue methods using SBSE, in which a great number of compounds with different chemical properties have to be analysed. During SBSE optimisation, some parameters can be observed to affect a group of compounds positively, while another group of compounds can respond negatively under the same conditions. Therefore, in most cases, the final SBSE conditions are selected as a compromise between obtaining the highest extraction efficiencies for most of the target analytes and extracting the highest number of compounds. To resolve this problem and ensure the highest extraction efficiencies for all of the studied compounds, several strategies have been proposed in

SBSE, such as multi-shot (or dual), sequential and dual-phase modes. In Figure 12, schematic representations of the different SBSE modes are shown.

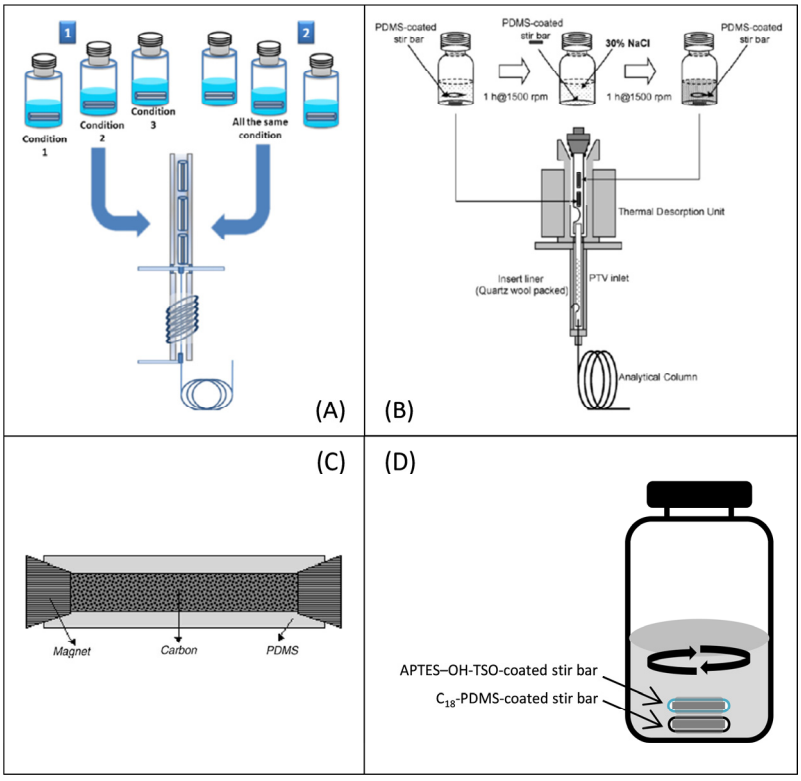


Figure 12. The schemes of the different SBSE modes: (A) multi-shot SBSE [90]; (B) sequential SBSE [306]; (C) dual-phase SBSE using one stir bar with different extracting phases [307] and (D) dual-phase SBSE using two stir bars with different extracting phases each one [276].

Multi-shot mode in SBSE consists of analysing different samples under the same or different extraction conditions using a stir bar per sample, and then all the stir bars are simultaneously desorbed in a TD unit followed by gas chromatography (GC) [90]. The procedure of the multi-shot SBSE is represented in Figure 12 (A). In 2004, Kawaguchi et al. [308] was the first to apply this approach to determine oestrogens in river waters by headspace sampling mode followed by SBSE(TD)-GC-MS. When five water samples of 10 mL were analysed under the same extraction conditions by multi-shot mode, lower LODs were achieved (0.2-1 ng L^{-1}) rather than analysing 50 mL of water sample by single-shot mode (0.5-2 ng L^{-1}), demonstrating an increase in sensitivity using this approach. Similarly, Van Hoeck et al. [309] developed a multi-residue screening method for

determining endocrine disrupting compounds (EDCs) and pharmaceuticals in water samples using a multi-shot SBSE(TD)-GC-MS. In this study, four different 10 mL aliquots were extracted under different conditions to enhance the extraction of different groups of compounds. Other studies have been reported in which two stir bars (dual SBSE) were used to extract different organic compounds from complex matrices under the same or different conditions [310,311]. More recently, an interesting approach was proposed by Arbulu et al. [312], in which two stir bars (dual SBSE) were used to extract organoleptic compounds from the same sample (wine), one extracting in immersion mode and the second extracting in headspace mode. As a result, the authors emphasised that the extraction of both volatile and semi-volatile compounds could be performed simultaneously, achieving adequate sensitivity.

In sequential SBSE mode, the same sample is sequentially extracted under different extraction conditions using one or more stir bars and then the stir bars are also simultaneously desorbed by SBSE(TD)-GC-MS (Figure 12 (B)). As an example, Ochiai et al. [306] were able to extract 80 pesticides (log $K_{O/W}$ values of 1.7–8.4) at ultra-trace levels from river water samples (5 mL) using sequential SBSE(TD)-GC-MS. In this study, while the first extraction was performed without salt addition, the second was conducted with 30% of NaCl. As a result, the most hydrophobic compounds were extracted in the first extraction, whereas the hydrophilic ones were extracted in the second case. Thus, using sequential SBSE, higher recovery values were obtained than using conventional SBSE. A similar study also reports the application of sequential SBSE(TD)-GC-MS for extracting pesticides from underground and surface waters [313]. Unlike multi-shot SBSE, in sequential SBSE, a lower sample volume is required, since the different extractions are performed on the same sample solution.

A few years ago, another way to improve the retention of compounds with different polarities was achieved using a dual-phase SBSE approach, which involved only one stir bar coated and packed with different sorptive materials (Figure 12 (C)) [307,314] or the use of two differently coated stir bars placed in the same sample solution for the simultaneous extraction of the target analytes (Figure 12 (D)) [276]. For instance, Bicchi's research group introduced dual-phase twisters, consisting of a short PDMS tube and packed with different types of adsorbents, such as activated carbons, Tenax GC or a bisphenol/PDMS copolymer, for extracting a group of compounds with widely differing physico-chemical properties from food samples [307,314] (Figure 12 (C)). The authors reported that the dual-phase twisters resulted in increased concentration capability in comparison to conventional twisters (PDMS stir bar), especially for volatile and polar compounds. The other dual-phase SBSE approach has recently been reported by Xu et al. [276], who developed two stir bars coated with different extracting phases: 3-

aminopropyltriethoxysilane (APTES)-hydroxyterminated silicone oil (OH-TSO)-coated stir bar and a C_{18} silica-polydimethylsiloxane (C_{18}-PDMS)-coated stir bar (Figure 12 (D)). Both in-house stir bars were immersed in the sample solution (10 mL) for extracting several preservatives with a wide range of polarities (log $K_{O/W}$ values of 1.3–3.4) from beverage samples. The different interactions established by two stir bars complemented each other and then, satisfactory recovery values (>75%) for this heterogeneous group of compounds were obtained. In contrast to previous studies, these in-house stir bars were simultaneously desorbed by LD and followed by LC-UV.

In light of the above, the use of the different SBSE modes enables improved SBSE performance due to a suitable adjustment of the extraction conditions depending on the extracted analytes. Moreover, other approaches have been proposed and widely applied for increasing the affinity of polar analytes towards the PDMS stir bar, as well as for analysis by GC, such as derivatisation. Different derivatisation procedures have been reported, such as in-situ, on-stir-bar and post-extraction, in order to reduce the polarity of some organic compounds and thereby improving their extraction efficiencies and/or chromatographic responses. In-situ derivatisation is the simplest approach and consists of the derivatisation of the analytes in the sample solution previously or simultaneously to the extraction step. The derivatised compounds are then retained onto the stir bar. With respect to on-stir-bar derivatisation, the derivatisation reagent can be impregnated onto the stir bar and the reaction starts when the analytes interact with the PDMS coating. Alternatively, the analytes can be concentrated in the PDMS phase first and then the stir bar is exposed to the vapour of the derivatisation reagent. Lastly, post-extraction derivatisation can be performed either in TD (in-tube derivatisation) or in LD (in-extract derivatisation) and its aim is basically to enhance the signal response in the GC system [90,249]. Of the derivatisation techniques, in-situ derivatisation by acetylation (using acetic anhydride under alkaline conditions) and in-tube derivatisation by silylation (usually using N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA)) are the most widely used [239,246,263,274,315,316]. As an example, Llorca-Pórcel et al. [316] proposed the extraction of phenolic organic compounds from soil samples using ultrasonic extraction (USE) followed by SBSE(TD)-GC-MS. In particular, prior to the SBSE, they evaluated the inclusion of a derivatisation reagent (acetylation with acetic anhydride) before SBSE (in-situ derivatisation). The results showed that the introduction of the acetyl group to the phenolic hydroxyl groups resulted in an increasing signal response, due to their better retention onto the stir bar as well as better chromatographic peaks. Therefore, the method detection limits (MDLs) using the in-situ derivatisation ranged from 0.2 to 1.7 μg kg^{-1}, while the MDLs without derivatisation increased, between 1.1 and 61 μg kg^{-1}. Another clear example of the improvement from introducing the derivatisation step prior to the SBSE is reported by

Casas et al. [315], who demonstrated that the in-situ acetylation of the target analytes for SBSE (a group of PCPs) resulted in a noticeable improvement in the extraction process, with recovery values higher than 78%, except for the methylparaben acetate, which was the most polar compound.

In SBSE using immersion sampling mode, the direct contact of the coating with the vessel containing the sample can cause physical damage to the surface of the coating, leading to a decrease in the extraction efficiencies of the analytes. To tackle this problem, novel sorptive extraction techniques with improved devices have been performed. These new approaches which improve the SBSE performance in terms of lifetime of the extracting phase are described in the review paper included in Section 1.2.2.1.

As well as the SBSE limitations presented and the corresponding suitable solutions proposed throughout this section, the availability of just a single commercial stir bar (based on PDMS) for many years is still the principal drawback. Moreover, due to the apolar nature of this commercial coating, poor extraction of polar compounds has been achieved. For this reason, since 2004, the synthesis and application of novel and polar coatings for SBSE has attracted great interest and many coatings with different polarities have been reported. An extensive review of the recent methodologies for synthesising more polar coatings as well as their subsequent application for SBSE in complex matrices has been compiled by the present author in a review paper recently published in the journal *Trends in Analytical Chemistry,* in the following Section 1.2.2.1. In 2011, a new commercial stir bar was introduced to the market, commercialised under the name EG Silicone Twister by Gerstel. This novel stir bar is coated with a more polar extracting phase, based on a poly(ethylene-glycol) (PEG) modified silicone. Another stir bar coated with polyacrylate (PA) with a proportion of PEG (Acrylate Twister), marketed by Gerstel, is not yet fully commercially available, but is undergoing pilot tests. Due to their recent emergence, few applications have been reported and they are included in the following review paper.

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

1.2.2.1. New coatings for stir-bar sorptive extraction of polar emerging organic contaminants

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

NEW COATINGS FOR STIR-BAR SORPTIVE EXTRACTION OF POLAR EMERGING ORGANIC CONTAMINANTS

Núria Gilart, Rosa Maria Marcé, Francesc Borrull*, Núria Fontanals

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

Abstract

Stir-bar sorptive extraction (SBSE) is a sample-preparation technique that allows the sorptive extraction and preconcentration of emerging organic contaminants (EOCs) from complex matrices. Since its introduction, this technique has been widely applied in environmental, food and biological research, followed by gas chromatography (GC) or liquid chromatography (LC). However, the single commercially available coating for SBSE, based on polydimethylsiloxane (PDMS), has become its principal limitation, so use of SBSE has been reduced to the extraction of apolar or moderately polar compounds. In recent years, there has been growing interest in developing more polar in-house coatings for SBSE and, therefore, extend the applicability of this sorptive extraction technique. Different approaches to synthesis of polar coatings for SBSE have been developed, with sol-gel technology and monolithic materials being notable examples. This review focuses on the commonest and novel strategies for synthesizing new coatings for SBSE to enhance the extraction of polar EOCs and their applications.

Keywords: *stir-bar sorptive extraction; polar coatings; sol-gel technology; monolithic materials; polar contaminants*

1. INTRODUCTION

Over the past few decades, scientific concern about environmental pollution has increased and environment-friendly methodologies have gained popularity, including modified and less hazardous sample pre-treatments [1]. In addition, environmental analysis has focused on the extraction and the determination of a wide range of emerging organic contaminants (EOCs) with an apolar or moderately polar character because sample-preparation techniques were incapable of extracting many compounds with such different chemical properties simultaneously. Thus, the aim of sample pre-treatments has been to extract more polar contaminants, simplify the manipulation of the sample, reduce the volumes of sample and organic solvent used, miniaturize the analytical devices, and remove the maximum of interferences from com-

plex matrices [2].

For the analysis of liquid samples, conventional liquid-liquid extraction (LLE) was further replaced by less time-consuming solid-phase extraction (SPE), these being most well-established techniques for preconcentration and clean-up from aqueous sample in many different research fields [3]. However, great efforts have been concentrated on the development of extraction techniques with lower solvent consumption and low sample handling, so novel sorption-based extraction techniques, such as solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE) or microextraction by packed sorbent (MEPS), and new miniaturized solvent-based extraction techniques, such as single-drop microextraction (SDME), dispersive liquid-liquid microextraction (DLLME) or hollow-fiber liquid-phase microextraction (HF-LPME), have been introduced and discussed in the past few decades [3–6].

While the main advantage of SPE is the high availability of commercial sorbents, other sorptive techniques, such as SPME and SBSE, are still restricted in this respect, so limiting the range of analyte classes that can be extracted. In particular, SBSE is an enrichment technique based on SPME principles [7], first introduced by Baltussen et al. [8]. The SBSE device comprises a magnetic stir bar covered with a polymeric coating that enables the distribution of the analytes between the sample and the small amount of extracting phase [9,10]. In contrast to SPME, the volumes of extraction phase found in SBSE stir bars (24–126 µL) are larger than in SPME fibers (maximum 0.5 µL), which leads to higher amounts of analytes being extracted from the samples [11]. Many publications have demonstrated the applicability of SBSE in different research areas due to its versatility in both sampling [immersion and headspace (HS)] and desorption modes [liquid (LD) or thermal desorption (TD)] [4,10,12].

In SBSE, to promote the transfer of the analytes onto the extracting phase, several variables affecting the extraction step should be evaluated, including extraction time and temperature, sample pH, addition of an inert salt, stirring rate and sample volume. As for TD, desorption time and temperature are the most important variables to be tested, while organic solvent nature, desorption time and volume are the most common variables studied in LD [4,7]. All factors affecting the development of SBSE have been extensively reviewed in several publications [4,7,13]. Moreover, another parameter to take into account in SBSE is the coating, it being an essential factor in enhancing the retention of the analytes. However, the only commercially available coating for SBSE, until recently, was polydimethylsiloxane (PDMS). This main disadvantage limits SBSE to apolar compounds [4,11].

So far, several SBSE-related reviews have been reported, mainly focusing on SBSE applications in environmental, food and biological analysis [4,11,12,14,15], on SBSE method optimization [4,15], novel sorptive extraction techniques related to SBSE [2,10], and, more recently, the development of new in-house coatings for SBSE [4,9,10]. This review extensively covers the state of novel commercial and in-house coatings for SBSE since 2004, and, in particular, their application in

the analytical field for the extraction of polar pollutants from complex matrices.

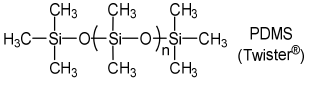
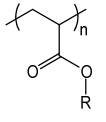
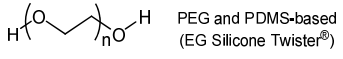
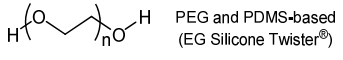
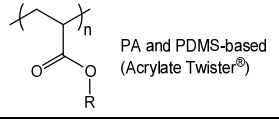
2. COMMERCIALLY AVAILABLE COATINGS FOR SBSE

For years, PDMS has been the only commercially available extracting phase for SBSE, commercialized under the name Twister by Gerstel. Current reviews [4,12,15,16] highlight a great number of publications in which the PDMS coating has been applied to the extraction of many EOCs from environmental, food and biological matrices. Nevertheless, this limitation has become the principal disadvantage of SBSE, focusing on the extraction of apolar or moderately polar analytes (generally for those with log $K_{o/w}$>3) [16]. As a consequence, the present trend in analytical chemistry and environmental analysis is control and determination of EOCs, mainly with polar behavior, in the environment.

In this respect, very recently, SBSE stir bars with polar coatings were marketed by Gerstel. These new stir bars are coated with poly(ethyleneglycol) (PEG)-modified silicone (EG Silicone Twister) and polyacrylate (PA) with a proportion of PEG (Acrylate Twister). We should note that the EG Silicone Twister is commercially available, while Acrylate Twister was still undergoing pilot tests at the time of writing. The structures of these new coatings are presented in Table 1, which shows the enhancement of polarity through hydroxyl and ester groups from PEG and PA structures, respectively. Although they have been synthesized to improve PDMS stir-bar performance, both Acrylate and EG Silicone Twisters are PDMS-based, which might influence the proper retention of polar compounds. Currently, few publications have reported the use of these polar commercial coatings. These are detailed in Table 1.

In 2011, Fries [17] was the first to use the Acrylate Twister for SBSE to extract benzothiazole from wastewaters, using TD coupled to gas chromatography-mass spectrometry (GC-MS). The authors emphasized that the proposed analytical method enabled the improvement of extraction efficiencies compared to SPME, as well as saving time and costs, since no filtering, clean-up steps or organic solvents were required. Recently, Sgorbini et al. [18] evaluated both EG Silicone and Acrylate Twisters for the SBSE of volatile organic compounds (VOCs) from food and cosmetic samples analyzed by GC-MS and subsequently compared them with the PDMS coating. When both polar coatings were used under immersion and HS modes, it was observed that these coatings provided better performance by immersion in comparison to HS mode. Moreover, EG Silicone Twister provided higher percentage concentration factors for most of the target analytes than Acrylate Twister, being able to extract a wider range of analytes with different polarities (log $K_{O/W}$ = -0.2–4.8) and improving PDMS limi-tations. In our research group, these novel polar coatings were first applied to the SBSE of pharmaceuticals and personal-care products (PPCPs) by immersion from wastewaters, followed by LC-tandem MS (LC-MS/MS) [19]. In this study, all three commercial stir bars were evaluated in terms of recovery values and matrix effect. While the matrix effect was generally low for most of the compounds using the three

Table 1. Structures and application of novel commercially available coatings for SBSE.

Coating Phase*	Structure	Analyte	Matrix	Sampling Mode	Desorption	Analysis	Ref.
PDMS (Twister®) PA (Acrylate Twister®)		VOCs	Food and cosmetic	Immersion/ HS	TD	GC-MS	[18]
PEG (EG Silicone Twister®) PDMS (Twister®)							
PA (Acrylate Twister®) PEG (EG Silicone Twister®)		PPCPs	Wastewater	Immersion	LD	LC-MS/MS	[19]
PEG (EG Silicone Twister®)		Bisphenols	PCPs	Immersion	TD	GC-MS	[20]
PA (Acrylate Twister®)		Benzothiazole	Untreated wastewater	Immersion	TD	GC-MS	[17]

*Commercial name in brackets
GC-MS: gas chromatography-mass spectrometry; HS: headspace; LD: liquid desorption; LC-MS/MS: liquid chromatography-tandem mass spectrometry; PA: polyacrylate; PCPs: personal care products; PEG: poly(ethylene)glycol; PPCPs: pharmaceuticals and personal care products; TD: thermal desorption; VOCs: volatile organic compounds;

stir bars, there were significant differences with the recovery values obtained between them, as shown in Table 2. EG Silicone Twister provided the best results when moderately polar and apolar compounds were extracted, compared to PDMS and Acrylate Twister, which were able to extract only the more apolar compounds [% recovery values (%R) = 14–43%]. However, it was demonstrated that even EG Silicone Twister is still limited in terms of the more polar analytes, achieving %R of 24–80% for compounds with log $K_{O/W}$ > 3 (except for diclofenac). Finally, a recent publication [20] also described the use of EG Silicone Twister for SBSE of a group of bisphenols in personal-care products (PCPs) by SBSE(TD)-GC-MS, avoiding the need for a derivatization step, which is frequently necessary using the PDMS coating.

Even though there are still few publications reporting the use of these polar commercial stir bars, their SBSE performance could be improved in order to increase polar compound retention. For this reason, the following sections present the new polymeric phases recently developed in-house with polar behavior and their analytical applications.

3. NOVEL IN-HOUSE COATINGS FOR SBSE

Over the past few years, interest in novel SBSE coatings has grown significantly in order to promote the retention of polar compounds from complex matrices. To overcome the limitation of the commercial PDMS stir bar, the main requirements of the new SBSE coatings are the polarity of the coatings and mechanical stability. The most common approaches for synthesizing in-house coatings for SBSE are sol-gel technology, the synthesis of monolithic materials and polyurethane foams (PUFs). Fig. 1 shows each approach to synthesis, and Table 3 shows the most relevant advantages and drawbacks of these approaches.

3.1. PDMS modified coatings

The first in-house SBSE coatings were based on PDMS and obtained, basically, by sol-gel technology. Using this methodology, very thermally and mechanically stable films with long lifetimes were obtained because of the strong chemical bond between the coating and the surface of the glass bar [4,9,21].

As shown Fig. 1(A), the sol-gel procedure comprises several reactions, starting with hydrolysis of the coating precursors, followed by polycondensation of the hydroxylated compounds, and, finally, chemical bonding of the coating to a glass bar, which is pretreated to generate the superficial silanol groups [10,21,22]. Moreover, depending on the precursors used in the sol-gel reaction, the chemical properties of the final coating can be influenced to achieve the desired polarity. However, the sol-gel SBSE coatings are generally PDMS-based and a decrease in the affinity to polar compounds may be observed because of its apolar nature. Table 4 shows the structure of the monomers used in the synthesis of several sol-gel SBSE coatings and their subsequent application.

Liu et al. [23] were the first to synthesize an SBSE coating using the sol-gel procedure. In this study, the low polarity of the hydroxyl-terminated PDMS led

Table 2. Recovery values (%) obtained when 50 mL of ultrapure water spiked with the analyte mixture were extracted with PDMS, Acrylate, EG Silicone and VPD/DVB coatings.

Analyte	Log $K_{O/W}$ [a]	Recovery values (%)			
		Commercial [b]			In-house [c]
		PDMS coating	PA coating	EG Silicone coating	VPD/DVB coating
Paracetamol	0.5	-	<1	-	9
Caffeine	-0.6	<1	-	-	20
Benzotriazole	0.4	<1	<1	<1	n.i.
Antipyrine	1.4	-	1	<1	42
Propranolol	2.9	<1	2	2	87
Pridinol	3.4	2	2	3	n.i.
Methylparaben	1.9	<1	2	1	91
Carbamazepine	1.9	<1	<1	<1	83
Propylparaben	2.9	<1	2	10	89
DHB	3.2	<1	9	24	50
Benzylparaben	3.6	1	14	39	n.i.
DHMB	4.3	8	9	26	93
Diclofenac	4.5	<1	<1	<1	80
Benzophenone-3	4.0	34	10	45	92
Triclocarban	6.1	16	43	59	81
Triclosan	5.3	40	42	80	n.i.

[a] Log $K_{o/w}$ calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2012 ACD/Labs)
[b] % RSD (*n*=3) were lower than 15% for %R > 16%. For the experimental conditions, see references [19].
[c] % RSD (*n*=3) were lower than 6% for %R > 20%. For the experimental conditions, see reference [48].
n.i. = analyte not included in the cited paper.

to the extraction of a group of apolar compounds [n-alkanes, polycyclic aromatic hydrocarbons (PAHs)] and organophosphorus pesticides (OPPs) from aqueous samples. The authors highlighted the thermal stability and the uniformity of the resulting coating, which are the principal advantages of this approach.

In order to promote the polarity of the SBSE coatings, Yu et al. [24,25] developed several in-house coatings by sol-gel technology using more polar sol-gel precursors. Among them, a novel combined stir bar coated with PDMS and poly(vinylalcohol) (PVA) was prepared to extract OPPs from honey [24]. With a larger surface area (21.25 m^2 g^{-1}) but lower thickness (30 μm) than the commercial PDMS stir bar (0.5 mm thick), this novel coating was able to reach equilibrium in just 15 min for all of the compounds studied, providing slightly more sensitive and less time-

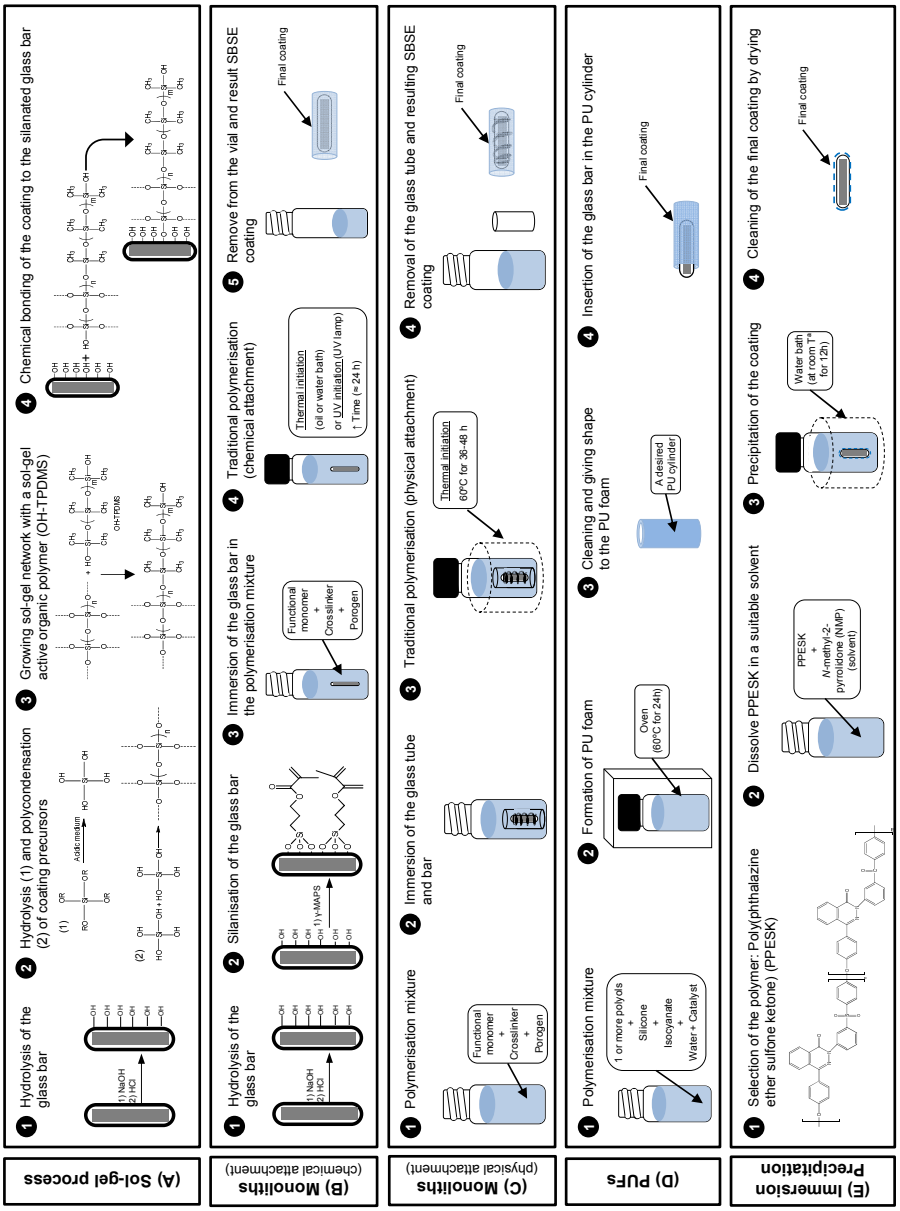


Fig. 1. The commonest strategies to obtain coatings for stir-bar sorptive extraction (SBSE).

Table 3. Advantages and drawbacks of new approaches to the synthesis of in-house coatings for SBSE.

Synthetic approach	Advantages	Drawbacks
Commercial stir bars	Mechanical and chemical stability	PDMS-based Low polarity
Sol-gel technology	Mechanical and chemical stability Strong chemical bonding Availability of polar monomers Selectivity through MISBSE	PDMS-based Tedious treatment of the glass bar
Monolithic coating (Chemical attachment)	Mechanical stability Strong chemical bonding Availability of polar monomers Not PDMS-based Selectivity through MISBSE	Tedious treatment of the glass bar Longer desorption times (>1h)
Monolithic coating (Physical attachment)	No treatment of the glass bar Availability of polar monomers Not PDMS-based Easy preparation Selectivity through MISBSE	Low physical stability
Polyurethane Foams	Easy preparation Mechanical and chemical stability	Longer extraction times (>4h) No MISBSE in this format
Immersion precipitation	Easy preparation Few reagents used Mechanical and chemical stability	Few papers related No MISBSE in this format

consuming performance than the SPME fiber performance coated with PDMS/PVA. In the same way, the same authors also synthesized a similar coating based on PDMS, PVA and Carbowax (CW) to determine volatile organic sulfur compounds (VOSCs) in waters [25]. The addition of CW and PVA enhanced the polarity of the coating and promoted the retention of more polar VOSCs. The authors stated that this novel coating showed a higher sorption capacity than Carboxen-PDMS SPME fibers or PDMS stir bars.

In another study, a novel sol-gel polar precursor, cyanopropyltriethoxysilane (CNPrTEOS), was combined with PDMS for the SBSE of two non-steroidal anti-inflammatory drugs (NSAIDs) from aqueous samples [26]. The cyano moieties present in the PDMS/CNPrTEOS coating were responsible for the extraction of relatively more polar compounds, such as diclofenac, while the PDMS moieties contributed to the extraction of a more apolar compound, such as ketoprofen.

Of the sol-gel precursors, a common PDMS modifier for enhancing polarity is β-cyclodextrin (β-CD). Several authors have frequently used PDMS and β-CD for the synthesis of coatings for the SBSE of brominated flame retardants (BFRs) in soil dust [27], steroid hormo-

Table 4. Structures, synthesis approach and applications of some in-house coatings for SBSE.

Polymer	Structure	Preparation technique	Analytes	Matrix	Analysis	Ref.
PDMS/MTMS	MTMS	Sol-gel	n-alkanes, PAHs, OPPs	Water	GC-FID	[23]
PDMS/PVA	PVA	Sol-gel	OPPs	Honey	GC-FPD	[24]
PDMS/CW/PVA	CW	Sol-gel	VOSCs	Water	GC-FPD	[25]
PDMS/CNPrTEOS	CNPrTEOS	Sol-gel	NSAIDs	Water	CE-UV	[26]
PDMS/β-CD/PTMS	β-CD PTMS	Sol-gel	Steroid hormones	Water	LDTD-APCI-MS/MS	[28]
PDMS/β-CD/DVB	DVB	Sol-gel	Estrogens	Pork and chicken	LC-UV	[29]

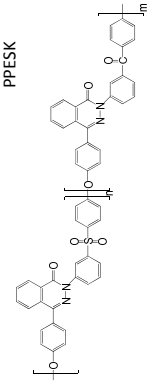
Table 4. Continued.

Polymer	Structure	Preparation technique	Analytes	Matrix	Analysis	Ref.
	PPY					
PDMS/PPY		Copolymerisation	Antidepressants	Plasma	LC-UV	[33]
	OcMA					
OcMA/EDMA	EDMA	Monolith (Chemical attachment)	PAHs/Steroid hormones	Sea /Urine	LC-UV	[38]
	VI					
VI/DVB		Monolith (Chemical attachment)	PAAs	Lake and sea	LC-UV	[40]
	VPD					
VPD/DVB		Monolith (Chemical attachment)	PAAs	Lake and sea	LC-UV	[40]
	VP					
VP/EDMA		Monolith (Chemical attachment)	PAAs	Lake and sea	LC-UV	[40]
	VPH					
VPH/MBAA	MBAA	Monolith (Chemical attachment)	Benzimidazoles Sulfonamides	Milk and honey Pork and chicken	LC-UV LC-MS/MS	[41] [42]

Table 4. Continued.

Polymer	Structure	Preparation technique	Analytes	Matrix	Analysis	Ref.
AA/EDMA	AA	Monolith (Chemical attachment)	Soluble cations	Milk	IC-ECD	[43]
MASPE/DVB	MASPE	Monolith (Chemical attachment)	Nitroimidazoles	Honey	LC-UV	[44]
VPD/DVB	VPD	Monolith (Physical attachment)	PPCPs	Environmental waters	LC-MS/MS	[48]
MAA/DVB	MAA	Monolith (Physical attachment)	PPCPs	Environmental waters	LC-MS/MS	[49]
PEGMA/PETRA	PEGMA PETRA	Monolith (Physical attachment)	PPCPs	Environmental waters	LC-MS/MS	[50]
PPG/TMPE/MDI	PPG TMPE MDI	PU foams (Physical attachment)	Triazine herbicides	Water samples	LC-UV	[53]

Table 4. Continued.

Polymer	Structure	Preparation technique	Analytes	Matrix	Analysis	Ref.
PPESK		Immersion precipitation	Organochlorine compounds/OPPs	Sea waters/Juices	GC-ECD/GC-TSD	[55]

ACB: activated carbon; β-CD: β-cyclodextrin; CE-UV: capillary electrophoresis-ultraviolet detection; CNPrTEOS: cyanopropyltriethoxysilane; CW: carbowax; DVB: divinylbenzene; EDMA: ethylene dimethacrylate; GC-ECD: gas chromatography-electron capture detector; GC-FID: gas chromatography-flame ionisation detection; GC-FPD: gas chromatography-flame photometric detection; GC-MS: gas chromatography-mass spectrometry; GC-TSD: gas chromatography- thermionic specified detector; IC-ECD: ion chromatography-electrochemical detector; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-UV: liquid chromatography-ultraviolet detection; LDTD-APCI-MS/MS: laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry; MAA: methacrylic acid; MASPE: methacrylic acid-3-sulphopropyl ester potassium salt; MBAA: N,N'-methylenebisacrylamide; MDI: 4,4'-methylene bisphenyl diisocyanate; MTMS: methyltrimethoxysilane; OcMA: octyl methacrylate; PDMS: polydimethylsiloxane; PEGMA: poly(ethyleneglycol) methacrylate; PETRA: pentaerythritol triacrylate; PPESK: polyphatalazine ether sulfone ketone; PPG: glycerol propoxylate; PPY: polypyrrole; PTMS: phenyltrimethylsiloxane; PVA: poly(vinylalcohol); TMPE: trimethylolpropane ethoxylate; VI: vinylimidazole; VOSCs: volatile organic sulphur compounds; VP: vinylpyridine; VPD: vinylpyrrolidone; VPH: vinylphthalimid

nes in aqueous samples [28] and estrogens in pork and chicken [29], among others. As shown in Table 4, the structure of β-CD, which is a cyclic oligosaccharide, has become the main functional component for extracting these EOCs from complex matrices by SBSE, thanks to its hydrophobic interior cavity and its hydrophilic exterior structure, which is full of hydroxyl groups. For instance, Yu et al. [27] compared the synthesized coating based on PDMS/β-CD with conventional PDMS and the synthesized coating provided higher extraction efficiencies for BFRs when soil-dust samples were analyzed.

Other authors have developed novel sol-gel SBSE coatings based on PDMS/β-CD/DVB to extract estrogens from pork and chicken samples [29], and based on PDMS/β-CD/phenyltrimethylsiloxane (PTMS) to extract steroid hormones from aqueous samples [28]. Using PDMS/β-CD/DVB to extract estrogens from tissue samples after a solid-liquid extraction, high recovery values were achieved (>70%) for all of the target analytes, due to the β-CD and DVB structures enabling both hydrophilic and π-π interactions, respectively. Furthermore, the sol-gel coating based on PDMS/β-CD/PTMS provided higher selectivity and polarity due to the β-CD and PTMS structures compared to the commercial PDMS stir bar for extracting steroid hormones from water samples [28]. To demonstrate this, Fig. 2 shows the recovery values for all of the target compounds in real water samples using the in-house coating (PDMS/β-CD/PTMS) in the range 46–99%, while, using the PDMS stir bar, the values were 3–22% in real water samples.

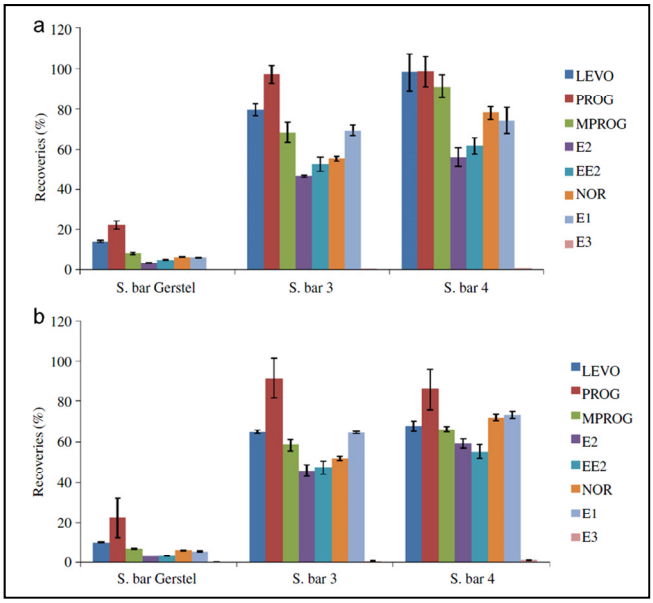


Fig. 2. Recovery values (%) of eight hormones in real water samples ((a) tap water and (b) wastewater) using the commercial PDMS stir bar (S. bar Gerstel) and two in-house coatings based on PDMS/β-CD/PTMS with different compositions (S. bar 3 and S. bar 4). Reprinted from [28] with permission of Elsevier.

Very recently, a sol-gel amino-modified multi-walled carbon nanotube-PDMS (AMMWCNT-PDMS) was first synthesized and used as a novel coating for the SBSE of phenols from environmental waters and soils [30]. Carbon nanotubes (CNTs) are very promising as adsorbents in SPE and SPME due to their thermal and chemical stability, their ease of functionalization and their large surface-area-to-volume ratio. The proposed SBSE coating offered higher extraction efficiencies for the studied analytes than the commercial PDMS coating, due to the π-π, electrostatic and hydrogen bonding interactions.

Moreover, recently, a sol-gel coating for SBSE, proposed by Hu et al. [31], was based on PDMS and a metal-organic framework (MOF) to determine estrogens in environmental water samples. MOFs are a new class of porous solid materials, which are self-assembled by metal ions and organic ligands. The large diversity of structures and pore sizes, high surface areas and good adsorption made MOFs attractive sorbents in SPE, SPME, and very recently a SBSE coating [31]. Due to MOF properties, when the PMDS/MOFs SBSE coating was compared to the comercial PDMS stir bar, higher extraction efficiencies of the target analytes were obtained using the in-house coating.

Apart from the sol-gel technique, other coatings based on PDMS have been

synthesized using a different strategy, in which a magnetic stirring rod was inserted into a Teflon mold containing the polymerization mixture, including PDMS, a modifier and a curing agent, for several hours at high temperature. For instance, Barletta et al. [32] used activated carbon (ACB) as a modifier to extract polar pesticides from juice samples, and Melo et al. [33] decided to use polypyrrole (PPY) as a modifier to determine antidepressants in plasma samples. In the latter study, the modifier PPY was demonstrated to be extremely suitable for the extraction of the compounds studied, as it was able to establish hydrogen bonding and π-π interactions with recovery values in 1 mL of plasma samples of 38–83% [33].

All of the above examples show how the polarity of the SBSE coating using modifiers with polar functional groups is enhanced. However, all of them lack selectivity, so sol-gel technology was also proposed for synthesizing molecularly imprinted polymers (MIPs) for SBSE, but few publications have been reported. For instance, Si et al. [34] prepared a selective coating for molecularly-imprinted SBSE (MISBSE) by the sol-gel technique using nicosulfuron (sulfonylurea herbicide) as a template, methacrylic acid (MAA) as the functional monomer and methacryloxypropyltrimethoxysilane (MPTMS) as the cross-linker. The authors emphasized the high mechanical strength and the efficiency of the sol-gel MIP coating developed, which provided good recoveries (96%) and high selectivity compared with non-imprinted polymer (NIP) when 25 mL of tap water were extracted over 2 h.

Another example of a MISBSE coating using sol-gel technique is a dummy MIP for the extraction of bisphenol A from tap water [35]. In this study, a structural analogue similar to bisphenol A was used as a template in order to avoid possible leakage of the residual template molecules, which could interfere with the determination of a trace amount of bisphenol A. As a result, a homogenous, stable coating surface was obtained with a thickness of 57 µm, showing high selectivity toward bisphenol A and high recovery values (>80%) compared with those obtained using the conventional PDMS coating (<20%). Although these two studies highlighted the promising MISBSE performance in terms of recovery values and selectivity, a washing or clean-up step was not included in their performance, making them susceptible to interfering compounds when analyzing complex matrices.

3.2. Monolithic coatings

Over the past few years, there has been growing interest in the use of monolithic materials as SBSE coatings due to their numerous advantages, such as large pore structures, high permeability, high availability of commercial monomers with different polarities and functionalities, and, in particular, simple, inexpensive preparation. Furthermore, monoliths have widely been applied as alternative stationary phases in LC and capillary electrochromatography (CEC) as well as extraction sorbents for several extraction techniques, such as SPE and SPME [36, 37].

The preparation of a monolith involves a polymerization mixture, including adequate monomers (functional monomers and cross-linker), porogenic solvents and initiators, in an appropriate

ratio, which is introduced into the desired mold and is initiated thermally or by UV radiation. Therefore, depending on the shape and size of the mold, SBSE coatings with a higher volume of extracting phase can be obtained, and, consequently, an increase in capacity and recovery values may be observed [4,9]. In the synthesis of a monolithic coating for SBSE, apart from the mold, other important parameters should be taken into account to obtain the desired final product, such as the choice of monomers for enhancing the polarity of the coating and the porogenic solvent used for forming large pores in the monolithic structure and promoting high permeability. The monolithic approach has therefore become a successful option for obtaining chemically and mechanically stable coatings for SBSE [10].

In the monolithic approach, two different strategies have been developed for fixing the coating to the glass bar (i.e. chemical or physical). The procedures for synthesizing monolithic coatings using chemical or physical attachment are detailed in Fig. 1 (B) and Fig. 1(C), respectively, and we discuss them in the following subsections.

3.2.1. Monolithic coatings by chemical attachment

Used extensively, the chemical attachment of a monolithic SBSE coating involves treatment of the glass surface of the bar in order to create double bonds through a silanization agent, and, then, subsequent growing of the polymer to obtain the final coating, as shown Fig. 1(B). Huang et al. [38] synthesized the first chemically-attached monolithic coating for SBSE of PAHs and steroid hormones from seawater and urine samples, respectively. The proposed monolithic coating was based on octyl methacrylate (OcMA) as the functional monomer and ethylene dimethacrylate (EDMA) as the cross-linker. Both monomers with ester groups in their structure (Table 4) favoured the transfer of the polar analytes to the coating. In this study, the final dimension of the monolithic coating was evaluated, and it was demonstrated that greater thickness led to higher adsorptive capacity. However, the desorption step took 2 h to release the extracted analytes, which was quite a long desorption time. Moreover, the monomer-to-porogen ratio was also studied [40/60, 45/55 and 50/50, (%, w/w)], and it was observed that a decrease of the porogen content led to a polymer with small pore size and low permeability, and, in view of this, the monomer-to-porogen ratio was kept to 40/60 (%, w/w).

Since 2007, Huang and co-workers have synthesized several polar monoliths as SBSE coatings by chemical bonding. For instance, a novel polar stir bar coated with vinylimidazole (VI) and DVB was developed for the extraction of sulfonamides from milk [39]. Due to the imidazole groups in the monomer structure, hydrogen bonds and hydrophobic interactions were established with the polar analytes (log $K_{O/W}$<1.6), providing higher extraction capacity and lower method detection limits (MDLs) than the commercial PDMS stir bar. Furthermore, Huang et al. [40] synthesized three in-house monolithic coatings for the SBSE of polar aromatic amines (PAAs) from lake and sea

waters. The proposed coatings were based on VI and DVB, vinylpyrrolidone (VPD) and DVB and, finally, vinylpyridine (VP) and EDMA. Their SBSE performances of these coatings were subsequently compared between themselves and with the commercial PDMS coating. Fig. 3 shows the extraction of PAAs from water samples using the in-house and the commercial PDMS SBSE coatings under the same conditions (2.5 h and 1 h as extraction and desorption times, respectively). The target compounds, PAAs with log $K_{O/W}$<2.7, were successfully extracted using all three in-house coatings, in contrast to the PDMS coating, which completely failed, since only hydrophobic interactions contributed to the extraction of PAAs. Of the three in-house coatings, VI/DVB provided the best extraction efficiencies for all PAAs (57–114%), due to the imidazole and phenyl groups in its structure.

More recently, a new monolithic SBSE coating was synthesized using two less common monomers but with high polar functionalities: vinylphthalimide (VPH) as the functional monomer and *N,N'*-methylenebisacrylamide (MBAA) as the cross-linker (Table 4) [41,42]. This novel coating was applied for the SBSE of two benzimidazoles (oxfendazole and mebendazole) in milk and honey samples and for the SBSE of 10 sulfonamides in pork and chicken samples. In the first study [41], thanks to the hydrophilic and π-π interactions established by these monomers, promising SBSE performance was achieved for benzoimidazole compounds with recovery values of 71–102% when 5 mL of milk and 2.5 g of honey were extracted for 2.5 h, with a significant improvement compared to the conventional PDMS coating. Moreover, the authors evaluated the porogen content and observed that lower porogen content in

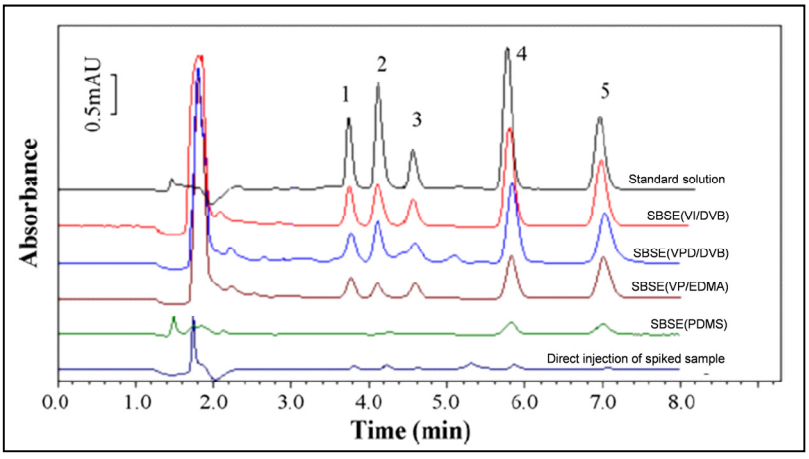


Fig. 3. HPLC chromatograms of five polar aromatic amines (PAAs) in a standard solution and being extracted using four in-house coatings and the commercial PDMS coating for SBSE. Peaks: 1 = *p*-Nitroaniline (*p*-NA); 2 = aniline (A); 3 = 2,4-dinitroaniline (2,4-DNA); 4 = *o*-chloroaniline (*o*-CA); 5 = 3,4-dichloroaniline (3,4-DCA). Reprinted from [40] with permission of Elsevier.

the polymerization mixture led to lower peak areas of the analytes due to a decrease in the pore size and, thus, in the permeability of the polymer.

In order to enhance the extraction of polar compounds with carboxyl or amino functional groups, anion-exchange or cation-exchange monolithic coatings have been required for SBSE over the past few years. In this respect, the combinations of the monomers acrylic acid (AA) with EDMA and methacrylic acid-3-sulphopropyl ester potassium salt (MASPE) with DVB (structures shown in Table 4) have been used to synthesize cation-exchange monolithic SBSE coatings and apply them to the analysis of soluble cations in milk [43] and nitroimidazole antibiotics in honey [44], respectively. Using the MASPE/DVB coating, the authors highlighted that the sulfonic and phenyl groups present in the monolithic structure were essential for the extraction of polar nitroimidazole antibiotics from honey, which had a log $K_{O/W}$ of -0.38–0.31. This novel coating with strong cation-exchange behavior enabled better SBSE performance than the PDMS coating, with high extraction efficiencies (%R > 71%), great enhancement of the peak height, and, as a result, lower MDLs. However, with the analytes being retained by ion-exchange interactions, no washing or clean-up step with organic solvent was included in the SBSE protocol to remove interferences from the matrix, which is the recommended protocol for ion-exchange materials.

As mentioned previously in sub-section 3.1., MIPs recently gained popularity as SBSE coatings in order to promote selectivity. In this respect, there were more studies in which MIPs were synthesized as monolithic coatings for MISBSE application rather than using the sol-gel methodology. The classic approach for obtaining MIPs was the copolymerization of an appropriate template, functional monomer and cross-linker in a suitable porogenic solvent. Subsequently, the MIP was chemically attached to a glass bar using a silanization agent. Thus, Xu et al. [45] were the first to synthesize a monolithic SBSE coating with a MIP for ractopamine chemically attached. In this study, the silanization reagent, the cross-linker, the functional monomer and the template were 3-(methacryloxy)propyltrimethoxysilane (MPTS), EDMA, MAA and ractopamine, respectively. Finally, the MISBSE coating was successfully applied for the extraction of a group of β_2-agonists from pork, liver and feed samples, achieving recovery values of 74–93%. The same research group applied this synthesis procedure to obtain several coatings for the MISBSE of triazine herbicides from food and soil samples [46], and trimethoprim from biological samples [47], among others. The authors highlighted the good mechanical and chemical stability, selectivity and physical properties (homogenous and porous) of the resulting MISBSE coatings. We highlight that, in these studies, the proposed MISBSE procedures were unable to offer a washing step to reduce interfering compounds, so losses in selectivity and sensitivity could be observed. Efforts should therefore focus on the development of completely selective MISBSE coatings through effective washing and loading steps.

3.2.2. Monolithic coatings by physical attachment

Synthesis of monolithic SBSE coatings can be also achieved using physical attachment. While the polymerization procedure for obtaining the desired product was the same as those described previously, immobilization of the coating on the glass bar was significantly easier than with the chemical attachment, without losing mechanical stability. As can be seen in Fig. 1(C), the stir-bar set-up comprised a glass bar introduced in an iron spring to give stability to the whole monolithic coating. Subsequently, the stir bar and the spring were together placed in a glass tube (with the desired dimensions) and immersed in the polymerization mixture.

To demonstrate the viability of this novel approach to monoliths, our research group pioneered this strategy and synthesized several monolithic SBSE coatings. Bratkowska et al. [48,49] developed two monolithic coatings for extracting polar PPCPs from environmental waters by SBSE(LD)-LC-MS/MS. The monolithic coatings were based on VPD [48] and MAA [49] as functional monomers and DVB as the cross-linker. As can be seen in Table 2, using the VPD/DVB coating, great results were obtained for most of the studied compounds in terms of recovery values in 50 mL of ultrapure water (42–110%) after being extracted for 4 h, compared to the commercial PDMS stir bar. In addition, better SBSE performance was achieved using the MAA/DVB coating, which provided excellent recovery values in ultrapure water for most of the compounds (61–107%). These promising results were compared with those obtained using the commercial PDMS coating, in which the studied analytes were hardly recovered. The high potential of these two monolithic coatings for the extraction of polar compounds was therefore demonstrated, and may be attributed to the nitrogen and oxygen atoms and to the phenyl groups being able to establish hydrogen bonds and hydrophobic interactions, as can be seen in Table 4.

Following the corresponding procedure, the same research group synthesized another monolithic SBSE coating using two novel monomers: poly(ethyleneglycol) methacrylate (PEGMA) as the functional monomer and pentaerythritol triacrylate (PETRA) as the cross-linker [50]. This monolithic material contained a great number of hydroxyl and ester groups, providing a polar SBSE coating for extracting PPCPs from wastewater samples. Using the PEGMA/PETRA coating, even the recovery values for the studied analytes in ultrapure water were slightly lower than those achieved with the MAA/DVB and VPD/DVB coatings, the analytes being almost fully extracted in just 1 h. Moreover, these results were much better than those from the commercial polar coatings (EG Silicone Twister and Acrylate Twister) under their optimized SBSE conditions.

Regarding MISBSE, an interesting study by Turiel et al. [51] proposed both physical and chemical attachment of a monolithic coating for the MISBSE of thiabendazole from citrus samples. While the chemical attachment of the coating was the same as those described above, physical attachment was achieved by pre-treating the glass bar with a commercial epoxy adhesive and then immersing it in a vial containing

MIP particles. Although the physical attachment of the MIP coating was easier to perform than the chemical one, the former procedure was not completely able to obtain a homogenous immobilization of the MIP particles onto the glass bar surface. Besides, the chemically attached MISBSE coating allowed the performance of selective loading and washing steps to remove all of the interferences bonded by non-specific interactions to the MIP.

3.3. Other sorptive materials and formats

Apart from these two main approaches, other novel strategies and synthesis routes have been developed to obtain coatings for SBSE, such as PUFs or the immersion precipitation technique.

PUFs are defined as plastics materials and are easily obtained by combining all of the required reagents (an isocyanate, a polyol (or polyalcohol), expansion agents, catalysts and surfactants), as described in Fig. 1(D). After polymerization, the PUFs were cleaned with an organic solvent and cut to the desired shape and dimensions. Thus, these materials offered high chemical stability, flexibility and simplicity, being really promising as SBSE coatings. Nogueira's research group has been the only one to develop and to apply PUFs, using different polyol types, in the SBSE of organic compounds from aqueous matrices [52]. In this study, high stability and mechanical resistance to organic solvents were the main advantages of PUF coatings, providing better SBSE recoveries for the studied compounds (25–70%) than the PDMS coating (5–25%). In addition, the same research group developed several SBSE coatings based on PUFs for extracting triazine herbicides [53] or acidic pharmaceuticals [54] from water samples.

A novel, simple synthesis of SBSE coatings was developed by Guan et al. [55], who were the first to develop a highly thermally-stable polar coating based on poly(phthalazine ether sulfone ketone) (PPESK) using the immersion-precipitation technique. As detailed in Fig 1(E), the synthesis involved the precipitation of the polymer (PPESK) when the glass bar was immersed in the polymer solution in a water bath at room temperature. The PPESK coating was applied to extract organochlorine compounds from seawaters and OPPs from juices. When the PPESK coating was evaluated, better selectivity towards both organochlorine compounds and OPPs was observed in comparison with the PDMS coating, due to its high number of carbonyl and aromatic groups enabling both hydrogen bonds and hydrophobic interactions. Moreover, the novel PPESK coating also presented higher extraction capacity for OPPs (%R = 7–66%) than an SPME fiber coated with PPESK (%R<7%), thanks to its greater thickness (250 μm for PPESK coating and 30 μm for PPESK SPME fiber).

Since 2009, other formats have been designed to solve one of the main drawbacks of the in-house coatings for SBSE, namely direct contact of the coating with the vessel when immersion sampling is applied. To solve this problem, several in-house and alternative sorptive extractions were proposed recently, such as rotating-disk sorptive extraction (RDSE), stir-rod sorptive extraction (SRSE), stir-cake sorptive extraction (SCSE) and two techniques of adsorptive microextrac-

tion (AμE) (bar adsorptive microextraction (BAμE) or multi-sphere adsorptive microextraction (MSAμE)). Fig. 4 details the different sorptive extraction devices of each novel technique.

The first attempt to improve SBSE was RDSE [Fig. 4(A)], in which the coating is immobilized onto the upper surface of the rotating disk. This novel format prevented damage by physical contact, and, consequently, could be used to perform at least 50 experiments. However, the only extracting phase used in RDSE was PDMS for extracting nonylphenol, pesticides and chromogenic organic compounds from waters [56–58]. With respect to SRSE [Fig. 4 (B)], a metal rod containing a magnet at the end is coated with the sorptive material, usually a monolithic polymer, and fixed in the sample vessel. For example, Luo et al. [59] synthesized a polar monolith based on VP and EDMA for the SRSE of NSAIDs from water and sewage-sludge samples, providing successful recovery values (>76%) and no damage to sorptive material after at least 60 experiments. More recently, in 2011, Huang et al. [60] also applied monolithic materials to develop the novel SCSE, similar to RDSE. As shown in Fig. 4(C), the monolith is synthesized in a circular mold with the desired thickness containing an iron wire to stir and to enhance the transfer of the analytes to the extracting phase without friction loss of the monolith. Different monoliths have been synthesized to be applied in SCSE, such as one based on VI and DVB for extracting steroid hormones from milk [60] and a polymeric ionic liquid-based monolith for extracting six preservatives from juices [61]. In both studies, the authors highlighted the feasibility of the novel technique, obtaining good recovery

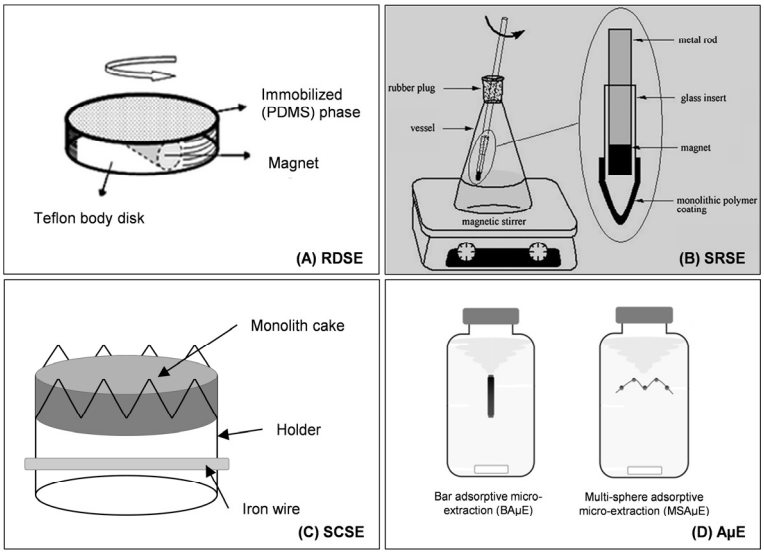


Fig. 4. Schematic representation of several novel sorptive extraction formats and devices: a) Rotating disk sorptive extraction (RDSE); b) Stir rod sorptive extraction (SRSE); c) Stir cake sorptive extraction (SCSE) and d) Adsorptive microextraction techniques (AμE).

values (63–117%), and, in particular, higher longevity of the extracting phase than in SBSE.

With respect to AμE techniques, Nogueira's research group has developed novel approaches, such as BAμE and MSAμE [2]. As detailed in Fig. 4(D), BAμE comprises a floating bar covered with a powdered sorbent, while MSAμE involves coating polystyrene spheres (attached by a thread) with powdered sorbent. The most commonly used sorptive materials are activated carbons and poly(styrene-DVB) for extracting polar solutes and metabolites from aqueous samples [62,63]. For example, when caffeine and acetaminophen, two extremely polar EOCS, were extracted using both MSAμE and BAμE with activated carbons, high recovery values were obtained (>80%), whereas the conventional PDMS coating for SBSE was completely unable to extract them [62]. However, these techniques have not been widely applied and few publications are available in the literature.

4. Conclusions

SBSE is a well-accepted sorptive extraction technique for preconcentrating a great variety of compounds from many different complex matrices. Despite the large number of SBSE applications in the literature, until very recently, the availability of commercial coatings for SBSE was limited to PDMS, with an apolar nature. In recent years, promising strategies were proposed to overcome this limitation, such as sol-gel technology and monolithic materials. These novel approaches provided high versatility to SBSE, since a broad range of commercial monomers with different polarities can be used during coating synthesis, promoting the extraction of more polar EOCs.

Moreover, other important factors to take into account in SBSE have been extensively evaluated, such as mechanical, thermal or chemical stability and the physical dimensions of the coatings, providing successful SBSE performance in comparison with the conventional PDMS coating. Although promising in-house polar coatings for SBSE have been developed, new polar monomers and novel formats need to be exploited more in order to extract polar EOCs from complex matrices.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] M. Farré, S. Pérez, C. Gonçalves, M.F. Alpendurada, D. Barceló, Green analytical chemistry in the determination of organic pollutants in the aquatic environment, TrAC, Trends Anal. Chem. 29 (2011) 1347-1362.

[2] J.M.F. Nogueira, Novel sorption-based methodologies for static microextraction analysis: A review on SBSE and related techniques, Anal. Chim. Acta 757 (2012) 1-10.

[3] L. Ramos, Critical overview of selected contemporary sample preparation techniques, J. Chromatogr. A 1221 (2012) 84-98.
[4] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions, J. Chromatogr. A 1217 (2010) 2642-2666.
[5] A. Sarafraz-Yazdi, A. Amiri, Liquid-phase microextraction, TrAC, Trends Anal. Chem. 29 (2010) 1-14.
[6] Y. Chen, Z.P. Guo, X.Y. Wang, C.G. Qiu, Sample preparation, J. Chromatogr. A 1184 (2008) 191-219.
[7] M. Kawaguchi, R. Ito, H. Nakazawa, A. Takatsu, Environmental and biological applications of stir bar sorptive extraction, in: J. Pawliszyn (Editor), Comprehensive Sampling and Sample Preparation, Elsevier Inc., USA, 2012, pp. 797-818.
[8] E. Baltussen, P. Sandra, F. David, C. Cramers, Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles, J. Microcol. Sep. 11 (1999) 737-747.
[9] I. Rykowska, W. Wasiak, Advances in stir bar sorptive extraction coating: A review, Acta Chromatogr. 25 (2013) 27-46.
[10] R. Lucena, Extraction and stirring integrated techniques: examples and recent advances, Anal. Bioanal. Chem. 403 (2012) 2213-2223.
[11] F. Sánchez-Rojas, C. Bosch-Ojeda, J. Cano-Pavón, A review of stir bar sorptive extraction, Chromatographia 69 (2009) 79-94.
[12] M. Kawaguchi, A. Takatsu, R. Ito, H. Nakazawa, Applications of stir-bar sorptive extraction to food analysis, TrAC, Trends Anal. Chem. 45 (2013) 280-293.
[13] Q. Osmani, H. Hughes, P. McLoughlin, Probing the recognition of molecularly imprinted polymer beads, J. Mater. Sci. 47 (2012) 2218-2227.
[14] F.M. Lancas, M.E.C. Queiroz, P. Grossi, I.R.B. Olivares, Recent developments and applications of stir bar sorptive extraction, J. Sep. Sci. 32 (2009) 813-824.
[15] F. David, P. Sandra, Stir bar sorptive extraction for trace analysis, J. Chromatogr. A 1152 (2007) 54-69.
[16] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, Novel stir bar sorptive extraction methods for environmental and biomedical analysis, J. Pharm. Biomed. Anal. 40 (2006) 500-508.
[17] E. Fries, Determination of benzothiazole in untreated wastewater using polar-phase stir bar sorptive extraction and gas chromatography mass spectrometry, Anal. Chim. Acta 689 (2011) 65-68.
[18] B. Sgorbini, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, M.R. Ruosi, C. Bicchi, New medium-to-high polarity twister coatings for liquid and vapour phase sorptive extraction of matrices of vegetable origin, J. Chromatogr. A 1265 (2012) 39-45.
[19] N. Gilart, N. Miralles, R.M. Marcé, F. Borrull, N. Fontanals, Novel coatings for stir bar sorptive

extraction to determine pharmaceuticals and personal care products in environmental waters by liquid chromatography and tandem mass spectrometry, Anal. Chim. Acta 774 (2013) 51-60.

[20] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC-MS, J. Pharm. Biomed. Anal. 78-79 (2013) 255-260.

[21] A. Kabir, K.G. Furton, A. Malik, Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry, TrAC, Trends Anal. Chem. 45 (2013) 197-218.

[22] W.A.W. Ibrahim, W.N.W. Ismail, A.S.A. Keyon, M.M. Sanagi, Preparation and characterization of a new sol-gel hybrid based tetraethoxysilane-polydimethylsiloxane as a stir bar extraction sorbent materials, J. Sol-Gel Sci. Technol. 58 (2011) 602-611.

[23] W.M. Liu, H.M. Wang, Y.F. Guan, Preparation of stir bars for sorptive extraction using sol-gel technology, J. Chromatogr. A 1045 (2004) 15-22.

[24] C.H. Yu, B. Hu, Sol-gel polydimethylsiloxane/poly(vinylalcohol)-coated stir bar sorptive extraction of organophosphorus pesticides in honey and their determination by large volume injection GC, J. Sep. Sci. 32 (2009) 147-153.

[25] C.H. Yu, X. Li, B. Hu, Preparation of sol-gel polyethylene glycol-polydimethylsiloxane-poly(vinyl alcohol)-coated sorptive bar for the determination of organic sulfur compounds in water, J. Chromatogr. A 1202 (2008) 102-106.

[26] W.A.W. Ibrahim, A.S.A. Keyon, N. Prastomo, A. Matsuda, Synthesis and characterization of polydimethylsiloxane-cyano-propyltriethoxysilane-derived hybrid coating for stir bar sorptive extraction, J. Sol-Gel Sci. Technol. 59 (2011) 128-134.

[27] C.H. Yu, B. Hu, Novel combined stir bar sorptive extraction coupled with ultrasonic assisted extraction for the determination of brominated flame retardants in environmental samples using high performance liquid chromatography, J. Chromatogr. A 1160 (2007) 71-80.

[28] S.V. Duy, P.B. Fayad, B. Barbeau, M. Prevost, S. Sauve, Using a novel sol-gel stir bar sorptive extraction method for the analysis of steroid hormones in water by laser diode thermal desorption/atmospheric chemical ionization tandem mass spectrometry, Talanta 101 (2012) 337-345.

[29] C. Hu, M. He, B.B. Chen, B. Hu, Determination of estrogens in pork and chicken samples by stir bar sorptive extraction combined with high-performance liquid chromatography-ultraviolet detection, J. Agric. Food Chem. 60 (2012) 10494-10500.

[30] C. Hu, B. Chen, M. He, B. Hu, Amino modified multi-walled carbon nanotubes/polydimethylsiloxane coated stir bar sorptive extraction coupled to high performance liquid chromatography-ultraviolet detection for

the determination of phenols in environmental samples, J. Chromatogr. A 1300 (2013) 165-172.

[31] C. Hu, M. He, B. Chen, C. Zhong, B. Hu, Polydimethylsiloxane/metal-organic frameworks coated stir bar sorptive extraction coupled to high performance liquid chromatography-ultraviolet detector for the determination of estrogens in environmental water samples, J. Chromatogr. A 1310 (2013) 21-30.

[32] J.Y. Barletta, P. Gomes, A.J. dos Santos-Neto, F.M. Lancas, Development of a new stir bar sorptive extraction coating and its application for the determination of six pesticides in sugarcane juice, J. Sep. Sci. 34 (2011) 1317-1325.

[33] L.R. Melo, A.M. Nogueira, F.M. Lancas, M.E.C. Queiroz, Polydimethylsiloxane/polypyrrole stir bar sorptive extraction and liquid chromatography (SBSE/LC-UV) analysis of antidepressants in plasma samples, Anal. Chim. Acta 633 (2009) 57-64.

[34] B.J. Si, J. Zhou, Non-hydrolytic sol-gel methodology to prepare a molecularly imprinted, organic-silica hybrid-based stir bar for recognition of sulfonylurea herbicides, Chin. J. Chem. 29 (2011) 2487-2494.

[35] N. Sheng, F. Wei, W. Zhan, Z. Cai, S. Du, X. Zhou, F. Li, Q. Hu, Dummy molecularly imprinted polymers as the coating of stir bar for sorptive extraction of bisphenol A in tap water, J. Sep. Sci. 35 (2012) 707-712.

[36] R.D. Arrua, M. Talebi, T.J. Causon, E.F. Hilder, Review of recent advances in the preparation of organic polymer monoliths for liquid chromatography of large molecules, Anal. Chim. Acta 738 (2012) 1-12.

[37] L. Xu, Z.G. Shi, Y.Q. Feng, Porous monoliths: sorbents for miniaturized extraction in biological analysis, Anal. Bioanal. Chem. 399 (2011) 3345-3357.

[38] X.J. Huang, D.X. Yuan, Preparation of stir bars for sorptive extraction based on monolithic material, J. Chromatogr. A 1154 (2007) 152-157.

[39] X.J. Huang, N.N. Qiu, D.X. Yuan, Simple and sensitive monitoring of sulfonamide veterinary residues in milk by stir bar sorptive extraction based on monolithic material and high performance liquid chromatography analysis, J. Chromatogr. A 1216 (2009) 8240-8245.

[40] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, Sensitive determination of strongly polar aromatic amines in water samples by stir bar sorptive extraction based on poly(vinylimidazole-divinylbenzene) monolithic material and liquid chromatographic analysis, J. Chromatogr. A 1216 (2009) 4354-4360.

[41] X.J. Huang, L.L. Chen, D.X. Yuan, X.B. Luo, Preparation, characterization and application of a new stir bar sorptive extraction based on poly(vinylphthalimide-co-N,N'-methylenebisacrylamide) monolith, J. Sep. Sci. 34 (2011) 3418-3425.

[42] X. Huang, L. Chen, D. Yuan, Development of monolith-based stir bar sorptive extraction and liquid chromatography tandem

mass spectrometry method for sensitive determination of ten sulfonamides in pork and chicken samples, Anal. Bioanal. Chem. 405 (2013) 6885-6889.

[43] X.J. Huang, J.B. Lin, D.X. Yuan, Preparation of cation-exchange stir bar sorptive extraction based on monolithic material and its application to the analysis of soluble cations in milk by ion chromatography, Analyst 136 (2011) 4289-4294.

[44] X.J. Huang, J.B. Lin, D.X. Yuan, Simple and sensitive determination of nitroimidazole residues in honey using stir bar sorptive extraction with mixed mode monolith followed by liquid chromatography, J. Sep. Sci. 34 (2011) 2138-2144.

[45] Z.G. Xu, Y.F. Hu, Y.L. Hu, G.K. Li, Investigation of ractopamine molecularly imprinted stir bar sorptive extraction and its application for trace analysis of beta(2)-agonists in complex samples, J. Chromatogr. A 1217 (2010) 3612-3618.

[46] Y.L. Hu, J.W. Li, Y.F. Hu, G.K. Li, Development of selective and chemically stable coating for stir bar sorptive extraction by molecularly imprinted technique, Talanta 82 (2010) 464-470.

[47] Z.G. Xu, Z. Du, Y.L. Hu, Y.F. Hu, Y.P. Pan, G.K. Li, Preparation of trimethoprim molecularly imprinted stir bar sorptive extraction and its application for trace analysis of trimethoprim and sulfonamides in complex samples, Chin. J. Anal. Chem. 40 (2012) 1002-1009.

[48] 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, Anal. Chim. Acta 706 (2011) 135-142.

[49] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, Preparation of a polar monolithic stir bar based on methacrylic acid and divinylbenzene for the sorptive extraction of polar pharmaceuticals from complex water samples, J. Chromatogr. A 1225 (2012) 1-7.

[50] N. Gilart, P.A.G. Cormack, R.M. Marcé, F. Borrull, N. Fontanals, Preparation of a polar monolithic coating for stir bar sorptive extraction of emerging contaminants from wastewaters, J. Chromatogr. A 1295 (2013) 42-47.

[51] E. Turiel, A. Martín-Esteban, Molecularly imprinted stir bars for selective extraction of thiabendazole in citrus samples, J. Sep. Sci. 35 (2012) 2962-2969.

[52] N.R. Neng, M.L. Pinto, J. Pires, P.M. Marcos, J.M.F. Nogueira, Development, optimisation and application of polyurethane foams as new polymeric phases for stir bar sorptive extraction, J. Chromatogr. A 1171 (2007) 8-14.

[53] F.C.M. Portugal, M.L. Pinto, J.M.F. Nogueira, Optimization of polyurethane foams for enhanced stir bar sorptive extraction of triazinic herbicides in water matrices, Talanta 77 (2008) 765-773.

[54] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, Advances in stir bar sorptive extraction for the determination of acidic pharma-

ceuticals in environmental water matrices Comparison between polyurethane and polydimethylsiloxane polymeric phases, J. Chromatogr. A 1209 (2008) 10-16.

[55] W. Guan, Y.J. Wang, F. Xu, Y.F. Guan, Poly(phthalazine ether sulfone ketone) as novel stationary phase for stir bar sorptive extraction of organochlorine compounds and organophosphorus pesticides, J. Chromatogr. A 1177 (2008) 28-35.

[56] A. Giordano, P. Richter, I. Ahumada, Determination of pesticides in river water using rotating disk sorptive extraction and gas chromatography-mass spectrometry, Talanta 85 (2011) 2425-2429.

[57] P. Richter, A. Canas, C. Munoz, C. Leiva, I. Ahumada, Rotating disk sorbent extraction for preconcentration of chromogenic organic compounds and direct determination by solid phase spectrophotometry, Anal. Chim. Acta 695 (2011) 73-76.

[58] P. Richter, C. Leiva, C. Choque, A. Giordano, B. Sepulveda, Rotating-disk sorptive extraction of nonylphenol from water samples, J. Chromatogr. A 1216 (2009) 8598-8602.

[59] Y.B. Luo, H.B. Zheng, J.X. Wan, Q. Gao, Q.W. Yu, Y.Q. Feng, An anionic exchange stir rod sorptive extraction based on monolithic material for the extraction of non-steroidal anti-inflammatory drugs in environmental aqueous samples, Talanta 86 (2011) 103-108.

[60] X.J. Huang, L.L. Chen, F.H. Lin, D.X. Yuan, Novel extraction approach for liquid samples: Stir cake sorptive extraction using monolith, J. Sep. Sci. 34 (2011) 2145-2151.

[61] F.H. Lin, S.Y. Nong, X.J. Huang, D.X. Yuan, Sensitive determination of organic acid preservatives in juices and soft drinks treated by monolith-based stir cake sorptive extraction and liquid chromatography analysis, Anal. Bioanal. Chem. 405 (2013) 2077-2081.

[62] N.R. Neng, A.R.M. Silva, J.M.F. Nogueira, Adsorptive microextraction techniques-Novel analytical tools for trace levels of polar solutes in aqueous media, J. Chromatogr. A 1217 (2010) 7303-7310.

[63] C. Almeida, J.M.F. Nogueira, Comparison of the selectivity of different sorbent phases for bar adsorptive microextraction-Application to trace level analysis of fungicides in real matrices, J. Chromatogr. A 1265 (2012) 7-16.

1.3. References

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

[1] A. Jurado, E. Vázquez-Sune, J. Carrera, M. López de Alda, E. Pujades, D. Barceló, Sci. Total Environ. 440 (2012) 82.
[2] S.D. Richardson, T.A. Ternes, Anal. Chem. 83 (2011) 4614.
[3] M. Farré, S. Pérez, L. Kantiani, D. Barceló, Trends Anal. Chem. 27 (2008) 991.
[4] R.L. Oulton, T. Kohn, D.M. Cwiertny, J. Environ. Monit. 12 (2010) 1956.
[5] A. Pal, K.Y.H. Gin, A.Y.C. Lin, M. Reinhard, Sci. Total Environ. 408 (2010) 6062.
[6] N. Ratola, A. Cincinelli, A. Alves, A. Katsoyiannis, J. Hazard. Mater. 239–240 (2012) 1.
[7] S. Suárez, M. Carballa, F. Omil, J.M. Lema, Rev. Environ. Sci. Biotechnol. 7 (2008) 125
[8] K. Fischer, E. Fries, W. Korner, C. Schmalz, C. Zwiener, Appl. Microbiol. Biotechnol. 94 (2012) 11.
[9] K. Kümmerer, in P. Wilderer (Editor), Treatise on Water Science, Elsevier, Oxford, 2011, p. 69.
[10] L.H.M.L.M. Santos, A.N. Araújo, A. Fachini, A. Pena, C. Delerue-Matos, M.C.B.S.M. Montenegro, J. Hazard. Mater. 175 (2010) 45.
[11] European Council Directive 2000/60/EC, Off. J. Eur. Commun. L327 (2000) 1.
[12] European Council Directive 98/83/EC, Off. J. Eur. Commun. L330 (1998) 32.
[13] European Council Directive 2008/105/EC, Off. J. Eur. Commun. L348 (2008) 84.
[14] Proposal for an European Council Directive 2011, Off. J. Eur. Commun. (2011) 1.
[15] J. Tadeo, C. Sánchez-Brunete, B. Albero, A. García-Valcárcel, R.A. Pérez, Cent. Eur. J. Chem 10 (2012) 480.
[16] D. Fatta-Kassinos, S. Meric, A. Nikolaou, Anal. Bioanal. Chem. 399 (2011) 251.
[17] M. Petrović, M. Farré, M. López de Alda, S. Pérez, C. Postigo, M. Kock, J. Radjenović, M. Gros, D. Barceló, J. Chromatogr. A 1217 (2010) 4004.
[18] M. Farré, L. Kantiani, M. Petrović, S. Pérez, D. Barceló, J. Chromatogr. A 1259 (2012) 86.
[19] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 34 (2011) 1091.
[20] S. Babić, D.M. Pavlović, D. Ašperger, M. Periša, M. Zrnčić, A.J.M. Horvat, M. Kaštelan-Macan, Anal. Bioanal. Chem. 398 (2010) 1185.
[21] B. Shao, D. Chen, J. Zhang, Y.N. Wu, C.J. Sun, J. Chromatogr. A 1216 (2009) 8312.
[22] K. Demeestere, M. Petrović, M. Gros, J. Dewulf, H. Van Langenhove, D. Barceló, Anal. Bioanal. Chem. 396 (2010) 825.
[23] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1216 (2009) 6994.
[24] M.A. Sousa, C. Gonçalves, E. Cunha, J. Hajslová, M.F. Alpendurada, Anal. Bioanal. Chem. 399 (2011) 807.
[25] S.S. Verenitch, C.J. Lowe, A. Mazumder, J. Chromatogr. A 1116 (2006) 193.

[26] A. Gheorghe, A. van Nuijs, B. Pecceu, L. Bervoets, P.G. Jorens, R. Blust, H. Neels, A. Covaci, Anal. Bioanal. Chem. 391 (2008) 1309.
[27] S. Grujić, T. Vasiljević, M. Laušević, J. Chromatogr. A 1216 (2009) 4989.
[28] R. Grabic, J. Fick, R.H. Lindberg, G. Fedorova, M. Tysklind, Talanta 100 (2012) 183.
[29] K. Nödler, T. Licha, K. Bester, M. Sauter, J. Chromatogr. A 1217 (2010) 6511.
[30] J. Radjenović, A. Jelić, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 393 (2009) 1685.
[31] E. Gracia-Lor, M. Martínez, J.V. Sancho, G. Peñuela, F. Hernández, Talanta 99 (2012) 1011.
[32] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[33] A. Agüera, M.J.M. Bueno, A.R. Fernández-Alba, Environ. Sci. Pollut. Res. Int. 20 (2013) 3496.
[34] M. Gros, T.M. Pizzolato, M. Petrović, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374.
[35] M. Gros, S. Rodríguez-Mozaz, D. Barceló, J. Chromatogr. A 1248 (2012) 104.
[36] M. Gros, S. Rodríguez-Mozaz, D. Barceló, J. Chromatogr. A 1292 (2013) 173.
[37] A. Jelić, M. Petrović, D. Barceló, Talanta 80 (2009) 363.
[38] M. Gros, M. Petrović, D. Barceló, Anal. Chem. 81 (2009) 898.
[39] L. Ferrando-Climent, S. Rodríguez-Mozaz, D. Barceló, Anal. Bioanal. Chem. 405 (2013) 5937.
[40] A.C. Hogenboom, J.A. van Leerdam, P. de Voogt, J. Chromatogr. A 1216 (2009) 510.
[41] H.-X. Wang, Y. Zhou, Q.-W. Jiang, Microchem. J. 100 (2012) 83.
[42] I. Ferrer, E.M. Thurman, J. Chromatogr. A 1259 (2012) 148.
[43] A. Pérez-Parada, M.d.M. Gómez-Ramos, M. Martínez Bueno, S. Uclés, A. Uclés, A. Fernández-Alba, Environ. Sci. Pollut. Res. 19 (2012) 467.
[44] M. Laven, T. Alsberg, Y. Yu, M. Adolfsson-Erici, H.W. Sun, J. Chromatogr. A 1216 (2009) 49.
[45] M. Petrović, M. Gros, D. Barceló, J. Chromatogr. A 1124 (2006) 68.
[46] I. González-Mariño, J.B. Quintana, I. Rodríguez, M. González-Díez, R. Cela, Anal. Chem. 84 (2012) 1708.
[47] J. Casado, I. Rodríguez, M. Ramil, R. Cela, J. Chromatogr. A 1299 (2013) 40.
[48] J.A. van Leerdam, A.C. Hogenboom, M.M.E. van der Kooi, P. de Voogt, Int. J. Mass Spectrom. 282 (2009) 99.
[49] L. Bijlsma, E. Emke, F. Hernández, P. de Voogt, Anal. Chim. Acta 768 (2013) 102.
[50] M.G. Cahill, B.A. Dineen, M.A. Stack, K.J. James, J. Chromatogr. A 1270 (2012) 88.
[51] M. Himmelsbach, J. Chromatogr. B 883–884 (2012) 3.

[52] K. Wille, H.F. De Brabander, L. Vanhaecke, E. De Wulf, P. Van Caeter, C.R. Janssen, Trends Anal. Chem. 35 (2012) 87.
[53] T.R. Covey, B.A. Thomson, B.B. Schneider, Mass Spectrom. Rev. 28 (2009) 870.
[54] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[55] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Talanta 74 (2008) 1299.
[56] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, Mass Spectrom. Rev. 30 (2011) 491.
[57] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, J. Chromatogr. A 1217 (2010) 3929.
[58] A. Wick, G. Fink, T.A. Ternes, J. Chromatogr. A 1217 (2010) 2088.
[59] K.T.N. Nguyen, C. Scapolla, M. Di Carro, E. Magi, Talanta 85 (2011) 2375.
[60] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, J. Chromatogr. A 1235 (2012) 178.
[61] A. García-Ac, P.A. Segura, L. Viglino, C. Gagnon, S. Sauvé, J. Mass Spectrom. 46 (2011) 383.
[62] G.-W. Lien, C.-Y. Chen, G.-S. Wang, J. Chromatogr. A 1216 (2009) 956.
[63] L. Ramos, J. Chromatogr. A 1221 (2012) 84.
[64] Y. Chen, Z.P. Guo, X.Y. Wang, C.G. Qiu, J. Chromatogr. A 1184 (2008) 191.
[65] L. Nováková, J. Chromatogr. A 1292 (2013) 25.
[66] J. Pawliszyn, H.L. Lord, in, John Wiley & Sons Inc., New Jersey, 2010, p. 491.
[67] J.R. Dean, Extraction techniques in analytical science, John Wiley & Sons Inc., Newcastle, UK, 2009.
[68] N. Fontanals, R.M. Marcé, F. Borrull, in J.C. Taylor (Editor), Advances in Chemistry Research, Nova Science Publishers, 2010.
[69] F. Augusto, L.W. Hantao, N.G.S. Mogollón, S.C.G.N. Braga, Trends Anal. Chem. 43 (2013) 14.
[70] N. Fontanals, F. Borrull, R.M. Marcé, Trends Anal. Chem. 41 (2012) 15.
[71] S.C. Cobzac, S. Gocan, J. Liq. Chromatogr. Relat. Technol. 34 (2011) 1157.
[72] D.E. Raynie, Anal. Chem. 82 (2010) 4911.
[73] A. Spietelun, Ł. Marcinkowski, M. de la Guardia, J. Namieśnik, J. Chromatogr. A 1321 (2013) 1.
[74] J.M.F. Nogueira, Anal. Chim. Acta 757 (2012) 1.
[75] J.Q. Xu, J. Zheng, J.Y. Tian, F. Zhu, F. Zeng, C.Y. Su, G.F. Ouyang, Trends Anal. Chem. 47 (2013) 68.
[76] D. Han, K. Row, Microchim. Acta 176 (2012) 1.
[77] F. Augusto, E. Carasek, R.G.C. Silva, S.R. Rivellino, A.D. Batista, E. Martendal, J. Chromatogr. A 1217 (2010) 2533.
[78] Z. Huang, H.K. Lee, Trends Anal. Chem. 39 (2012) 228.

[79] N. Fontanals, P.A.G. Cormack, R.M. Marcé, F. Borrull, Trends Anal. Chem. 29 (2010) 765.
[80] L. Vidal, M.L. Riekkola, A. Canals, Anal. Chim. Acta 715 (2012) 19.
[81] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[82] L. Novakova, H. Vlckova, Anal. Chim. Acta 656 (2009) 8.
[83] S. Risticevic, V. Niri, D. Vuckovic, J. Pawliszyn, Anal. Bioanal. Chem. 393 (2009) 781.
[84] H. Kataoka, K. Saito, J. Pharm. Biomed. Anal. 54 (2011) 926.
[85] G. Ouyang, J. Pawliszyn, Anal. Bioanal. Chem. 386 (2006) 1059.
[86] C.F. Duan, Z. Shen, D.P. Wu, Y.F. Guan, Trends Anal. Chem. 30 (2011) 1568.
[87] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[88] M. Kawaguchi, R. Ito, H. Nakazawa, A. Takatsu, in J. Pawliszyn (Editor), Comprehensive Sampling and Sample Preparation, Elsevier Inc., USA, 2012, p. 797.
[89] R. Lucena, Anal. Bioanal. Chem. 403 (2012) 2213.
[90] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642.
[91] M. Kawaguchi, A. Takatsu, R. Ito, H. Nakazawa, Trends Anal. Chem. 45 (2013) 280.
[92] M. Abdel-Rehim, Anal. Chim. Acta 701 (2011) 119.
[93] M. Abdel-Rehim, J. Chromatogr. A 1217 (2010) 2569.
[94] A. Prieto, A. Vallejo, O. Zuloaga, A. Paschke, B. Sellergen, E. Schillinger, S. Schrader, M. Möder, Anal. Chim. Acta 703 (2011) 41.
[95] A. Prieto, S. Schrader, C. Bauer, M. Moder, Anal. Chim. Acta 685 (2011) 146.
[96] P.L. Kole, G. Venkatesh, J. Kotecha, R. Sheshala, Biomed. Chromatogr. 25 (2010) 199.
[97] L. Kovatsi, K. Rentifis, D. Giannakis, S. Njau, V. Samanidou, J. Sep. Sci. 34 (2011) 1716.
[98] H. Guan, W.E. Brewer, S.T. Garris, S.L. Morgan, J. Chromatogr. A 1217 (2010) 1867.
[99] S.J. Lehotay, K. Mastovska, A.R. Lightfield, A. Nuñez, T. Dutko, C. Ng, L. Bluhm, J. Chromatogr. A 1313 (2013) 103.
[100] S.T. Ellison, W.E. Brewer, S.L. Morgan, J. Anal. Toxicol. 33 (2009) 356.
[101] M.A. Jeannot, A. Przyjazny, J.M. Kokosa, J. Chromatogr. A 1217 (2010) 2326.
[102] L. Xu, C. Basheer, H.K. Lee, J. Chromatogr. A 1152 (2007) 184.
[103] L. Vidal, A. Chisvert, A. Canals, A. Salvador, Talanta 81 (2010) 549.
[104] M. Tobiszewski, A. Mechlińska, B. Zygmunt, J. Namieśnik, Trends Anal. Chem. 28 (2009) 943.
[105] F. Pena-Pereira, I. Lavilla, C. Bendicho, Trends Anal. Chem. 29 (2010) 617.

[106] A. Jain, K.K. Verma, Ana. Chim. Acta 706 (2011) 37.
[107] M. Ghambarian, Y. Yamini, A. Esrafili, Microchim. Acta 177 (2012) 271.
[108] J. Lee, H.K. Lee, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 624 (2008) 253.
[109] X. Jiang, H.K. Lee, Anal. Chem. 76 (2004) 5591.
[110] M.B. Melwanki, S.-D. Huang, M.-R. Fuh, Talanta 72 (2007) 373.
[111] L. Guo, H.K. Lee, J. Chromatogr. A 1235 (2012) 26.
[112] L. Guo, H.K. Lee, J. Chromatogr. A 1286 (2013) 9.
[113] A. Zgoła-Grześkowiak, T. Grześkowiak, Trends Anal. Chem. 30 (2011) 1382.
[114] C.F. Poole, Trends Anal. Chem. 22 (2003) 362.
[115] J.S. Fritz, Analytical solid-phase extraction, John Wiley & Sons Inc., USA, 1999.
[116] S. Mitra, Sample preparation techniques in analytical chemistry, John Wiley & Sons Inc., New Jersey, 2003.
[117] M.-C. Hennion, J. Chromatogr. A 856 (1999) 3.
[118] V. Camel, Spectrochim. Acta, Part B 58 (2003) 1177.
[119] P. Vázquez-Roig, C. Blasco, Y. Picó, Trends Anal. Chem. 50 (2013) 65.
[120] W.W. Buchberger, J. Chromatogr. A 1218 (2011) 603.
[121] N. Fontanals, F. Borrull, R.M. Marcé, J. Chromatogr. A 1286 (2013) 16.
[122] S. Rodríguez-Mozaz, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1152 (2007) 97.
[123] V. Pichon, J. Chromatogr. A 885 (2000) 195.
[124] M.E. León-González, L.V. Pérez-Arribas, J. Chromatogr. A 902 (2000) 3.
[125] A. Zwir-Ferenc, M. Biziuk, Pol. J. Environ. Stud. 15 (2006) 677.
[126] O. Rozita, S. Norashikin, A. Md. Pauzi, Res. J. Chem. Environ. 13 (2009) 7.
[127] O. Zuloaga, P. Navarro, E. Bizkarguenaga, A. Iparraguirre, A. Vallejo, M. Olivares, A. Prieto, Anal. Chim. Acta 736 (2012) 7.
[128] N. Masqué, R.M. Marcé, F. Borrull, Trends Anal. Chem. 17 (1998) 384.
[129] M.C. Hennion, J. Chromatogr. A 885 (2000) 73.
[130] K. Pyrzyńska, Chem. Anal. (Warsaw) 48 (2003) 781.
[131] M.C. Bruzzoniti, C. Sarzanini, G. Costantino, M. Fungi, Anal. Chim. Acta 578 (2006) 241.
[132] L.M. Ravelo-Pérez, A.V. Herrera-Herrera, J. Hernández-Borges, M.A. Rodríguez-Delgado, J. Chromatogr. A 1217 (2010) 2618.
[133] M.F.L. De Volder, S.H. Tawfick, R.H. Baughman, A.J. Hart, Science 339 (2013) 535.
[134] R. Sitko, B. Zawisza, E. Malicka, Trends Anal. Chem. 51 (2013) 33.
[135] V.A. Lemos, L.S.G. Teixeira, M.D.A. Bezerra, A.C.S. Costa, J.T. Castro, L.A.M. Cardoso, D.S. de Jesus, E.S. Santos, P.X. Baliza, L.N. Santos, Appl. Spectrosc. Rev. 43 (2008) 303.

[136] H. Niu, Y. Cai, Y. Shi, F. Wei, J. Liu, S. Mou, G. Jiang, Anal. Chim. Acta 594 (2007) 81.
[137] Y.S. Al-Degs, M.A. Al-Ghouti, Int. J. Environ. Anal. Chem. 88 (2008) 487.
[138] J. Wu, L. Chen, P. Mao, Y. Lu, H. Wang, J. Sep. Sci. 35 (2012) 3586.
[139] N. Fontanals, R.M. Marcé, F. Borrull, J. Chromatogr. A 1152 (2007) 14.
[140] N. Fontanals, R.M. Marcé, F. Borrull, Contrib. Sci. 6 (2010) 199.
[141] M. Vosough, N.R. Mojdehi, Talanta 85 (2011) 2175.
[142] H.M. Koch, J. Angerer, J. Chromatogr. B 759 (2001) 43.
[143] N. Fontanals, R.M. Marcé, F. Borrull, Trends Anal. Chem. 24 (2005) 394.
[144] V.A. Davankov, M.P. Tsyurupa, React. Polym. 13 (1990) 27.
[145] M.P. Tsyurupa, L.A. Maslova, A.I. Andreeva, T.A. Mrachkovskaya, V.A. Davankov, React. Polym. 25 (1995) 69.
[146] M.P. Tsyurupa, V.A. Davankov, React. Funct. Polym. 66 (2006) 768.
[147] P. Veverka, K. Jeřábek, React. Funct. Polym. 41 (1999) 21.
[148] N. Fontanals, J. Cortés, M. Galià, R.M. Marcé, P.A.G. Cormack, F. Borrull, D.C. Sherrington, J. Polym. Sci., Part A: Polym. Chem. 43 (2005) 1718.
[149] J.-H. Ahn, J.-E. Jang, C.-G. Oh, S.-K. Ihm, J. Cortez, D.C. Sherrington, Macromolecules 39 (2005) 627.
[150] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. A 1215 (2008) 21.
[151] N. Fontanals, P. Manesiotis, D.C. Sherrington, P.A.G. Cormack, Adv. Mater. 20 (2008) 1298.
[152] S. Guenu, M.-C. Hennion, J. Chromatogr. A 737 (1996) 15.
[153] A.A. D'Archivio, M. Fanelli, P. Mazzeo, F. Ruggieri, Talanta 71 (2007) 25.
[154] G. Sirvent, J.M. Sánchez, M. Hidalgo, V. Salvadó, Int. J. Environ. Anal. Chem. 89 (2009) 293.
[155] G.W. Muna, V. Quaiserová-Mocko, G.M. Swain, Anal. Chem. 77 (2005) 6542.
[156] D. Puig, D. Barceló, J. Chromatogr. A 733 (1996) 371.
[157] M. Scheurer, H.J. Brauch, F.T. Lange, Anal. Bioanal. Chem. 394 (2009) 1585.
[158] A. Zygler, A. Wasik, J. Namiesnik, Talanta 82 (2010) 1742.
[159] A. Salemi, M. Vosough, E. Mazlumi, J. Liq. Chromatogr. Relat. Technol. 35 (2012) 2113.
[160] L. Culleré, M. Bueno, J. Cacho, V. Ferreira, J. Chromatogr. A 1217 (2010) 1557.
[161] N. Fontanals, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, J. Chromatogr. A 1191 (2008) 118.
[162] J.S. Fritz, P.J. Dumont, L.W. Schmidt, J. Chromatogr. A 691 (1995) 133.
[163] C.W. Huck, G.K. Bonn, J. Chromatogr. A 885 (2000) 51.
[164] N. Masqué, M. Galià, R.M. Marcé, F. Borrull, Analyst 122 (1997) 425.
[165] M. Lee, S.Y. Oh, T.S. Pathak, I.R. Paeng, B.-Y. Cho, K.-J. Paeng, J. Chromatogr. A 1160 (2007) 340.

[166] S.R. Dave, H. Kaur, S.K. Menon, React. Funct. Polym. 70 (2010) 692.
[167] C. Karadaş, O. Turhan, D. Kara, Food Chem. 141 (2013) 655.
[168] A. Jahnke, S. Huber, C. Temme, H. Kylin, U. Berger, J. Chromatogr. A 1164 (2007) 1.
[169] E.S. Majzik, F. Tóth, L. Benke, Z. Kiss, Chromatographia 63 (2006) S105.
[170] Á. Kovács, M. Mörtl, A. Kende, Microchem. J. 99 (2011) 125.
[171] M. Metafa, A. Economou, Cent. Eur. J. Chem. 11 (2013) 228.
[172] E. Lendoiro, Ó. Quintela, A. de Castro, A. Cruz, M. López-Rivadulla, M. Concheiro, Forensic Sci. Int. 217 (2012) 207.
[173] F. Busetti, K.L. Linge, A. Heitz, J. Chromatogr. A 1216 (2009) 5807.
[174] T. Kosjek, E. Heath, A. Krbavčič, Environ. Int. 31 (2005) 679.
[175] A. García-Ac, P.A. Segura, L. Viglino, A. Fürtös, C. Gagnon, M. Prévost, S. Sauvé, J. Chromatogr. A 1216 (2009) 8518.
[176] A. Stafiej, K. Pyrzynska, F. Regan, J. Sep. Sci. 30 (2007) 985.
[177] M. Bjørk, K. Simonsen, D. Andersen, P. Dalsgaard, S. Sigurðardóttir, K. Linnet, B. Rasmussen, Anal. Bioanal. Chem. 405 (2013) 2607.
[178] G. Sirvent, M. Hidalgo, V. Salvadó, J. Sep. Sci. 27 (2004) 613.
[179] J. Coughlin, B. Winnik, B. Buckley, Anal. Bioanal. Chem. 401 (2011) 995.
[180] R. Fernández-Torres, M.O. Consentino, M.A.B. López, M.C. Mochón, Talanta 81 (2010) 871.
[181] E.Y. Ordóñez, J.B. Quintana, R. Rodil, R. Cela, J. Chromatogr. A 1256 (2012) 197.
[182] J.Y. Pailler, A. Krein, L. Pfister, L. Hoffmann, C. Guignard, Sci. Total Environ. 407 (2009) 4736.
[183] P. Vázquez-Roig, V. Andreu, C. Blasco, Y. Picó, Anal. Bioanal. Chem. 397 (2010) 2851.
[184] J. Ma, R. Xiao, J. Li, X. Zhao, B. Shi, S. Li, J. Chromatogr. Sci. 47 (2009) 110.
[185] T. Ogawa, H. Hattori, R. Kaneko, K. Ito, M. Iwai, Y. Mizutani, T. Arinobu, A. Ishii, H. Seno, Anal. Bioanal. Chem. 400 (2011) 1959.
[186] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Water Air Soil Pollut. 217 (2011) 267.
[187] L. Maldaner, I.C.S.F. Jardim, Talanta 100 (2012) 38.
[188] I. Botello, F. Borrull, M. Calull, C. Aguilar, G.W. Somsen, G.J. De Jong, Anal. Bioanal. Chem. 403 (2012) 777.
[189] N. Stoilova, A. Surleva, G. Stoev, Food Anal. Methods 6 (2013) 803.
[190] Y. Wang, X. Liu, C. Xiao, Z. Wang, J. Wang, H. Xiao, L. Cui, Q. Xiang, T. Yue, Food Control 28 (2012) 131.
[191] A. Azzouz, E. Ballesteros, Sci. Total Environ. 419 (2012) 208.
[192] I. Kosarac, C. Kubwabo, K. Lalonde, W. Foster, J. Chromatogr. B 898 (2012) 90.
[193] I. Baranowska, B. Kowalski, Water Air Soil Pollut. 211 (2010) 417.

[194] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678.
[195] S. Weigel, R. Kallenborn, H. Huhnerfuss, J. Chromatogr. A 1023 (2004) 183.
[196] R. Liu, J.L. Zhou, A. Wilding, J. Chromatogr. A 1022 (2004) 179.
[197] D.M. Pavlović, D. Ašperger, D. Tolić, S. Babić, J. Sep. Sci. 36 (2013) 3042.
[198] Z. Ma, Q. Wu, D.Y.W. Lee, M. Tracy, S.E. Lukas, J. Chromatogr. B 823 (2005) 108.
[199] H. He, C. Sun, X.-R. Wang, C. Pham-Huy, N. Chikhi-Chorfi, H. Galons, M. Thevenin, J.-R. Claude, J.-M. Warnet, J. Chromatogr. B 814 (2005) 385.
[200] V.F. Samanidou, E.D. Tsochatzis, I.N. Papadoyannis, Microchim. Acta 160 (2008) 471.
[201] A. Zotou, C. Vasiliadou, Chromatographia 64 (2006) 307.
[202] G.M. Momplaisir, C.G. Rosal, E.M. Heithmar, K.E. Varner, L.A. Riddick, D.F. Bradford, N.G. Tallent-Halsell, Talanta 81 (2010) 1380.
[203] M. Borecka, A. Białk-Bielińska, G. Siedlewicz, K. Kornowska, J. Kumirska, P. Stepnowski, K. Pazdro, J. Chromatogr. A 1304 (2013) 138.
[204] K.A. Krogh, K.V. Vejrup, B.B. Mogensen, B. Halling-Sørensen, J. Chromatogr. A 957 (2002) 45.
[205] M. Castillo, D. Puig, D. Barceló, J. Chromatogr. A 778 (1997) 301.
[206] U. Divrikli, A. Akdogan, M. Soylak, L. Elci, J. Hazard. Mater. 149 (2007) 331.
[207] H. Elvan, D. Ozdes, C. Duran, D. Sahin, M. Tufekci, Z. Bahadir, Quim. Nova 36 (2013) 831.
[208] H. Bagheri, M. Saraji, J. Chromatogr. A 910 (2001) 87.
[209] H. Bagheri, M. Saraji, D. Barceló, Chromatographia 59 (2004) 283.
[210] H. Bagheri, M. Saraji, J. Chromatogr. A 986 (2003) 111.
[211] H. Bagheri, A. Mohammadi, A. Salemi, Anal. Chim. Acta 513 (2004) 445.
[212] A.W. Trochimczuk, M. Streat, B.N. Kolarz, React. Funct. Polym. 46 (2001) 259.
[213] D. Drechny, A.W. Trochimczuk, React. Funct. Polym. 66 (2006) 323.
[214] A.W. Trochimczuk, S. Aoki, K. Yamabe, A. Jyo, Eur. Polym. J. 38 (2002) 941.
[215] A.W. Trochimczuk, S. Aoki, K. Yamabe, A. Jyo, Eur. Polym. J. 38 (2002) 1175.
[216] K. Bielicka-Daszkiewicz, A. Voelkel, M. Szejner, J. Osypiuk, Chemosphere 62 (2006) 890.
[217] N. Fontanals, P. Puig, M. Galià, R.M. Marcé, F. Borrull, J. Chromatogr. A 1035 (2004) 281.
[218] N. Fontanals, M. Galià, R.M. Marcé, F. Borrull, Chromatographia 60 (2004) 511.
[219] N. Fontanals, M. Galià, R.M. Marcé, F. Borrull, J. Chromatogr. A 1030 (2004) 63.
[220] D. Bratkowska, N. Fontanals, F. Borrull, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, J. Chromatogr. A 1217 (2010) 3238.
[221] M. Gao, H. Yan, N. Sun, Anal. Chim. Acta 800 (2013) 43.
[222] A. Furey, M. Moriarty, V. Bane, B. Kinsella, M. Lehane, Talanta 115 (2013) 104.
[223] P.J. Taylor, Clin. Biochem. 38 (2005) 328.

[224] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 877 (2009) 2198.
[225] F. Peters, D. Remane, Anal. Bioanal. Chem. 403 (2012) 2155.
[226] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, J. Chromatogr. A 1309 (2013) 22.
[227] A. Jia, Y. Xiao, J. Hu, M. Asami, S. Kunikane, J. Chromatogr. A 1216 (2009) 4655.
[228] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, J. Chromatogr. A 1216 (2009) 3078.
[229] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, J. Chromatogr. A 1224 (2012) 19.
[230] A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Anal. Bioanal. Chem. 395 (2009) 819.
[231] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.
[232] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 40 (2006) 500.
[233] F. Sánchez-Rojas, C. Bosch-Ojeda, J. Cano-Pavón, Chromatographia 69 (2009) 79.
[234] I. Carpinteiro, M. Ramil, I. Rodríguez, J.M.F. Nogueira, J. Sep. Sci. 35 (2012) 459.
[235] F.M. Lancas, M.E.C. Queiroz, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813.
[236] L. Abdulra'uf, G. Tan, Chromatographia 77 (2014) 15.
[237] M. He, B. Chen, B. Hu, Anal. Bioanal. Chem. (2014) DOI: 10.1007/s00216.
[238] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chim. 397 (2010) 2833.
[239] N. Ramírez, F. Borrull, R.M. Marcé, J. Sep. Sci. 35 (2012) 580.
[240] D.R. Klein, D.F. Flannelly, M.M. Schultz, J. Chromatogr. A 1217 (2010) 1742.
[241] C. Margoum, C. Guillemain, X. Yang, M. Coquery, Talanta 116 (2013) 1.
[242] P. Tölgyessy, B. Vrana, K. Šilhárová, Chromatographia 76 (2013) 177.
[243] M. Martínez-Parreño, J. Llorca-Pórcel, I. Valor, J. Sep. Sci. 31 (2008) 3620.
[244] V.M. León, J. Llorca-Pórcel, B. Álvarez, M.A. Cobollo, S. Muñoz, I. Valor, Anal. Chim. Acta 558 (2006) 261.
[245] X. Mao, B. Hu, M. He, W. Fan, J. Chromatogr. A 1260 (2012) 16.
[246] M.G. Pintado-Herrera, E. González-Mazo, P.A. Lara-Martín, Anal. Bioanal. Chem. 405 (2013) 401.
[247] F.J. Camino-Sánchez, A. Zafra-Gómez, J.P. Pérez-Trujillo, J.E. Conde-González, J.C. Marques, J.L. Vílchez, Chemosphere 84 (2011) 869.
[248] C. Almeida, J.M.F. Nogueira, J. Pharm. Biomed. Anal. 41 (2006) 1303.
[249] A. Iparraguirre, A. Prieto, P. Navarro, M. Olivares, L.-Á. Fernández, O. Zuloaga, Anal. Bioanal. Chem. 401 (2011) 339.
[250] N. Ochiai, T. Ieda, K. Sasamoto, Y. Takazawa, S. Hashimoto, A. Fushimi, K. Tanabe, J. Chromatogr. A 1218 (2011) 6851.
[251] A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria, L.A. Fernández, Anal. Bioanal. Chem. 390 (2008) 739.

[252] A. Sánchez-Ortega, N. Unceta, A. Gómez-Caballero, M.C. Sampedro, U. Akesolo, M.A. Goicolea, R.J. Barrio, Anal. Chim. Acta 641 (2009) 110.
[253] P.M.Á. do Rosário, J.M.F. Nogueira, Electrophoresis 27 (2006) 4694.
[254] G. Zhao, Y. Chen, S. Wang, J. Yu, X. Wang, F. Xie, H. Liu, J. Xie, Talanta 116 (2013) 822.
[255] C. Fernandes, E. Van Hoeck, P. Sandra, F.M. Lanças, Anal. Chim. Acta 614 (2008) 201.
[256] A. Stopforth, C.J. Grobbelaar, A.M. Crouch, P. Sandra, J. Sep. Sci. 30 (2007) 257.
[257] P.L. Kole, J. Millership, J.C. McElnay, Talanta 85 (2011) 1948.
[258] A.R. Chaves, S.M. Silva, R.H.C. Queiroz, F.M. Lanças, M.E.C. Queiroz, J. Chromatogr. B 850 (2007) 295.
[259] Y. Fang, M.C. Qian, J. Agric. Food. Chem. 54 (2006) 8567.
[260] A. Juan-García, J. Mañes, G. Font, Y. Picó, J. Chromatogr. A 1050 (2004) 119.
[261] B.T. Weldegergis, A.M. Crouch, J. Agric. Food. Chem. 56 (2008) 10225.
[262] V.G. Zuin, M. Schellin, L. Montero, J.H. Yariwake, F. Augusto, P. Popp, J. Chromatogr. A 1114 (2006) 180.
[263] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, J. Chromatogr. A 1315 (2013) 21.
[264] L.R. Melo, A.M. Nogueira, F.M. Lancas, M.E.C. Queiroz, Anal. Chim. Acta 633 (2009) 57.
[265] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1217 (2010) 2667.
[266] V.M. León, B. Álvarez, M.A. Cobollo, S. Muñoz, I. Valor, J. Chromatogr. A 999 (2003) 91.
[267] F. Hernández, J. Beltran, F.J. López, J.V. Gaspar, Anal. Chem. 72 (2000) 2313.
[268] X.J. Huang, N.N. Qiu, D.X. Yuan, J. Chromatogr. A 1216 (2009) 8240.
[269] N.S. Chary, S. Herrera, M. Gómez, A. Fernández-Alba, Anal. Bioanal. Chem. 404 (2012) 1993.
[270] P. Serôdio, J.M.F. Nogueira, Anal. Bioanal. Chem. 382 (2005) 1141.
[271] M. Barriada-Pereira, P. Serôdio, M.J. González-Castro, J.M.F. Nogueira, J. Chromatogr. A 1217 (2010) 119.
[272] X. Huang, N. Qiu, D. Yuan, B. Huang, Talanta 78 (2009) 101.
[273] S.V. Duy, P.B. Fayad, B. Barbeau, M. Prevost, S. Sauve, Talanta 101 (2012) 337.
[274] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1174 (2007) 27.
[275] X.J. Huang, D.X. Yuan, B.L. Huang, Talanta 75 (2008) 172.
[276] J. Xu, B. Chen, M. He, B. Hu, J. Chromatogr. A 1278 (2013) 8.
[277] C. Bicchi, C. Cordero, P. Rubiolo, P. Sandra, J. Sep. Sci. 26 (2003) 1650.
[278] J. Vercauteren, C. Pérès, C. Devos, P. Sandra, F. Vanhaecke, L. Moens, Anal. Chem. 73 (2001) 1509.

[279] X.J. Huang, D.X. Yuan, J. Chromatogr. A 1154 (2007) 152.
[280] W.M. Liu, H.M. Wang, Y.F. Guan, J. Chromatogr. A 1045 (2004) 15.
[281] W.M. Liu, Y. Hu, J.H. Zhao, Y. Xu, Y.F. Guan, J. Chromatogr. A 1095 (2005) 1.
[282] H. Faraji, S.W. Husain, M. Helalizadeh, J. Chromatogr. Sci. 49 (2011) 482.
[283] A. Zalacain, J. Marín, G.L. Alonso, M.R. Salinas, Talanta 71 (2007) 1610.
[284] A. Giordano, M. Fernández-Franzón, M.J. Ruiz, G. Font, Y. Picó, Anal. Bioanal. Chem. 393 (2009) 1733.
[285] N. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Daishima, A. Heiden, A. Hoffman, Analyst 126 (2001) 1652.
[286] S. Montesdeoca-Esponda, A. del Toro-Moreno, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, J. Sep. Sci. 36 (2013) 2168.
[287] E. Magi, M. Di Carro, C. Scapolla, K.N. Nguyen, Chromatographia 75 (2012) 973.
[288] A. Silva, J.M.F. Nogueira, Anal. Bioanal. Chem. 396 (2010) 1853.
[289] C. Hu, M. He, B.B. Chen, B. Hu, J. Agric. Food Chem. 60 (2012) 10494.
[290] X.J. Huang, J.B. Lin, D.X. Yuan, J. Sep. Sci. 34 (2011) 2138.
[291] X.J. Huang, J.B. Lin, D.X. Yuan, J. Chromatogr. A 1217 (2010) 4898.
[292] X.J. Huang, N.N. Qiu, D.X. Yuan, J. Chromatogr. A 1194 (2008) 134.
[293] X. Huang, L. Chen, M. Chen, D. Yuan, S. Nong, J. Sep. Sci. 36 (2013) 907.
[294] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, Anal. Chim. Acta 706 (2011) 135.
[295] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, J. Chromatogr. A 1225 (2012) 1.
[296] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1216 (2009) 4354.
[297] C.H. Yu, B. Hu, J. Sep. Sci. 32 (2009) 147.
[298] W.A.W. Ibrahim, A.S.A. Keyon, N. Prastomo, A. Matsuda, J. Sol-Gel Sci. Technol. 59 (2011) 128.
[299] M. Kawaguchi, R. Ito, N. Endo, N. Sakui, N. Okanouchi, K. Saito, N. Sato, T. Shiozaki, H. Nakazawa, Anal. Chim. Acta 557 (2006) 272.
[300] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, J. Chromatogr. A 1241 (2012) 21.
[301] R. Rodil, M. Moeder, J. Chromatogr. A 1179 (2008) 81.
[302] P. Tölgyessy, B. Vrana, Z. Krascsenits, Talanta 87 (2011) 152.
[303] E. Van Hoeck, F. David, P. Sandra, J. Chromatogr. A 1157 (2007) 1.
[304] R. Rodil, M. Moeder, J. Chromatogr. A 1178 (2008) 9.
[305] J.P. Lambert, W.M. Mullett, E. Kwong, D. Lubda, J. Chromatogr. A 1075 (2005) 43.
[306] N. Ochiai, K. Sasamoto, H. Kanda, E. Pfannkoch, J. Chromatogr. A 1200 (2008) 72.
[307] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, F. David, P. Sandra, J. Chromatogr. A 1094 (2005) 9.

[308] M. Kawaguchi, Y. Ishii, N. Sakui, N. Okanouchi, R. Ito, K. Inoue, K. Saito, H. Nakazawa, J. Chromatogr. A 1049 (2004) 1.

[309] E. Van Hoeck, F. Canale, C. Cordero, S. Compernolle, C. Bicchi, P. Sandra, Anal. Bioanal. Chem. 393 (2009) 907.

[310] F. Mellano, M. Bujalance, I. Giráldez, P. Ruiz-Azcona, D. Sánchez-Rodas, E. Morales, J. Chromatogr. A 1300 (2013) 151.

[311] N. Ochiai, K. Sasamoto, H. Kanda, S. Nakamura, J. Chromatogr. A 1130 (2006) 83.

[312] M. Arbulu, M.C. Sampedro, A. Sánchez-Ortega, A. Gómez-Caballero, N. Unceta, M.A. Goicolea, R.J. Barrio, Anal. Chim. Acta 777 (2013) 41.

[313] M.C. Sampedro, M.A. Goicolea, N. Unceta, A. Sánchez-Ortega, R.J. Barrio, J. Sep. Sci. 32 (2009) 3449.

[314] C. Bicchi, C. Cordero, E. Liberto, B. Sgorbini, F. David, P. Sandra, P. Rubiolo, J. Chromatogr. A 1164 (2007) 33.

[315] A.M. Casas Ferreira, M. Möder, M.E. Fernández Laespada, Anal. Bioanal. Chem. 399 (2011) 945.

[316] J. Llorca-Pórcel, M. Martínez-Parreño, E. Martínez-Soriano, I. Valor, J. Chromatogr. A 1216 (2009) 5955.

CHAPTER 2

OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

The main aim of the present Doctoral Thesis is to develop analytical methodologies for determining organic contaminants in environmental waters using novel materials for different sorptive extraction techniques, such as solid-phase extraction and stir bar sorptive extraction.

This principal objective can be divided into two parts:

- Development and evaluation of selective sorbents for solid-phase extraction, such as molecularly imprinted polymers and strong cation-exchange polymers.

- Preparation and application of novel polar, commercial and in-house coatings for stir bar sorptive extraction.

CHAPTER 3

EXPERIMENTAL, RESULTS AND DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

As mentioned throughout the Introduction to this Doctoral Thesis, emerging organic contaminants (EOCs) include a great variety of compounds with a wide range of chemical properties, such as pharmaceuticals, personal care products (PCPs), surfactants, hormones and illicit drugs, among others. The continuous presence of these compounds in environmental waters is mainly due to the high consumption and use of products containing these compounds, as well as the possible ineffectiveness of the wastewater treatment plants (WWTPs) in terms of removing these compounds from wastewaters, thereby causing adverse effects on aquatic and terrestrial organisms [1,2]. Therefore, it is important to control their presence and also their concentration levels in the environment in order to prevent these compounds reaching drinking water supplies and, as a result, affecting human health. Due to the low concentrations of EOCs found, as well as the high complexity of environmental waters, selective and sensitive analytical methodologies are required. Such methodologies are the main aim of the research presented in this Doctoral Thesis.

This chapter includes the experimental part, results and discussion of the different studies that have been carried out throughout this Doctoral Thesis. These studies have been classified into two sections and, in each case, a brief introduction is included to establish the context of the research. In addition, the most noteworthy results are also discussed at the end of each section. The results included in these studies have already been published or are in the process of being published and they are presented in journal paper format. The list of articles resulting from this Doctoral Thesis is included in Annex II.

The first section reports three novel analytical methods in which selective solid-phase extraction (SPE) is used for the extraction of pharmaceuticals, PCPs and illicit drugs from environmental waters by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Two of these methods involve the use of a molecularly imprinted polymer (MIP) for the selective extraction of a group of pharmaceuticals belonging to the group of non-steroidal anti-inflammatory drugs (NSAIDs) from wastewater samples. In the first study, a selective analytical method was developed based on molecularly imprinted solid-phase extraction (MISPE) followed by LC-MS/MS. In contrast, the second study proposed, for first time, the direct coupling between MISPE specifically for NSAIDs and MS/MS without LC. In this section, another type of selective materials for SPE was synthesised and evaluated, consisting of strong cation-exchange (SCX) sorbents. Due to the acidic functionalities present in the structures of the sorbents, a group of basic pharmaceuticals and illicit drugs was successfully retained and further determined in environmental waters by LC-MS/MS. The performances of the SPE sorbents presented in this section included a selective clean-up of the matrix to eliminate interfering

compounds in order to reduce the matrix effect. The syntheses of these SCX sorbents 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, United Kingdom) during my research placement there.

The second section focuses on the improvement of stir bar sorptive extraction (SBSE) of polar organic compounds through novel polar coatings for SBSE. Firstly, two new commercially available coatings for SBSE were evaluated and compared to the classic polydimethylsiloxane (PDMS)-coated stir bar for extracting a group of pharmaceuticals and PCPs. Moreover, different in-house polar coatings were synthesised in the laboratory of the Polymer Research Group (University of Strathclyde, Glasgow) and evaluated as monolithic materials for SBSE. The best in-house polar SBSE coating was applied to the determination of several organic contaminants in environmental waters by LC-MS/MS.

References

[1] M. Farré, S. Pérez, L. Kantiani, D. Barceló, Trends Anal. Chem. 27 (2008) 991.
[2] M. Stuart, D. Lapworth, E. Crane, A. Hart, Sci. Total Environ. 416 (2012) 1.

3.1. Determination of pharmaceuticals and illicit drugs by selective solid-phase extraction followed by liquid chromatography-tandem mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

The large number of organic compounds considered emerging organic contaminants (EOCs) detected in environmental waters, as well as their different physico-chemical properties, makes it difficult to analyse them with a single analytical method. In spite of the great variety of EOCs, many scientists have made many efforts to achieve the determination of a wide number of compounds in a single run thanks to multi-residue analytical methods. Nowadays, many multi-residue methods can be found in the literature, reporting the determination of several pharmaceuticals belonging to different therapeutic classes, such as personal care products (PCPs) or illicit drugs, among others, in environmental waters [1-5]. In these studies, solid-phase extraction (SPE) is the most preferred extraction technique for enriching the analytes and cleaning up the matrix from interferences. When a multi-residue analytical method is proposed, the selected SPE sorbent must be versatile and universal to retain numerous compounds with different polarities. To achieve this aim, the most successful SPE sorbent has been and still remains Oasis HLB, due to its hydrophilic-lipophilic balanced structure, with it being able to retain both apolar and polar compounds present in different matrices. Despite its universality in enriching a wide range of compounds, this sorbent and other SPE sorbents with a similar structure are also prone to retaining matrix interferences, resulting in their co-elution with the analytes of interest and reducing or enhancing the analyte signal responses [3,6].

To tackle the problem of matrix interferences, which is a common issue discussed in analytical chemistry, the use of selective materials for sorptive extraction techniques has gained popularity, especially in SPE. Since the introduction of SPE, there has been a continuous need to improve the currently available sorbents for this extraction technique, with either commercial or in-house sorbents. The most well-known selective materials for SPE are molecularly imprinted polymers (MIPs), which present a high selectivity towards an analyte or a group of analytes (template/s) due to the specific interactions established between the template/s and the polymer matrix. The main advantage of these materials is their selectivity towards the template, offering the possibility of cleaning the matrix with an organic solvent to remove interferences while the analyte/s of interest are still retained. For many years, MIPs as SPE sorbents were only obtained in the laboratory and they displayed great selectivity towards many templates (pharmaceuticals, pesticides, PCPs, etc.) to be applied in the environmental, food and biological fields [7-9]. The successful results provided by this type of materials in establishing specific interactions encouraged the manufacturers to produce and commercialise MIPs. Currently, there are a few manufacturers that commercialise MIPs as SPE sorbents, such as Biotage, Supelco and Polyintell, and, as a result, a few applications have reported the use of commercial MIPs. These companies offer MIPs for the selective SPE of non-steroidal and anti-inflammatory drugs (NSAIDs) [10],

clenbuterol, fluoroquinolones, amphetamines [11,12], β-blockers [13], oestrogens [14,15], antidepressants [16] and triazines, among others. In general, MIPs provide high selectivity and clean extracts with the elimination of many interferences. However, these materials sometimes suffer from low capacity, loading lower sample volumes, which may turn out to be a critical issue for quantifying EOCs at low concentration levels.

Apart from MIPs, other selective materials were synthesised to offer both selectivity through specific interactions, such as ion-exchange materials, and capacity through a polymeric structure with a high specific surface area. These properties define a new class of selective materials, known by the name of mixed-mode polymeric sorbents. These sorbents are commercially available and have a macroporous structure with basic or acidic functionalities that enable the selective retention of acidic or basic compounds through ion-exchange interactions, respectively. As shown in the review paper included in Section 1.1.2.1, mixed-mode sorbents for SPE have been widely applied for extracting mainly pharmaceuticals, PCPs and illicit drugs, due to their basic and acidic functional groups, from environmental waters. Nonetheless, continuous advances in the synthesis of novel mixed-mode polymeric sorbents have been performed in order to improve the physico-chemical properties (higher specific surface areas, different functionalities, etc.) of these sorbents. Our research group has made significant progresses in this field, synthesising a series of hypercrosslinked mixed-mode polymers with a weak anion-exchange (WAX) [17,18], weak cation-exchange (WCX) [19] and strong anion-exchange (SAX) [20] behaviours. The promising results obtained with these sorbents showed selectivity towards basic or acidic compounds and encouraged us to synthesise two different porous strong cation-exchange (SCX) sorbents, using the simple traditional polymerisation.

In this chapter, the first two studies presented the molecularly imprinted solid-phase extraction (MISPE) performance of a commercial MIP selective for a group of NSAIDs. Therefore, a large group of basic and acidic pharmaceuticals (including NSAIDs and other acidic pharmaceuticals) were selected to evaluate the selectivity of the commercial MISPE sorbent towards acidic pharmaceuticals. Moreover, MISPE performance was compared to the classic Oasis HLB and two mixed-mode sorbents, Oasis MAX and Oasis WAX, to evaluate their effectiveness in removing interferences and reducing the matrix effect when wastewater samples were analysed by liquid chromatography-ultraviolet-visible (LC-UV) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Due to the clean extracts provided by MISPE, in the second study, a novel approach was reported, in which the extracts from the MISPE procedure were directly injected into tandem mass spectrometry (MS/MS), without chromatography. The results derived

from these two studies regarding selective MISPE performance were published in the *Journal of Separation Science* 35 (2012) 875-882 and *Talanta* 110 (2013) 196-201, respectively.

Finally, the third study included in this chapter reported the preparation and characterisation of two SCX sorbents and their applicability for the selective extraction of a group of basic pharmaceuticals and illicit drugs from wastewater samples. The SPE performances of both in-house SCX sorbents were compared to a SCX commercial sorbent, Oasis MCX, in terms of recovery values and matrix effect. Subsequently, of these two in-house sorbents, the best in-house SCX sorbent was used for determining the studied compounds in wastewater samples by LC-MS/MS. The results of this study have recently been published in the *Journal of Chromatography A* 1325 (2014) 137-146.

References

[1] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Anal. Bioanal. Chem. 391 (2008) 1293.
[2] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[3] R. Grabic, J. Fick, R.H. Lindberg, G. Fedorova, M. Tysklind, Talanta 100 (2012) 183.
[4] R. Loos, S. Tavazzi, B. Paracchini, E. Canuti, C. Weissteiner, Anal. Bioanal. Chem. 405 (2013) 5875.
[5] S. Idder, L. Ley, P. Mazellier, H. Budzinski, Anal. Chim. Acta 805 (2013) 107.
[6] E. Gracia-Lor, M. Martínez, J.V. Sancho, G. Peñuela, F. Hernández, Talanta 99 (2012) 1011.
[7] E. Turiel, A. Martín-Esteban, Anal. Chim. Acta 668 (2010) 87.
[8] A. Beltran, F. Borrull, P.A.G. Cormack, R.M. Marcé, Trends Anal. Chem. 29 (2010) 1363.
[9] N. Gilart, F. Borrull, N. Fontanals, R.M. Marcé, Trends Environ. Anal. Chem. 1 (2014) 8.
[10] S. Zorita, B. Boyd, S. Jönsson, E. Yilmaz, C. Svensson, L. Mathiasson, S. Bergström, Anal. Chim. Acta 626 (2008) 147.
[11] W. Wongniramaikul, A. Choodum, L. Dennany, N.N. Daeid, J. Sep. Sci. 35 (2012) 3332.
[12] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Rodil, J. González-Penas, R. Cela, J. Chromatogr. A 1216 (2009) 8435.
[13] M. Gros, T.M. Pizzolato, M. Petrović, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374.
[14] P. Lucci, O. Núñez, M.T. Galceran, J. Chromatogr. A 1218 (2011) 4828.

[15] D. Matějíček, A. Grycová, J. Vlček, J. Sep. Sci. 36 (2013) 1097.
[16] K. Demeestere, M. Petrović, M. Gros, J. Dewulf, H. Van Langenhove, D. Barceló, Anal. Bioanal. Chem. 396 (2010) 825.
[17] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, F. Borrull, J. Chromatogr. A 1217 (2010) 2855.
[18] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. A 1215 (2008) 21.
[19] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N. Fontanals, J. Chromatogr. A 1217 (2010) 1575.
[20] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C. Sherrington, R.M. Marcé, J. Sep. Sci. 35 (2012) 2621.

3.1.1. Determination of pharmaceuticals in wastewaters using solid-phase extraction-liquid chromatography-tandem mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

DETERMINATION OF PHARMACEUTICALS IN WASTEWATERS USING SOLID-PHASE EXTRACTION-LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Núria Gilart, Rosa Maria Marcé, Francesc Borrull, Núria Fontanals

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo s/n, 43007 Tarragona, Spain

Abstract

Four different commercial sorbents for solid-phase extraction have been evaluated for the extraction of a group of acidic pharmaceuticals in terms of selectivity and capacity: Oasis HLB (hydrophilic-lipophilic balance), Oasis MAX (strong anion-exchange), Oasis WAX (weak anion-exchange) and a commercial available molecularly imprinted polymer specific for non-steroidal anti-inflammatory drugs. Among the sorbents studied, molecularly imprinted polymer proved to be very effective in the reduction of matrix interferences and the selective extraction of acidic pharmaceuticals, such as salicylic acid, ibuprofen, fenoprofen, diclofenac and naproxen, among others, from effluent wastewater samples. Moreover, molecularly imprinted solid-phase extraction protocol was applied to liquid chromatography coupled to tandem mass spectrometry with the purpose of evaluating the clean-up effect on ion suppression/enhancement when the complexity of the samples increases and a reduction of this effect was observed. Molecularly imprinted solid-phase extraction followed by liquid chromatography coupled to UV detection and liquid chromatography coupled to tandem mass spectrometry validation methodologies with effluent wastewaters were developed, obtaining recoveries between 70% and 85% and limits of detection at low levels of μg/L (0.15-1 μg/L) and ng/L (0.5-2 ng/L), respectively. The final application of molecularly imprinted solid-phase extraction and liquid chromatography coupled to UV detection showed the presence of acidic pharmaceuticals studied in this work in effluent wastewaters (< limit of quantification-1493 ng/L).

Keywords: *pharmaceuticals; solid-phase extraction; molecularly imprinted polymer; liquid chromatography-tandem mass spectrometry; wastewaters*

1. INTRODUCTION

The recent detection of emerging organic contaminants (EOCs), such as pharmaceuticals and personal care products, in the environment has attracted the attention of researchers [1,2]. Currently, several studies are underway that focus on the fate of these EOCs and their metabolites [3,4]. Due to their low concentration (*i.e.* ng/L) and the complexity of the matrix in which they are found, powerful techniques and effective methodologies have been developed for their determination in different environmental matrices, such as wastewaters [5], sewage sludge [6] and soils [7].

Within the group of EOCs, pharmaceuticals include a variety of compounds, including non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, psychoactive drugs, lipid regulators and β-blockers, among others. Pharmaceuticals have been mainly analysed by liquid chromatography coupled to mass spectrometry or tandem mass spectrometry (LC-MS/MS) in different environmental matrices providing low limits of detection (LODs) [8,9]. However, the major drawback of this technique in quantitative analysis, especially with electrospray ionisation (ESI), is ion suppression/enhancement when analysing complex samples. Particularly, ion suppression/enhancement for some pharmaceuticals in wastewater samples has been reported to be as high as 70% [10]. The use of highly selective extraction materials and effective clean-up procedures can contribute to decreasing this problem [11].

Solid-phase extraction (SPE) is the most common technique for sample enrichment and clean-up of aqueous samples. The most commonly-used sorbents for SPE for the extraction of EOCs from environmental waters are either polymeric ones such as Oasis HLB [8] for high capacity or mixed-mode polymeric sorbents (e.g. Oasis MAX, Oasis WCX or Strata-X-C) [12-14] for high selectivity towards basic or acidic compounds.

Over the last few years, in order to reduce the level of co-extracted compounds and improve selectivity and thus achieve lower LODs, molecularly imprinted polymers (MIPs) have been applied as SPE sorbents, known as molecularly imprinted SPE (MISPE) [15]. MIPs are synthetic polymeric materials with specific molecular-recognition properties that can specifically rebind a target molecule. For this reason, they have been widely used to extract a great number of substances selectively, such as pharmaceuticals [16], personal care products [17] and herbicides [18], in different sample matrices. Thanks to their successful applications, commercially available MIPs for antidepressants [19], amphetamine drugs [20], NSAIDs [21] and β-blockers [11] have recently been tested. The combination of MISPE with LC-MS/MS has showed a great reduction of ion suppression/enhancement, compared to other common SPE sorbents [20].

The goal of the present paper is to evaluate different SPE procedures for the selective extraction of several acidic pharmaceuticals from wastewater samples for subsequent analysis by LC-MS/MS, using four commercial sorbents: Oasis HLB, a conventional macroporous copolymer which allows high capacity; Oasis MAX and Oasis WAX, two mixed-mode sorbents which provide anionic selectivity and finally a

commercial MIP for NSAIDs (Affinilute MIP - NSAIDs).

2. MATERIALS AND METHODS

2.1. Reagents and standards

Atenolol, metoprolol, propranolol, acetaminophen, caffeine, fluoxetine, carbamazepine, oxcarbazepine, diazepam, salicylic acid, naproxen, ibuprofen, fenoprofen, diclofenac and gemfibrozil were purchased from Sigma-Aldrich (Steinheim, Germany). All pharmaceutical standards used were of high purity grade (> 90%).

Stock solutions of individual standard were prepared by dissolving each compound in methanol at a concentration of 1000 mg/L, except for fluoxetine and oxcarbazepine, which were prepared at a concentration of 500 mg/L in methanol. A mix of all the compounds in methanol at a concentration of 50 mg/L was prepared weekly. Working solutions were prepared daily from these stock solutions diluted in MeOH/H_2O (15:85 v/v). These solutions were stored at 4 °C. The structures and pK_a values of these substances are presented in Table 1.

HPLC grade methanol (MeOH), acetonitrile (ACN) and acetic acid (CH_3COOH) (≥99.8%) were supplied by SDS (Peypin, France). Ultrapure-water was obtained from a water purification system (Veolia, Sant Cugat del Vallés, Spain) and nitrogen (N_2) (≥99.9%) came from Carburos Metálicos (Tarragona, Spain). Phosphoric acid (H_3PO_4) (85%) from Merck (Darmstadt, Germany), formic acid (HCOOH) (≥95%) from Sigma-Aldrich (Steinheim, Germany), sodium hydroxide (NaOH) (≥98%) from Prolabo (Fontenai S/Bois, France) and ammonium hydroxide (NH_4OH) (25%) from Panreac (Barcelona, Spain) were used to adjust the pH of the mobile phase and the sample and in SPE procedures.

2.2. Sample preparation

Wastewater samples were collected from two urban sewage treatments plants (STPs) in two cities with populations of about 120,000 habitants each (Tarragona and Reus). All samples were collected in pre-cleaned amber glass bottles, acidified to pH 3 with H_3PO_4 and stored at 4°C until analysis. Before the extraction, wastewater samples were filtered through 0.45 µm nylon membranes (Supelco, Bellefonte, PA, USA).

2.3. Solid-phase extraction

Different commercially available sorbents were tested in the SPE procedure: Oasis HLB 150 mg (Waters, Milford, MA, USA), Oasis MAX 150 mg (Waters), Oasis WAX 150 mg (Waters) and Affinilute MIP – NSAIDs (Biotage, Uppsala, Sweden), which was manually packed by weighting 150 mg of sorbent and then placed into 6 mL polyethylene cartridge with 2 polypropylene frits (~10 µm) (Symta, Madrid, Spain). The cartridges were placed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. SPE procedures for each sorbent are presented in Table 2. The collected fractions from the elution step were evaporated to dryness under a stream of N_2 and reconstituted to 1 mL of MeOH/H_2O (15:85 v/v) before LC injection.

Table 1. Structures, pK_a, retention time and experimental parameters employed for the MRM acquisition.

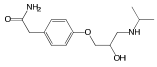
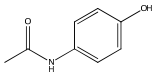
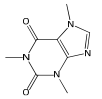
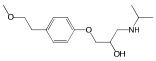
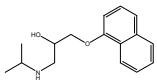
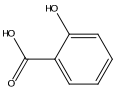
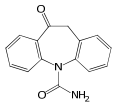
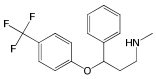
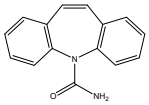
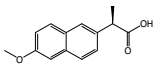
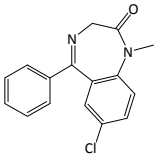
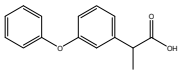
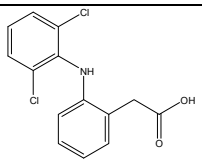
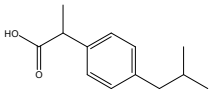
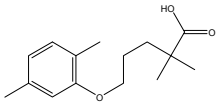
	Analyte	Structure	pK_a [a]	t_R (min)	MRM transition	Ions. mode	Cone volt. (V)	Col. ener. (V)
1	Atenolol		9.4	2.0	267 > 145 267 > 190	PI	130	25 25
2	Acetaminophen		9.9	3.0	152 > 93 152 > 110	PI	100	25 15
3	Caffeine		13.4	4.8	195 > 110 195 > 138	PI	125	25 15
4	Metoprolol		9.4	6.2	268 > 116 268 > 159	PI	125	15 20
5	Propranolol		9.5	8.3	260 > 116 260 > 183	PI	125	15 15
6	Salicylic acid		3.0	9.2	137 > 93 137 > 65	NI	75	15 30
7	Oxcarbazepine		13.7	10.1	253 > 208 253 > 180	PI	100	25 20
8	Fluoxetine		10.1	10.8	310 > 44 310 > 148	PI	90	10 3
9	Carbamazepine		13.9	11.5	237 > 179 237 > 193	PI	150	35 35
10	Naproxen		4.8	14.3	229 > 140 229 > 185	NI	50	30 5
11	Diazepam		3.4	15.0	285 > 154 285 > 193	PI	150	25 35
12	Fenoprofen		4.2	16.1	241 > 197 241 > 93	NI	100	10 40

Table 1. Continued.

	Analyte	Structure	pK_a [a]	t_R (min)	MRM transition	Ions. mode	Cone volt. (V)	Col. ener. (V)
13	Diclofenac		4.2	16.9	294 > 214 294 > 250	NI	75	20 10
14	Ibuprofen		4.4	17.5	205 > 161	NI	75	5
15	Gemfibrozil		4.8	19.1	249 > 121 249 > 106	NI	75	20 40

[a] pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 for Solaris (© 1994–2011 ACD/Labs).

2.4. Instrumentation

For the SPE optimisation, a LC-10AD binary liquid chromatograph equipped with a DGU-14A degasser, a 20 µL sample loop, a CTO-10A column oven and a SPD-10A UV detector, all from Shimadzu Corporation (Tokyo, Japan), was used.

For the analysis of real samples, evaluation of matrix effects and the method validation, an LC-MS/MS system was used. The instrument consisted of an Agilent 1200 series LC, equipped with an automatic injector (volume injected was 50 µL), a degasser, a quaternary pump and a column oven; and a 6410 series triple quadrupole mass spectrometer using an ESI interface from Agilent Technologies (Waldbronn, Germany).

2.5. Chromatographic conditions

The chromatographic column was a Fused-Core™ Ascentis Express C_{18} (100 mm × 4.6 mm) with 2.7 µm particle size (Supelco). Gradient elution was performed with acidified ultrapure-water at pH 2.8 (with H_3PO_4 for UV detection or CH_3COOH for MS/MS detection) as solvent A and ACN as solvent B. The gradient was initially 10% solvent B which was maintained for 2 min and then increased to 100% solvent B over 25 min (held for 1 min). Finally, the gradient decreased back to the initial conditions (10% solvent B) in 4 min. The flow rate was 0.6 mL/min and the temperature was set at 40 °C.

Using UV detection, the wavelengths program was as follows: 230 nm (0 - 15.5 min) and 220 nm (15.5 - stop time). As for MS/MS parameters, they were adapted from a previous study [22]. N_2 was used as the collision gas and its flow rate was set at 12 L/min. A source temperature of 300 °C, a nebuliser pressure of 40 psi (N_2) and a capillary potential of 4000 V were applied. All analytes were determined in multiple reaction monitoring (MRM)

Table 2. Optimised SPE conditions for the different sorbents tested.

	SPE			
	Oasis HLB	**Oasis MAX**	**Oasis WAX**	**Affinilute MIP - NSAIDs**
Conditioning	5 mL MeOH 5 mL H_2O	5 mL MeOH 5 mL H_2O	5 mL MeOH 5 mL H_2O	5 mL ACN, 5mL MeOH 5 mL H_2O pH~3
Sample*	pH~7	pH~7	pH~7	pH~3
Washing	5 mL 5% MeOH in H_2O	5 mL MeOH	5 mL MeOH	5 mL H_2O 5 mL ACN:H_2O (40:60)
Elution	5 mL MeOH:ACN (50:50)	5 mL 5% HCOOH in MeOH	5 mL 5% NH_4OH in MeOH	5 mL 1% CH_3COOH in MeOH:acetone (80:20)

*Sample volumes were 5 mL of ultra-pure water for SPE optimisation and 250 mL of effluent wastewater for method validation.

mode, in both positive and negative ionisation. Table 1 details MRM transitions, retention time, cone voltage and collision energy for each compound.

3. RESULTS AND DISCUSSION

3.1. LC conditions

In order to provide the best separation in the shortest time, a Fused-Core™ Ascentis Express C_{18} column (particle size 2.7 µm) was used, which allowed the separation of 15 compounds within 20 minutes. The chromatographic conditions (separation gradient, flow rate and temperature) were optimised using a common binary mobile phase: ACN and acidified ultrapure-water. In the first instance, when using UV detection, H_3PO_4 was selected to acidify aqueous mobile phase due to its non-absorption in the working wavelengths (220 - 230 nm). In contrast, when working with LC-MS/MS, CH_3COOH was used in order to avoid the formation of phosphate salts from phosphoric acid in the ionisation source, as well as to facilitate the evaporation of the solvent.

The optimisation of MS/MS parameters were performed by injecting each compound individually in flow injection analysis (FIA) mode. As can be seen in Table 1, all analytes were divided individually or in groups into six windows depending on their ESI ionisation (positive or negative) in accordance to their basic or acidic functionalities. Two MRM transitions were necessary for the identification and confirmation of all analytes, which were optimised for each compound. Finally, values of the cone voltage and the collision energy for each MRM transition were studied and optimised. It is worth mentioning that for ibuprofen, only one MRM transition was achieved because its MS/MS spectrum contained only one diagnostic ion, but this compound was not excluded from the study due to its occurrence in environmental water samples [22]. Using LC-UV instrument, all target analytes showed good linearity from 0.5 mg/L to 50 mg/L (r^2>0.998). LODs

were calculated as signal-to-noise ratio (S/N) ≥ 3, ranging from 0.015 mg/L to 0.100 mg/L for all studied compounds. Moreover, using LC-MS/MS technique in MRM mode, all target analytes presented a satisfactory linear range from 1 to 500 μg/L (r^2>0.996). LODs ranged between 0.025 μg/L and 0.200 μg/L for both acidic and basic analytes.

3.2. SPE optimisation

For the SPE performance, four different commercial sorbents were tested in order to check which is more effective at preconcentrating and selectively extracting acidic pharmaceuticals from environmental waters. Oasis HLB and two mixed-mode sorbents (Oasis MAX and Oasis WAX) have been selected for enabling high capacity and for providing selectivity because of anion exchange, respectively. Finally, a commercial MIP for the extraction of NSAIDs has been used, which is expected to extract NSAIDs more selectively from different matrices. In order to promote selectivity and capacity of these four sorbents, different parameters, such as sample pH, washing and elution solvents were first optimised. After SPE, a evaporation step was required before LC injection. It should be pointed out that fluoxetine, in all SPE protocols, provided the lowest recoveries because there was a 15% loss during the evaporation step.

Sample pH was studied to ensure the most suitable conditions to retain all the analytes and the behaviour of the sorbents was evaluated at different pH values, according to the pK_a values of the studied analytes: 3, 7 and 11. This parameter is affected to a greater degree when we are working with mixed-mode sorbents, such as Oasis MAX and WAX, because depending on the sample pH, different ionic forms of the target analytes and the sorbent have to be considered. In this case, the most suitable pH for the loading and activation steps was 7, without any losses. For Oasis HLB, sample pH was also fixed at 7 to promote hydrophobic interactions. Finally, for the NSAID MIP, the sample was adjusted to pH 3 to ensure interactions between the analytes and the sorbent. All the target analytes showed high recoveries (over 95%) for all tested sorbents, with the exception of atenolol which was lost when loading through the MIP (~80%).

The elution of the compounds through the cartridges was optimised using different volumes (2, 3 and 5 mL) of a suitable solvent. For Oasis MAX, MeOH containing 2% and 5% of HCOOH were tested in order to protonate the acidic compounds and disrupt the ionic interactions. 5 mL of 5% HCOOH in MeOH were selected, since it provided recoveries over 86%, except for fluoxetine (64%). With regard to Oasis WAX, 2% and 5% of NH_4OH in MeOH were evaluated in order to change the sorbent to its neutral form. The highest recoveries for the studied analytes (78 - 100%) were achieved using 5 mL of 5% of NH_4OH in MeOH. For Oasis HLB, MeOH, ACN and combinations of both were tested to break down the hydrophobic interactions. Finally, 5 mL of MeOH:ACN (50:50) was selected since it provides better recoveries (95-100%) for all acidic and basic analytes. In order to disrupt the interactions between the analytes and MIP, mainly hydrophobic, MeOH:acetone (80:20) containing 1% of CH_3COOH, in line with the manufacturer's recommendations,

and 1% of CH_3COOH in ACN were evaluated. Since there were no significant differences between them, 5 mL of 1% CH_3COOH in MeOH:acetone (80:20) was used, achieving recoveries for all the pharmaceuticals between 70% and 100%.

In order to enhance the selectivity towards acidic pharmaceuticals from the group of compounds studied, a washing step was included in each SPE protocol according to its sorbent nature. To this end, for Oasis MAX and Oasis WAX, 5 mL of MeOH were enough to elute all basic compounds, whereas acidic analytes were still retained on the sorbents (%R over 80%). For Oasis HLB, including a washing step of MeOH in ultrapure-water might cause losses of the studied analytes due to its lack of selectivity. Therefore, 5 mL of 5% MeOH in ultrapure-water were adopted as washing solvent. A selective clean-up was achieved with the MIP, when the cartridge was washed with 5 mL of ACN:H_2O (40:60) after its optimisation. This solution enabled the removal of all basic compounds and retained the rest of the acidic analytes on the sorbent (70 - 92%). It should be mentioned that diazepam was the only basic compound which was not completely eluted in this step (22%).

Once the protocol for each sorbent has been established, we then evaluated the maximum volume of sample that could be loaded in each cartridge. Different sample volumes from 5 to 1000 mL of ultrapure-water spiked with the analytes mixture were loaded. When 1000 mL of ultrapure-water were percolated through the different sorbents, the recoveries for all acidic pharmaceuticals ranged between 83% and 105% for all four sorbents, except diazepam and gemfibrozil, whose recoveries (~70%) were slightly lower when loading through the MIP.

3.3. SPE application to real samples

To assess the effectiveness of including a washing step into the SPE protocols, it was decided to apply the four optimised SPE performances to extract acidic pharmaceuticals selectively from effluent wastewater samples followed by LC-UV. To achieve similar recoveries to ultrapure-water, the volume of real samples was reduced due the complexity of effluent wastewaters. Thus, 250 mL of effluent wastewater spiked at 20 μg/L were tested for all four cartridges. Table 3 shows the recovery values (%) of the four sorbents under the conditions detailed above.

Although the recoveries obtained for all pharmaceuticals by Oasis HLB were similar to those obtained in ultrapure-water, this sorbent did not enable selective washing of real samples. In the case of the two mixed-mode sorbents, the washing step was able to eliminate all basic compounds, as well as gemfibrozil, which was almost eluted in this step (69 - 75%), in contrast to ultrapure-water experiments. Similar behaviour for gemfibrozil was observed in a previous study [23]. Finally, the recoveries obtained by MIP were slightly lower than those obtained with ultrapure-water, but this sorbent allowed the selective extraction of acidic analytes from real samples through the clean-up step developed (5 mL ACN:H_2O (40:60)). However, diazepam provided the lowest recovery value in real sample (washing, 46% and elution, 45%). This outcome was also followed

Table 3. Recovery values (%) for 250 mL effluent wastewater, spiked at 20 µg/L using Oasis HLB, Oasis MAX, Oasis WAX and Affinilute NSAIDs followed by LC-UV.

Analyte	Oasis HLB		Oasis MAX		Oasis WAX		Affinilute NSAIDs	
	W	E	W	E	W	E	W 2	E
Atenolol	-	100	70	-	68	-	-	-
Metoprolol	-	103	75	-	105	-	89	-
Propranolol	-	94	86	-	100	-	102	-
Caffeine	-	101	100	-	93	-	91	-
Paracetamol	-	95	60	-	70	-	95	-
Oxcarbazepine	-	93	84	-	98	-	98	-
Carbamazepine	-	91	92	-	87	-	93	-
Fluoxetine	-	71	81	-	85	-	75	-
Diazepam	-	94	88	-	93	12	46	45
Salicylic acid	-	92	-	91	-	76	-	81
Naproxen	-	98	-	92	-	98	-	85
Fenoprofen	-	100	-	96	-	99	-	83
Diclofenac	-	89	-	84	-	105	-	63
Ibuprofen	-	86	-	85	14	95	-	71
Gemfibrozil	-	92	75	25	69	25	-	70

W: Wash; E: Elution
%RSD (n=3) < 15% when %R > 25%

with ultrapure-water experiments, in which this compound was partially eluted in the washing step.

Apart from the %R itself, it is also very interesting to see the chromatogram profile for each sorbent. Figure 1 shows the chromatograms obtained after the loading of 250 mL of effluent wastewater samples spiked at 20 µg/L samples through each sorbent, following the optimised procedure. It demonstrates that the effective clean-up was achieved for real samples when MIP was applied (Figure 1a). Although MIP provided slightly lower recoveries than the rest of the sorbents, the extracts obtained were highly clean and no interferences from the matrix were observed. However, using Oasis MAX (Figure 1c) and Oasis WAX (Figure 1d), a broad band at the beginning of the chromatograms appeared. This fact may be explained by the elution steps in basic and acidic media, which promote the removal of acidic interferences from the matrix. Finally, the chromatogram obtained applying Oasis HLB (Figure 1b) was cleaner than those obtained by Oasis MAX and Oasis WAX. Initially, it was thought that Oasis HLB was the least selective sorbent for acidic compounds, but it became apparent that it was not able to interact with acidic interferences from the matrix due to the lack of ionic exchange. Therefore, it provided cleaner extracts.

Since MISPE-LC-UV provided a selective performance when real samples were analysed, the next step was the method validation with a sample volume of 250 mL of effluent wastewater. It should be

mentioned that diclofenac and gemfibrozil were tentatively found in real samples and their signals were subtracted in the spiked samples. The linear range was between 2 and 100 µg/L for all acidic compounds (r^2 > 0.992), except for naproxen (0.5 - 40 µg/L). For target compounds without blank signals, LODs were determined as the concentrations corresponding to SN≥3. For target compounds which present a signal in the blank, LODs were determined as the average (n=3) of the blank signal of each analyte plus three times the standard deviation of this signal. LODs (S/N≥3) were 1 µg/L for salicylic acid, diclofenac and gemfibrozil, 0.50 µg/L for diazepam, fenoprofen and ibuprofen, and 0.15 µg/L for naproxen. The repeatability (n=3) and reproducibility between days (n=3), expressed as % relative standard deviation (% RSD), were lower than 6% and 9%, respectively.

3.4. LC-MS/MS

In LC-(ESI)MS/MS, interference effects may lead to a suppression or enhancement of the analyte response, due to co-eluting matrix components. Several alternatives have been applied to deal

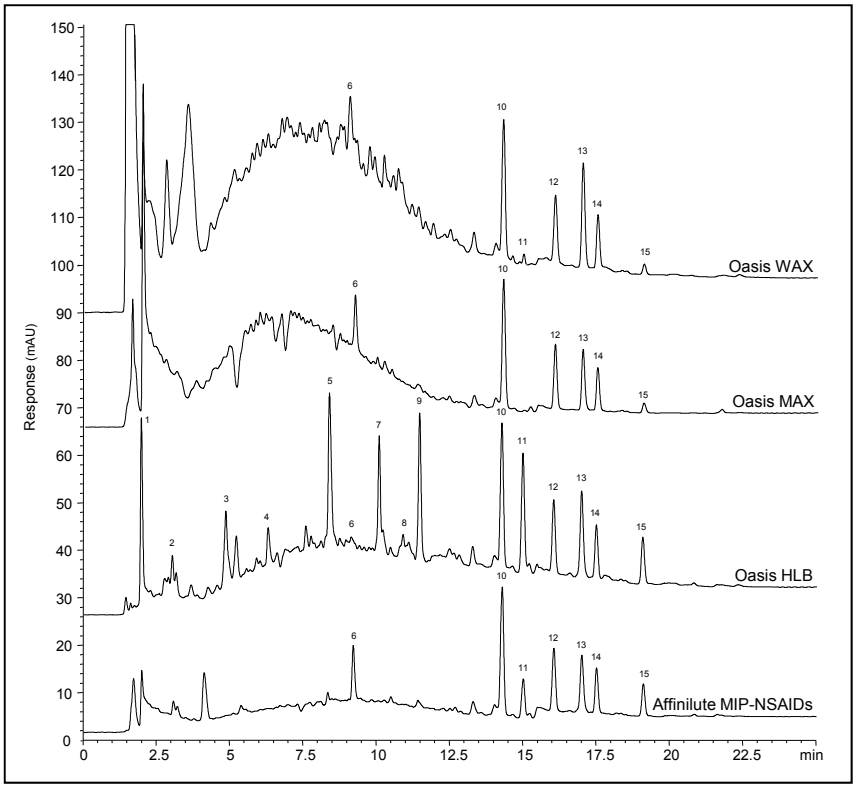


Fig. 1. LC-UV chromatograms of 250 mL of effluent wastewater samples spiked at 20 µg/L obtained in the elution step for all tested SPE sorbents. Peak assignment in Table 1.

with matrix effects, such as sample dilution or the use of an internal standard [24]. Nevertheless, an improved and effective sample pre-treatment may lead to a reduction of this effect.

Therefore, after the selective performance obtained by the MISPE-LC-UV method for the extraction of acidic pharmaceuticals from effluent wastewater samples in comparison with the other sorbents studied, it was decided to apply the MISPE protocol followed by LC-MS/MS in order to evaluate ion suppression/enhancement. This effect was calculated as the percentage decrease in the signal intensity obtained by the target analytes spiked at 100 µg/L after the extraction of 250 mL of effluent wastewaters *versus* the intensity of the same amount of the analytes in ultra-pure water [25].

The ion suppression/enhancement was also compared to that of Oasis HLB, which is a sorbent widely used in the extraction of pharmaceuticals followed by LC-MS/MS [8]. Figure 2 shows the signal suppression/enhancement values for the acidic pharmaceuticals obtained using the MISPE protocol, which were lower than those obtained using Oasis HLB, with a decrease of 20% for almost all the analytes. Diazepam showed the lowest ion suppression with the MIP, whereas with Oasis HLB this compound underwent an enhancement (15%). The findings coincide with Gros *et al.* [11], whose study demonstrated a decrease of the ion suppression/enhancement provided by a commercial MIP, in comparison with Oasis HLB, for the extraction of β–blockers from wastewater samples.

For the purpose of analysing real samples, MISPE-LC-MS/MS method was validated for 250 mL of effluent wastewater. All acidic pharmaceuticals presented good linearity with a linear range from 7 to 1000 ng/L (r^2>0.991). In addition, LODs (S/N≥3) ranged from 0.5 to 2 ng/L and limits of quantification (LOQs), calculated as S/N≥10, were between 1.5 and 5 ng/L for all the

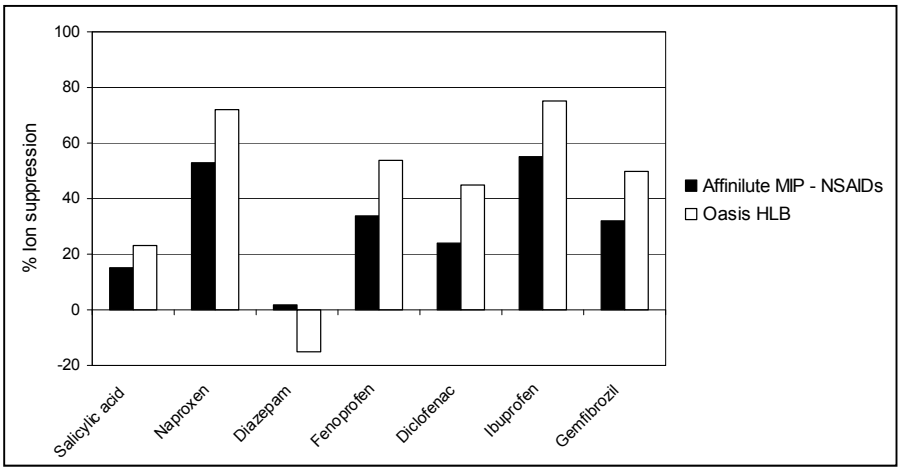


Fig. 2. Signal suppression of acidic pharmaceuticals in 250 mL effluent wastewater spiked after extraction at 100 µg/L using Affinilute MIP – NSAIDs and Oasis HLB followed by LC-MS/MS.

studied analytes, except for naproxen (7 ng/L). Besides, these quantification levels are satisfactory because of the environmental concentrations found [26].

3.5. Analysis of real samples

The MISPE-LC-MS/MS method, which showed great effectiveness in the clean-up of the sample matrices and so low LODs, was then applied to determine the presence of acidic pharmaceuticals in effluent wastewater.
Different effluent wastewater samples were collected from two different STPs and were analysed using the developed procedure. All acidic pharmaceuticals studied were detected (<LOQ to 1493 ng/L). It is important to mention that the highest concentrations corresponded to diclofenac (492 – 996 ng/L) and gemfibrozil (548–1493 ng/L), which were also detected by the MISPE-LC-UV method developed previously. In contrast, diazepam, fenoprofen and ibuprofen were found at low levels (<LOQ–205 ng/L). These results agree with those reported by Pedrouzo *et al.* [22] in which the highest values of ng/L were attributed to naproxen, diclofenac and ibuprofen.

4. CONCLUDING REMARKS

An effective clean-up of the sample has been showed for the selective extraction of several acidic pharmaceuticals in effluent wastewater samples. Among the different commercial SPE sorbents tested, commercial MIP enabled the cleanest extracts and gave good recoveries, except in the case of diazepam.
When MISPE protocol is followed by the LC-UV technique, clean chromatograms are obtained and enabled LODs at low levels of µg/L (0.15-1 µg/L). The combination of MISPE with LC-MS/MS achieves an improvement of ion suppression/enhancement, in comparison with Oasis HLB, when real samples were analysed. The validation of the MISPE-LC-MS/MS method for effluent wastewaters provided low LODs (0.5–2 ng/L). Finally, almost all acidic pharmaceuticals were found in effluent wastewater samples, above all, diclofenac and gemfibrozil which presented the highest concentrations (171–1493 ng/L).

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2008-00825/BQU) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] Fatta-Kassinos, D., Meric, S., Nikolaou, A., *Anal. Bioanal. Chem.*, 2011, *399*, 251-275.
[2] Nödler, K., Licha, T., Bester, K., Sauter, M., *J. Chromatogr. A*, 2010, *1217*, 6511-6521.
[3] Fortuna, A., Sousa, J., Alves, G., Falcão, A., Soares-da-Silva, P., *Anal. Bioanal. Chem.*, 2010, *397*, 1605-1615.
[4] Schröder, H. F., Gebhardt, W., Thevis, M., *Anal. Bioanal. Chem.*, 2010, *398*, 1207-1229.

[5] Pedrouzo, M., Borrull, F., Marcé, R. M., Pocurull, E., *TrAC, Trends Anal. Chem.*, 2011, *30*, 749-760.
[6] Nieto, A., Borrull, F., Pocurull, E., Marcé, R. M., *TrAC, Trends Anal. Chem.*, 2010, *29*, 752-764.
[7] Verma, K. S., Xia, K., *J. AOAC Int.*, 2010, *93*, 1313-1321.
[8] van Nuijs, A. L. N., Tarcomnicu, I., Simons, W., Bervoets, L., Blust, R., Jorens, P. G., Neels, H., Covaci, A., *Anal. Bioanal. Chem.*, 2010, *398*, 2211-2222.
[9] García-Galán, M. J., Garrido, T., Fraile, J., Ginebreda, A., Díaz-Cruz, M. S., Barceló, D., *Anal. Bioanal. Chem.*, 2011, *399*, 795-806.
[10] Shao, B., Chen, D., Zhang, J., Wu, Y. N., Sun, C. J., *J. Chromatogr. A*, 2009, *1216*, 8312-8318.
[11] Gros, M., Pizzolato, T. M., Petrovic, M., López de Alda, M. J., Barceló, D., *J. Chromatogr. A*, 2008, *1189*, 374-384.
[12] Sousa, M. A., Gonçalves, C., Cunha, E., Hajslová, J., Alpendurada, M. F., *Anal. Bioanal. Chem.*, 2011, *399*, 807-822.
[13] Zorita, S., Larsson, L., Mathiasson, L., *J. Sep. Sci.*, 2008, *31*, 3117-3121.
[14] Fontanals, N., Cormack, P. A. G., Marcé, R. M., Borrull, F., *TrAC, Trends Anal. Chem.*, 2010, *29*, 765-779.
[15] Beltran, A., Borrull, F., Cormack, P. A. G., Marcé, R. M., *TrAC, Trends Anal. Chem.*, 2010, *29*, 1363-1375.
[16] Hoshina, K., Horiyama, S., Matsunaga, H., Haginaka, J., *J. Chromatogr. A*, 2009, *1216*, 4957-4962.
[17] Núñez, L., Turiel, E., Martín-Esteban, A., Tadeo, J. L., *Talanta*, 2010, *80*, 1782-1788.
[18] Sambe, H., Hoshina, K., Haginaka, J., *J. Chromatogr. A*, 2007, *1152*, 130-137.
[19] Demeestere, K., Petrovic, M., Gros, M., Dewulf, J., Van Langenhove, H., Barceló, D., *Anal. Bioanal. Chem.*, 2010, *396*, 825-837.
[20] González-Mariño, I., Quintana, J. B., Rodríguez, I., Rodil, R., González-Penas, J., Cela, R., *J. Chromatogr. A*, 2009, *1216*, 8435-8441.
[21] Zorita, S., Boyd, B., Jönsson, S., Yilmaz, E., Svensson, C., Mathiasson, L., Bergström, S., *Anal. Chim. Acta*, 2008, *626*, 147-154.
[22] Pedrouzo, M., Borrull, F., Pocurull, E., Marcé, R. M., *Water Air Soil Pollut.*, 2011, *217*, 267-281.
[23] Fontanals, N., Cormack, P. A. G., Sherrington, D. C., *J. Chromatogr. A*, 2008, *1215*, 21-29.
[24] Bijlsma, L., Sancho, J. V., Pitarch, E., Ibáñez, M., Hernández, F., *J. Chromatogr. A*, 2009, *1216*, 3078-3089.
[25] Kasprzyk-Hordern, B., Dinsdale, R. M., Guwy, A. J., *Talanta*, 2008, *74*, 1299-1312.
[26] Pedrouzo, M., Reverté, S., Borrull, F., Pocurull, E., Marcé, R. M., *J. Sep. Sci.*, 2007, *30*, 297-303.

3.1.2. A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

A RAPID DETERMINATION OF ACIDIC PHARMACEUTICALS IN ENVIRONMENTAL WATERS BY MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION COUPLED TO TANDEM MASS SPECTROMETRY WITHOUT CHROMATOGRAHPY

Núria Gilart, Rosa Maria Marcé, Núria Fontanals, Francesc Borrull

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

Abstract

This study presents a rapid analytical method that involves an off-line molecularly imprinted solid-phase extraction (MISPE) specific for non-steroidal anti-inflammatory drugs (NSAIDs) as a selective sample pretreatment coupled directly to tandem mass spectrometry (MS/MS). The developed methodology provided sensitive and selective detection and quantification of six acidic pharmaceuticals in wastewaters without the chromatographic separation.

The optimised MISPE procedure enabled to extract effectively the studied analytes from effluent and influent wastewaters with satisfactory recovery values (from 62% to 103%). The analytical method developed was validated using 50 mL of effluent wastewaters, obtaining limits of detection (LODs) lower than 0.1 μg L^{-1} for all the compounds studied. The method was successfully applied for the determination of these acidic pharmaceuticals in effluent and influent wastewaters. The analytes and their concentration are in line with other studies in which these analytes are determined by SPE-LC-MS/MS in similar samples.

Keywords: *pharmaceuticals; direct coupling; molecularly imprinted solid-phase extraction; polymer; tandem mass spectrometry; wastewaters*

1. INTRODUCTION

Nowadays, rapid analytical methods are required in order to analyse the maximum number of samples in the minimum time period. Up to now, many analytical methods have been developed to determine acidic pharmaceuticals, such as non-steroidal anti-inflammatory drugs (NSAIDs), among others, in complex matrices, mainly using solid-phase extraction (SPE) followed by liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) [1-4]. However, these methodologies usually involve time-consuming procedures from the sample collection right through to their quantification. In order to reduce analysis time, it should be worthy to improve on sample pretreatments and couple them

directly to a specific and sensitive detection system, without chromatographic analysis.

MS or tandem MS is currently the most commonly used detection technique for the identification and quantification of pharmaceuticals in complex matrices due to its high sensitivity, selectivity and speed [5]. Despite its numerous advantages, this technique using an electrospray ionisation (ESI) source may suffer from ion suppression/enhancement caused by interferences present in complex matrices [6]. For this reason, removing as much as possible interfering matrix compounds in order to minimise these matrix effects is a challenge.

In recent years, few studies have reported the direct coupling of an extraction technique to a detection technique. For instance, the on-line SPE-MS system has been applied for the determination of clenbuterol in urine [7,8] and prednisolone in serum [9], while the coupling between SPE and MS/MS enabled to determine antihypertensive drugs in human plasma and urine [10]. These studies have been developed using non-selective SPE sorbents, whose protocols did not include an effective clean-up step, and many matrix compounds were still present in the SPE eluate, obtaining higher limits of detection (LODs) than expected.

To tackle this problem, it is necessary to purify the samples as much as possible in order to eliminate interferences. Molecularly imprinted solid-phase extraction (MISPE) has been defined as a selective extraction technique because of its molecular-recognition technology, which allows specific binding between the target molecule or template and the polymer structure [11,12]. Currently, new approaches are being developed in this field which apply these selective molecularly imprinted polymers (MIPs) coupled directly to detection techniques in order to eliminate as many of the interferences as possible without the losses of target analytes.

On this point, a few studies have been reported using MISPE-MS, such as for the determination of fluoroquinolones in urine [13], benzodiazepines in human plasma [14] and phenothiazines in urine [15], as well as another that used a MISPE-fluorescence detector (FD) to determine ochratoxin A in wheat samples [16]. These studies emphasised the selectivity and simplicity of the methodology in comparison with the classical methods which included SPE followed by LC prior to MS/MS for the determination of different drugs in environmental and biological matrices [17-23]. However, to the best of our knowledge, MISPE has never been coupled directly to MS/MS, which might significantly improve the sensitivity and selectivity of the methodology.

In view of this, the aim of the present work is to develop a rapid and selective analytical method for the determination of six acidic pharmaceuticals in wastewaters by MISPE-MS/MS.

2. MATERIALS AND METHODS

2.1. Materials

Clofibric acid, naproxen, ibuprofen, fenoprofen, diclofenac and gemfibrozil were purchased from Sigma-Aldrich (Steinheim, Germany). All pharmaceutical standards used were of high purity grade (>97%). As internal standard (IS),

gemfibrozil-d_6 (98%) (100 mg L^{-1} in dioxane) from Cambridge Isotope Laboratories (Andover, USA) was used.
Stock solutions of individual standards were prepared by dissolving each compound in methanol (MeOH) at a concentration of 1000 mg L^{-1}. A mixture of all compounds in MeOH at a concentration of 50 mg L^{-1} was prepared weekly. Working solutions were prepared daily from these stock solutions diluted in MeOH/H_2O at pH 7 (60:40) (v/v). These solutions were stored at 4 ºC. The structures and pK_a values of these substances are presented in Table 1.
HPLC grade MeOH and acetonitrile (ACN) were purchased from SDS (Peypin, France). Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain) and nitrogen (N_2) (99%) was supplied by Carburos Metálicos (Tarragona, Spain). Acetic acid (CH_3COOH) (≥99.8%) from SDS (Peypin, France), hydrochloric acid (HCl) (37%) from Prolabo (Bois, France) and ammonium hydroxide (NH_4OH) (25%) from Panreac (Barcelona, Spain) were used to adjust the pH of the carrier liquid and the samples.

2.2. Sample collection

The wastewater samples were collected from the influent and effluent of two domestic sewage treatment plants (STPs), which are located in two cities with populations of around 120,000 habitants each, by using pre-cleaned amber glass bottles. All the samples were filtered using a 0.45 µm nylon membrane (Supelco, Bellefonte, PA, USA), acidified to pH 3 (HCl) and stored at 4 ºC until analysis.

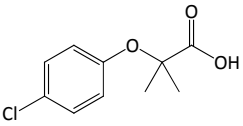
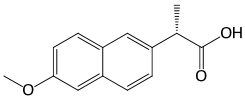
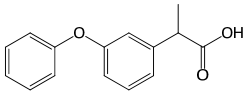
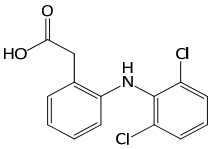
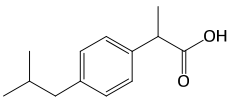
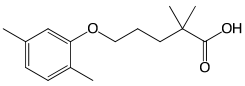
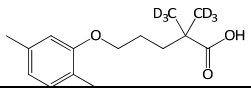
2.3. Molecularly imprinted solid-phase extraction

150 mg of a commercially available MIP, namely Affinilute MIP - NSAIDs (Biotage, Barcelona, Spain), were packed manually and placed into 6 mL polyethylene cartridge with 2 polypropylene frits (~10 µm) (Symta, Madrid, Spain). The cartridges were placed in an SPE manifold (Teknokroma, Barcelona, Spain) and connected to a vacuum pump. They were conditioned with 5 mL of ACN, 5 mL of MeOH and 5 mL of H_2O adjusted to pH 3. The samples adjusted to pH 3 were loaded through the MIP. A clean-up step was then performed with 5 mL of ACN/H_2O (40:60, v/v). In order to elute the retained analytes, 10 mL of MeOH/Acetone (80:20, v/v) with 1% CH_3COOH was passed through the cartridge. Elution extracts were evaporated to dryness under a gentle flow of N_2. Before MS/MS injection, the elution fractions were reconstituted to a final volume of 1 mL of MeOH/H_2O at pH 7 (60:40, v/v), to which gemfibrozil-d_6 (IS) was added at 50 µg L^{-1}, in order to correct LC injection and ionisation variability.

2.4. Instrumentation

All extracts were injected by flow injection analysis (FIA) using an Agilent quaternary pump 1200 series and an automatic injector (the volume injected was 50 µL) connected to a 6410 series triple quadrupole mass spectrometer using ESI from Agilent Technologies

Table 1. Structures, pK_a and experimental parameters employed for the MRM acquisition for all the studied analytes.

Analyte	Structure	pK_a [a]	MRM Transition	Cone Volt. (V)	Col. Ener. (V)
Clofibric acid		3.2	**213 > 127** 213 > 85	75	10 5
Naproxen		4.8	**229 > 170** 229 > 185	50	10 5
Fenoprofen		4.2	**241 > 197** 241 > 93	75	5 45
Diclofenac		4.2	**294 > 250** 294 > 214	75	10 20
Ibuprofen		4.4	**205 > 161**	75	5
Gemfibrozil		4.8	**249 > 121** 249 > 127	75	10 10
Gemfibrozil-d_6		4.8	**255 > 121** 255 > 133	50	10 5

[a] pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 for Solaris (© 1994–2011 ACD/Labs). The quantification MRM transitions are shown in bold.

(Waldbronn, Germany).

The optimised carrier liquid, used to push the extracts from the injector to MS/MS, was composed of MeOH/H_2O at pH 7 (60:40, v/v). The flow rate was set at 0.8 mL min^{-1}.

With respect to MS/MS detection, N_2 was used as the collision gas and its flow rate was set at 12 L min^{-1}. A source temperature of 300 ºC, a nebuliser pres-sure of 40 psi (N_2) and a capillary po-tential of 4000 V were applied. Multiple reaction monitoring (MRM) in negative ionisation mode was used to determine all analytes. Table 1 details MRM transitions, cone voltage and collision energy for each compound.

3. RESULTS AND DISCUSSION

3.1. MS/MS conditions

The different MS/MS parameters were adjusted, injecting each compound at 250 µg L^{-1} individually by FIA. Table 1 shows the optimum MS/MS conditions for each analyte in negative ESI. It was possible to obtain two different MRM transitions (selected as quantifier and qualifier) for all target analytes, except for ibuprofen, the MS/MS spectrum of which only contained one diagnostic ion and, hence, only one MRM transition was achieved for this compound. However, this compound was not initially excluded from the study due to its high prevalence in environmental water samples at high concentration levels [23]. Moreover, we injected a mixture of all the analytes and we checked that the same response was obtained for each analyte, rather than injecting them individually by FIA mode. Thus, it means that the signal of each analyte did not interfere with the signal of the rest of analytes. Therefore, MS/MS can be considered selective for the studied compounds under these conditions.

Next, the composition of the carrier liquid was optimised in order to enhance the analyte response. In case that the analytes were first separated using LC and then detected by MS/MS, the mobile phase had to be selected to obtain both a successful separation and proper ionisation of the compounds. However, when working with the direct coupling MISPE-MS/MS, the only requirement of the carrier liquid composition is to achieve the best solvent for ionisation in ESI interface. With this in mind, different solutions of MeOH or ACN (as organic solvent) combined with acidic or basic water were tested as the carrier liquid. To be specific, the carrier liquid compositions were: MeOH/H_2O at pH 3 (80:20, v/v), ACN/H_2O at pH 3 (80:20, v/v), MeOH/H_2O at pH 7 (80:20, v/v), ACN/H_2O at pH 7 (80:20, v/v), MeOH/H_2O at pH 7 (60:40, v/v) and ACN/H_2O at pH 7 (60:40, v/v). It should be mentioned that, in all instances, the injected solution containing the analytes and IS were prepared in the same composition as the carrier liquid. Fig. 1 shows the response achieved for all the analytes studied with the different carrier liquids tested.

First of all, MeOH/H_2O at pH 3 (80:20, v/v) and ACN/H_2O at pH 3 (80:20, v/v) were tested. These are typical mobile phases applied in LC since at pH 3 these analytes are in the neutral form, which would be appropriate for separation along the LC column. Nevertheless, solutions at pH 3 are not the most suitable for promoting the ionisation in the negative ESI interface, as shown in Fig. 1, in which the lowest areas were obtained. When the aqueous phase was adjusted to pH 7, maintaining the composition of the carrier liquid (80:20, v/v) and in both MeOH and ACN, the response increased for all target analytes. This fact could be explained because, under these conditions, acidic pharmaceuticals were mostly deprotonated and arrived at the ESI interface as negatively charged ions. Moreover, slightly better results were achieved using MeOH instead of ACN, since the protic nature of the former provided a strong solvation of negative ions [24]. In addition, in order to obtain the highest possible sensitivity, a different

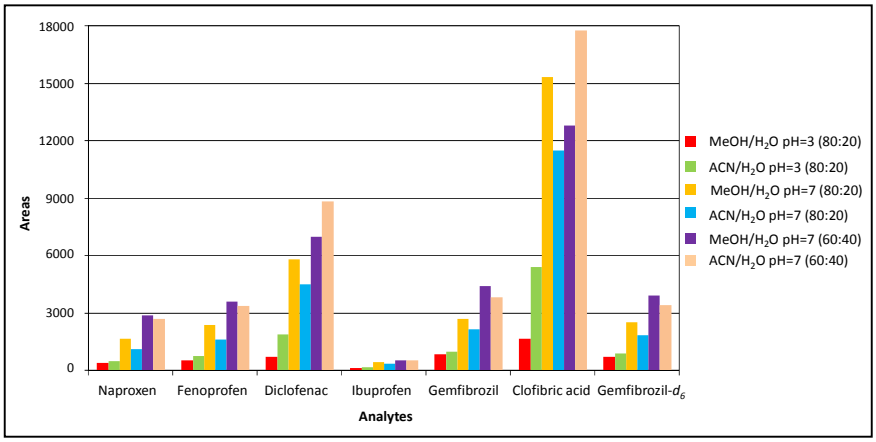


Fig. 1. Optimisation of carrier liquid used in MS/MS.

composition of carrier liquid (60:40, v/v) was evaluated, using both MeOH and ACN with H_2O adjusted to pH 7. Again, the areas obtained in MeOH/H_2O at pH 7 (60:40, v/v) were slightly higher than those in ACN/H_2O at pH 7 (60:40, v/v), except in the case of diclofenac and clofibric acid. Finally, MeOH/H_2O at pH 7 (60:40, v/v) was selected as a carrier liquid because this composition provided the best overall results.

The (ESI)-MS/MS procedure in MRM was validated for simultaneous determination of all the compounds. The instrumental method presented a good linear range (n=6) of 0.15-200 µg L^{-1} for all target analytes, except for ibuprofen (2-200 µg L^{-1}) by direct injection. LODs, calculated as the concentration which give a response corresponding a signal-to-noise ratio (S/N) of 3, ranged between 0.015 and 0.050 µg L^{-1} for all compounds, with the exception of ibuprofen (0.500 µg L^{-1}).

3.2. MISPE optimisation

MISPE performance was developed using a commercially available MIP, which was selective for NSAIDs and enabled the effective extraction of these compounds from complex matrices. This commercial MIP was used in a previous study in our research group [18] and its MISPE protocol was adapted to this work.

First, to promote the interactions between the analytes and the sorbents during the loading step, the sample pH was adjusted at 3, as we reported previously [18]. Once the analytes were retained onto the sorbent, the elution step was crucial to remove the analytes from the cartridge and the eluate extract also had to be a suitable solvent for the MS/MS system. As mentioned before, MeOH/H_2O at pH 7 (60:40, v/v) enabled a proper ionisation of all the analytes obtaining their highest response. However, this solvent was not able to release the interactions established between the sorbent and the analytes

and an acidic elution solvent, different from the optimum carrier liquid, was necessary. For this reason, the evaporation step was included. Therefore, the same elution solvent used in the previous study, MeOH/Acetone (80:20, v/v) with 1% CH_3COOH, was applied with an elution volume of 10 mL, and then, the evaporation procedure resulted in a 25-fold concentration as well as a solvent switch. When 25 mL of ultrapure water at pH 3 spiked at 2 µg L^{-1} was percolated through the cartridge using the above protocol, recovery values nearly of 100% for all target analytes were obtained.

Finally, a clean-up step is essential when analysing real samples using a MIP. Particularly, for this MIP, its high specificity towards the NSAIDs allows the removal of interferences present in the sample. As we detailed in our previous study [18], 5 mL of ACN/H_2O (40:60, v/v) was selected as the clean-up solvent and also applied in the present work. After loading 25 mL of ultrapure water at pH 3 spiked at 2 µg L^{-1}, we checked that the clean-up solution enabled to keep retained the studied analytes without losses, obtaining recovery values over 84%.

After the MISPE optimisation, the breakthrough volume percolated through the MIP was evaluated. Good recovery values were achieved with volumes up to 1000 mL of ultrapure water loaded (over 70%, except for diclofenac (56%)).

3.3. Application to real samples

Once the MISPE-MS/MS method was successfully applied in ultrapure water, the same protocol was applied to the analysis of environmental waters. In complex matrices such as wastewaters, ion suppression/enhancement is a common effect when ESI is used as MS interface because the analytes' response may vary depending on the matrix interferences [6]. Including the LC in the analytical methodology usually enables to separate the analytes from the matrix interferences during the chromatographic separation preventing their entrance into the ionisation interface at the same time. Therefore, if LC is not included, an effective sample pretreatment is needed to minimise this effect.

The ion suppression/enhancement effect was calculated as the percentage decrease in the signal obtained by the target analytes and IS spiked at 100 µg L^{-1} after a real sample extraction *versus* the intensity of the same amount of analytes in ultrapure water [18,25]. This effect was evaluated for effluent and influent wastewaters. When effluent and influent wastewaters were analysed, all the compounds showed high signal suppression with values up to 70%, except for clofibric acid and fenoprofen which presented the highest ion suppression (84%). This high effect could be explained due to the absence of the chromatographic separation in this method and, above all, the high content of organic matter present in these real samples. However, it should be pointed out that without the clean-up step almost all compounds were completely suppressed (nearly 100%). Thus, the clean-up step was necessary to remove as many interferences as possible.

Moreover, the applicability of the method was assessed in terms of extrac-

Table 2. Recovery values (%) obtained when the acidic pharmaceuticals from different water samples were determined by MISPE-MS/MS.

Analyte	% Recovery value		
	Ultrapure-water[a]	Effluent wastewater[b]	Influent wastewater[c]
Clofibric acid	70	73	69
Naproxen	94	102	83
Fenoprofen	106	88	87
Diclofenac	56	62	88
Ibuprofen	130	94	84
Gemfibrozil	105	100	103

%RSD < 19% (n=3) [a] 100 mL spiked at 0.5 μg L^{-1} [b] 50 mL spiked at 1 μg L^{-1}
[c] 10 mL spiked at 3 μg L^{-1}

tion recoveries for target analytes in effluent and influent wastewaters, using 50 mL and 10 mL, respectively. The volume of the real samples was reduced as the complexity of the matrices increased in order to obtain similar recoveries as for ultrapure water. Table 2 shows the recovery values of the analytes in ultrapure water, effluent and influent wastewaters. These recovery values in environmental waters were evaluated by comparing them with a blank sample spiked before and after the MISPE procedure at the same final concentration. The recovery values (n=3) of the target analytes in effluent wastewaters ranged from 62% to 102% (Table 2). When influent wastewaters were passed through the MIP cartridge, the recoveries were between 83% and 103%, except for clofibric acid (69%).It should be mentioned that the recovery values for ibuprofen cannot be taken as definitive because one MRM transition was found for its quantification. So, the present method can only be considered semi-quantitative for ibuprofen. These recovery values are higher than those presented in previous methods [19,20] where similar group of analytes from the same type of samples were determined using procedures that involve off-line SPE/LC-MS/MS.

Bearing in mind the results from the ion suppression/enhancement study, the next step was to validate the MISPE-MS/MS method using a matrix-matched calibration in order to perform an accurate quantification of real samples due to this effect. Therefore, the method validation was developed using 50 mL of effluent wastewaters. It should be mentioned that gemfibrozil was found in real samples and its signal was subtracted from the spiked samples. The linear ranges were between 0.50 and 50 μg L^{-1} for all compounds, except for fenoprofen (0.15 to 50 μg L^{-1}) (r^2 > 0.993 and for ibuprofen (r^2 > 0.987)). For the target analytes without blank signals, LODs were determined as the concentrations corresponding to S/N of 3. However, for gemfibrozil that was present in the blank, the LOD was tentatively calculated as three times the standard deviation of the analyte signal in the blank (n=3). LODs were 0.10 μg L^{-1} for all pharmaceuticals, except for fenoprofen, which had a LOD

Table 3. Concentrations found for the acidic pharmaceuticals in effluent and influent wastewaters analysed from two STPs.

Analyte	Concentrations found (μg L^{-1})	
	Effluent wastewaters	Influent wastewaters
Clofibric acid	<0.10-0.82	<0.10
Naproxen	<0.50-1.55	0.68-6.40
Fenoprofen	<0.05	<0.05
Diclofenac	<0.50-0.81	<0.50
Ibuprofen	2.64-5.46*	0.54-20.17*
Gemfibrozil	0.78-4.06	<0.50-2.39

*Tentatively quantified since the method is semi-quantitative for ibuprofen

of 0.05 μg L^{-1}. The repeatability and reproducibility between days of three samples spiked at 3 μg L^{-1}, expressed as % relative standard deviation (%RSD), were lower than 9% and 14%, respectively.

3.3.1. Analysis of real samples

To demonstrate the applicability of the MISPE-MS/MS, effluent and influent wastewaters from two STPs on different dates were analysed in triplicate. All the acidic pharmaceuticals were detected in both effluent and influent wastewaters, except fenoprofen (Table 3). For instance, Fig. 2 shows MRM chromatograms of an effluent wastewater sample. As expected, the most frequently detected compounds in wastewaters at high concentrations levels were gemfibrozil (<0.50 to 4.06 μg L^{-1}), naproxen (<0.50 to 6.40 μg L^{-1}) and ibuprofen, the concentration of the latter was tentatively quantified in the present method, as explained in Section 3.1. Moreover, higher concentrations of gemfibrozil were found in effluent than influent wastewaters. A possible explanation could be that the sampling of the influent and effluent wastewaters was not performed in the

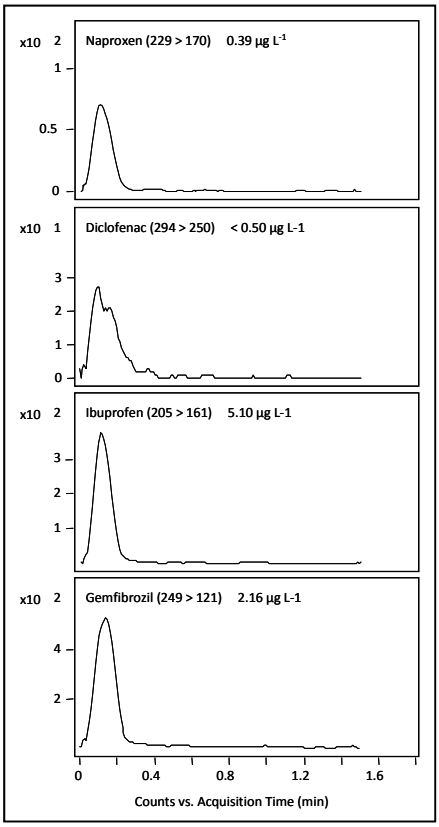


Fig. 2. MRM transitions of an effluent wastewater sample.

same time period as well as for a possible conversion of its conjugated metabolite to the original substance after the treatment processes [21,24]. These concentrations were similar to those obtained in other studies [19,21,23] performed in similar urban STPs, where the highest concentrations were also attributed to ibuprofen and naproxen. These levels showed the presence of these pharmaceuticals in environmental wastewaters.

4. CONCLUSIONS

A rapid analytical method consisting of off-line MISPE coupled directly to MS/MS (omitting the chromatographic separation) is able to determine six acidic pharmaceuticals in effluent and influent wastewaters with good recoveries (62% to 103%). Although considerable signal suppression/enhancement was obtained, using a matrix-matched calibration curve helped to balance this effect and obtain a reliable quantification of environmental water samples.

This coupling enabled to develop an analytical method which gave LODs at low levels of μg/L (0.05 to 0.10 μg L^{-1}) for effluent wastewater, despite of the absence of chromatographic separation. The promising results achieved with MISPE-MS/MS allowed the quantification of acidic pharmaceuticals in wastewaters from two different STPs.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] D.R. Baker, B. Kasprzyk-Hordern, J. Chromatogr. A 1218 (2011) 7901-7913.
[2] B. Shao, D. Chen, J. Zhang, Y.N. Wu, C.J. Sun, J. Chromatogr. A 1216 (2009) 8312-8318.
[3] A. Jelic, M. Petrović, D. Barceló, Talanta 80 (2009) 363-371.
[4] J. Radjenovic, A. Jelic, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 393 (2009) 1685-1695.
[5] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, TrAC, Trends Anal. Chem. 30 (2011) 749-760.
[6] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Talanta 74 (2008) 1299-1312.
[7] C.H.P. Bruins, C.M. Jeronimus-Stratingh, K. Ensing, W.D. van Dongen, G.J. de Jong, J. Chromatogr. A 863 (1999) 115-122.
[8] M.W.J. van Hout, C.M. Hofland, H.A.G. Niederlander, G.J. de Jong, Rapid Commun. Mass Spectrom. 14 (2000) 2103-2111.
[9] M.W.J. van Hout, C.M. Hofland, H.A.G. Niederlander, A.P. Bruins, R.A. de Zeeuw, G.J. de Jong, J. Chromatogr. B 794 (2003) 185-192.
[10] M. Levi, G. Wuerzner, E. Ezan, A. Pruvost, J. Chromatogr. B 877 (2009) 919-926.
[11] A. Beltran, F. Borrull, P.A.G. Cormack, R.M. Marcé, TrAC,

Trends Anal. Chem. 29 (2010) 1363-1375.
[12] J. Haginaka, J. Sep. Sci. 32 (2009) 1548-1565.
[13] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, J. Sep. Sci. 29 (2006) 1230-1236.
[14] E.C. Figueiredo, R. Sparrapan, G.B. Sanvido, M.G. Santos, M.A.Z. Arruda, M.N. Eberlin, Analyst 136 (2011) 3753-3757.
[15] E.C. Figueiredo, G.B. Sanvido, M.A.Z. Arruda, M.N. Eberlin, Analyst 135 (2010) 726-730.
[16] J.C. Vidal, P. Duato, L. Bonel, J.R. Castillo, Anal. Lett. 45 (2012) 51-62.
[17] T. Ishida, K. Kudo, M. Hayashida, N. Ikeda, J. Chromatogr. B 877 (2009) 2652-2657.
[18] N. Gilart, R.M. Marcé, F. Borrull, N. Fontanals, J. Sep. Sci. 35 (2012) 875-882.
[19] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958-2969.
[20] M. Gros, M. Petrović, D. Barceló, Talanta 70 (2006) 678-690.
[21] M.A. Sousa, C. Gonçalves, E. Cunha, J. Hajslová, M.F. Alpendurada, Anal. Bioanal. Chem. 399 (2011) 807-822.
[22] M.J. Gómez, M. Petrović, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1114 (2006) 224-233.
[23] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Water Air Soil Pollut. 217 (2011) 267-281.
[24] T. Henriksen, R.K. Juhler, B. Svensmark, N.B. Cech, J. Am. Soc. Mass. Spectrom. 16 (2005) 446-455.
[25] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019-3030.

3.1.3. Selective determination of pharmaceuticals and illicit drugs in wastewaters using a novel strong cation-exchange solid-phase combined with liquid chromatography-tandem mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

SELECTIVE DETERMINATION OF PHARMACEUTICALS AND ILLICIT DRUGS IN WASTEWATERS USING A NOVEL STRONG CATION-EXCHANGE SOLID-PHASE EXTRACTION COMBINED WITH LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Núria Gilart[a], P.A.G. Cormack[b], Rosa Maria Marcé[a], Núria Fontanals[a], Francesc Borrull[a]

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

[b] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Abstract

In this study, two materials are presented with strong cation-exchange (SCX) behaviour synthesised by two different approaches and then crushed for their application as sorbents for solid-phase extraction (SPE) to extract a group of pharmaceuticals and illicit drugs selectively from wastewater samples. The first SCX polymer was obtained by copolymerisation of three monomers: 2-acrylamido-2-methylpropane sulphonic acid (AMPSA), 2-hydroxyethyl methacrylate (HEMA) and pentaerythritol triacrylate (PETRA); while the second was obtained by post-modification with sulphuric acid (H_2SO_4) of a copolymer based on HEMA and divinylbenzene (DVB).

After their syntheses, both polymers were evaluated as SPE sorbents, with all parameters affecting SPE being optimised, such as sample pH, washing and elution solvents and volumes. Thanks to the sulphonic groups present in the structure of the polymers, all of the compounds with basic functionalities were retained on the sorbents after the washing step, removing the acidic analytes and other interfering compounds, providing successful results in terms of ion suppression/enhancement (-12% and 21%) when wastewater samples were analysed. However, AMPSA/HEMA/PETRA (20/60/20) failed to retain the analytes after loading wastewater samples (25 or 50 mL), decreasing analyte recovery values significantly, whereas the sulphonated HEMA/DVB (50/50) enabled good SPE performance with recovery values between 70% and 98%, except for ranitidine and EDDP (39% and 43%, respectively). Therefore, this polymer was selected for further method validation and quantification of wastewater samples, providing low method detection limits (MDLs) in this matrix (from 2 to 40 ng L^{-1}). Finally, most of the studied compounds were detected and quantified in wastewater samples, especially atenolol, ranitidine, cocaine and its metabolite benzoylecgonine.

Keywords: *selectivity; strong cation-exchange; mixed-mode solid-phase extraction; pharmaceuticals and illicit drugs; wastewaters*

1. INTRODUCTION

Sample pre-treatment is considered an essential step in the development of an analytical methodology, since it enables the reduction of the matrix interferences and preconcentration to achieve the lowest possible method detection limits (MDLs) [1]. In last decades, solid-phase extraction (SPE) has become the most popular technique for extracting emerging organic contaminants (EOCs) when analysing liquid samples due to the high variety of sorbents available with different chemical and physical properties and the wide range of formats [2, 3].

As well as the classic SPE sorbents, hydrophilic polymeric sorbents have become increasingly popular due to their high surface area and polar functionalities in their structures. Among them, Oasis HLB (Waters, Milford, MA, USA), which is based on a macroporous poly(*N*-vinylpyrrolidone-divinylbenzene) copolymer, has been one of the most widely used and applied for the determination of compounds with different polarities in complex matrices, such as environmental [4-6], food [7, 8], and biological [9, 10] samples. Although capacity and high retention of a broad type of compounds are the main advantages of this sorbent, it still lacks selectivity, as it extracts many interferences from the matrix together with the analytes of interest. Therefore, selectivity has been one of the most desired requirements for the development of new improved sorbents for SPE, with great progress being achieved with the synthesis of molecularly imprinted polymers (MIPs) [11, 12]. Another type of selective sorbents are known as dual-phase or mixed-mode polymeric sorbents, which are able to establish reversed-phase and ion-exchange interactions with the analytes. Their polymeric skeleton chemically modified with cationic or anionic functional groups has provided the extraction of both charged and neutral compounds from many real samples. Depending on the acidic or basic properties of the target analytes, strong or weak ion-exchange sorbents are commercially available, such Oasis MAX, Oasis MCX, Strata X-C, Strata X-WA or Bond Elut Plexa PCX, which are widely applied for the extraction of different EOCs, such as pharmaceuticals and personal care products (PPCPs) [1, 13-15], artificial sweeteners [6] and illicit drugs [16, 17] in environmental, biological and food samples.

Several approaches in the synthesis and application of novel in-house ion-exchange polymers as SPE sorbents have recently been reported with the aim of increasing the specific surface area, and thus enhancing the retention of a broad range of compounds. Our research group was the first to synthesise hypercrosslinked polymers with different mixed-mode properties: strong anion-exchange (SAX) (modified with dimethylbutylamine) [18], weak anion-exchange (WAX) (with 1,2-ethylenediamine and piperazine) [19] and weak cation-exchange (WCX) (with methacrylic acid) [20] for further off- and on-line SPE applications to extract a group of pharmaceuticals in environmental matrices [18-20]. These studies showed an outstanding increase in terms of retention of the target analytes by both reversed-phase and ion-exchange interactions due to higher specific surfaces areas than the commercial macroporous mixed-mode sor-

bents. Within our group, different SCX hypercrosslinked polymer microspheres were also synthesised using two different alkyl sulphate reagents (acetyl and lauryl sulphate) [21]. However, these particles have not yet been applied as SPE sorbents. A similar study [22] has recently been reported in which magnetic polystyrene microspheres were synthesised with SCX behaviour using acetyl sulphate as the sulphonating agent for the extraction of melamine from egg samples.

Bearing in mind the simplicity in terms of the polymer synthesis and sample pre-treatment, two polymeric materials have first been synthesised to be used as SCX sorbents for SPE, using two different approaches. The first extraction material was prepared by the copolymerisation of three monomers: 2-acrylamido-2-methylpropane sulphonic acid (AMPSA), 2-hydroxyethyl methacrylate (HEMA) and pentaerythritol triacrylate (PETRA). The second material was obtained by post-modification with sulphuric acid (H_2SO_4) of a copolymer based on HEMA and divinylbenzene (DVB). Both polymers were evaluated as SCX-SPE sorbents for the extraction of a group of pharmaceuticals and illicit drugs with acidic and basic properties from environmental waters by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

The reagents used for the polymer syntheses were AMPSA (99%), HEMA (97% grade), PETRA (technical grade), DVB (80% grade), cyclohexanol (99%), 1-dodecanol (98%), methanol (MeOH) (99.7%) and H_2SO_4 (98%) supplied by Sigma-Aldrich (Steinheim, Germany). HEMA, PETRA and DVB were purified by passing them through short columns packed with neutral alumina, provided by Sigma-Aldrich. The 2-2'-azobis(isobutyronitrile) (AIBN), used as the initiator, was supplied by BDH (Poole, UK) and purified by recrystallisation at low temperature with MeOH prior to use. The purification steps were described previously [23]. For the post-sulphonation, dichloromethane (DCM) from Sigma-Aldrich was used to swell the polymer.

The analytes selected to evaluate the SCX sorbents were a group of illicit drugs and pharmaceuticals with basic and acidic functional groups. As for illicit drugs, morphine (MOR), cocaine (COC), methadone (META), codeine (COD) and their metabolites (6-acetylmorphine (6-AM), benzoylecgonine (BE), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and dihydrocodeine (DCOD), respectively) were obtained from Cerilliant (Round Rock, TX, USA) as solutions in MeOH at a concentration of 1000 mg L^{-1}. For pharmaceuticals, atenolol (ATE), propranolol (PROP), metoprolol (MET), ranitidine (RANI), trimethoprim (TRIM), salicylic acid (SALI), clofibric acid (CLO), diclofenac (DICLO) and ibuprofen (IBU) were purchased from Sigma-Aldrich (>97%) and standard stock solutions of each analyte were prepared at 1000 mg L^{-1} in MeOH. A mixed solution of all analytes in MeOH at 50 mg L^{-1} was prepared weekly. All standard solutions were stored at -20 ºC. Working solutions were prepared daily by appropriate dilution of the mixed solution with water. The structures and pK_a values of these analytes are presented

in Table 1.

Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain). Acetonitrile (ACN) and MeOH were HPLC grade from Prolabo (Llinars del Vallès, Spain). Nitrogen (N_2) was supplied by Carburos Metálicos (Tarragona, Spain). Hydrochloric acid (HCl) (37%), formic acid (HCOOH) (≥95%), acetic acid (CH_3COOH) (99%), ammonium hydroxide solution (NH_4OH) (28%) and sodium hydroxide (NaOH) (≥98%) were purchased from Sigma-Aldrich.

2.2. Polymerisation synthesis and characterisation

The synthesis of the polymeric materials for SPE applications was developed by traditional polymerisation. The first SPE sorbent was prepared by the copolymerisation of three monomers: AMPSA, HEMA and PETRA. Firstly, all three monomers were dissolved in a ternary porogenic solution consisting of 60% MeOH, 5% ultrapure water and 35% cyclohexanol (w/w/w). Subsequently, the initiator (AIBN, 1 mol% relative to polymerisable double bonds) was added to the polymerisation solution and it was mixed ultrasonically into a homogenous solution. The ratio between monomers was 20% AMPSA, 60% HEMA and 20% PETRA and the ratio of total monomers to porogenic solvent was 40/60 (%, w/w). After sonication, the polymerisation mixture was poured into a 25 mL thick-walled glass Kimax culture tube and the polymerisation solution was placed on an ice-bath and purged with oxygen-free nitrogen for 5 min before being sealed under N_2. Polymerisation was carried out by leaving the sealed glass tube in an oil bath set at 60 ºC for 24 h. Once the polymerisation was completed, the culture tube was crushed carefully and the monolith obtained was ground, sieved and the fraction ranging from 32 to 50 µm collected, Soxhlet extracted overnight in MeOH and dried in readiness for the SPE experiments. Fig. 1 (a) shows the structure of the SCX terpolymer.

Regarding the second synthesis, the polymeric material was also prepared by the copolymerisation of two monomers: HEMA and DVB, with a ratio between monomers of 50/50 (%, w/w). In this case, the monomers were dissolved in the porogen 1-dodecanol with a ratio between the total monomers and porogen of 38/62 (%, w/w). Lastly, AIBN (1 mol% relative to polymerisable double bonds) was used as the initiator. The polymerisation process was as described in the previous paragraph. The resulting monolith was ground and washed by Soxhlet extraction with MeOH to remove unreacted monomers, initiator and porogen, and finally, dried in a vacuum oven at 40 ºC for 24h. Prior to sulphonation, the dried material (approximately 1 g) was swollen through contact with DCM (10 mL) for 30 min. Once the particles were swollen, 10 mL of concentrated H_2SO_4 was added and it was kept in an oil bath at 70 ºC for 4 h. The sulphonated material was washed by filtration with excess deionised water until a neutral pH was reached. Finally, it was dried and sieved between 32 and 50 µm for further SPE applications. The structure of the sulphonated HEMA/DVB copolymer is detailed in Fig. 1 (b).

The polymeric materials were characterised by Fourier-Transform Infrared

Table 1. Structures, pK_a, retention times and MS/MS parameters employed for the MRM acquisition of the studied analytes.

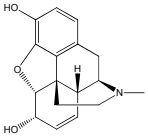
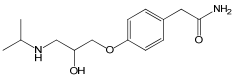
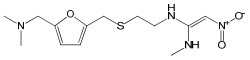
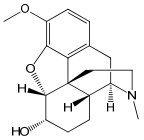
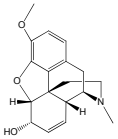
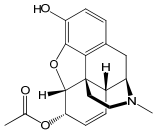
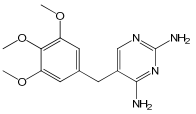
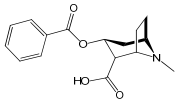
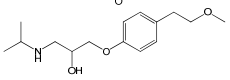
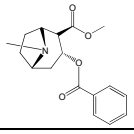
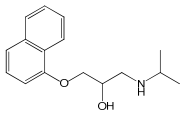
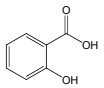
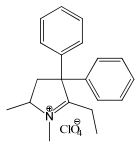
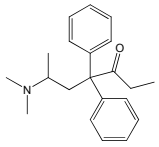
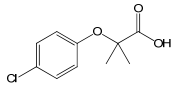
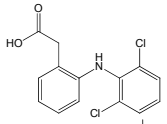
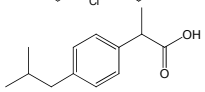
Analyte	Structure	pK_a [a]	t_R (min)	MRM transition	Ionis. mode	Cone volt. (V)	Colli. ener. (V)
MOR		8.3	2.2	286>152 286>165	+	125	50 50
ATE		9.4	2.9	267>145 267>190	+	125	25 10
RANI		8.4	3.3	315>176 315>130	+	100	5 15
DCOD		8.4	4.2	302>199 302>128	+	150	25 50
COD		8.3	4.5	300>165 300>153	+	150	50 50
6-AM		8.3	5.7	328>165 328>211	+	150	50 25
TRIM		7.0	6.2	291>230 291>123	+	125	15 25
BE		10.8 3.2	7.9	290>168 290>105	+	125	15 25
MET		9.4	8.1	268>116 268>159	+	125	15 15
COC		8.0	9.1	304>182 304>82	+	125	15 25

Table 1. Continued.

Analyte	Structure	pK_a [a]	t_R (min)	MRM transition	Ionis. mode	Cone volt. (V)	Colli. ener. (V)
PROP		9.5	11.2	260>116 260>183	+	125	15 15
SALI		3.1	12.3	137>93 137>65	-	75	15 30
EDDP		7.7	12.6	278>234 278>249	+	150	25 15
METAD		9.1	13.0	310>265 310>105	+	100	5 25
CLO		3.2	14.7	213>127 213>85	-	75	10 5
DICLO		4.2	15.4	294>250 294>214	-	75	5 15
IBU		4.4	15.6	205>161	-	75	5

[a] pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2012 ACD/Labs)

(FTIR) spectroscopic analyses using a Perkin-Elmer Spectrum One FT-IR spectrometer (Massachusetts, USA). The ion-exchange capacity (IEC) was calculated by the titration method. Finally, the surface area measurements of both polymers were performed using a Brunauer-Emmett-Teller (BET) treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 sorptometer (Norcross, GA, USA).

2.3. LC-(ESI)-MS/MS

The chromatographic system was an Agilent 1200 series LC coupled to a 6410 series triple quadrupole mass spectrometer with an electrospray ionisation (ESI) interface, an automatic injector (volume injected was 50 µL), a

degasser, a quaternary pump and a column oven from Agilent Technologies (Waldbronn, Germany). The analytical column was an Ascentis® Express C_{18} (100 mm x 4.6 mm i.d.) with 2.7 µm particle size from Sigma-Aldrich.

A binary mobile phase with a gradient elution was used. Solvent A was ultrapure water adjusted to pH 3 with HCOOH and solvent B was ACN and the flow rate was 0.6 mL min^{-1}. The column temperature was kept at 30 ºC. The gradient was as follows: 7% B to 28% B in 9 min increased to 100% B in 5 min, constant for 2 min and then decreased to initial conditions in 3 min. The chromatographic separation of 17 analytes was achieved in 16 min.

In order to obtain the highest sensitivity in LC-MS/MS analyses, a flow injection of standard solutions of each compound was performed to optimise all MS/MS parameters. Both positive and negative ionisation modes were applied to enable the simultaneous determination of the studied analytes. The optimal source conditions were as follows: nebuliser pressure of 45 psi, drying gas (N_2), flow rate of 12 L min^{-1}, source temperature of 350 ºC and a capillary potential of 4000 V. To obtain two multiple reaction monitoring (MRM) transitions for each compound, cone voltage and collision energies were optimised, the values of which are detailed in Table 1.

2.4. Solid-phase extraction

SPE cartridges were manually packed with 200 mg of each SCX polymer and placed into 6 mL polyethylene cartridges with two polypropylene frits (~10 µm) (Symta, Madrid, Spain). The cartridges were placed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. The SPE procedure was the same for both sorbents. First of all, the cartridges were preconditioned with 5 mL of MeOH and 5 mL of ultrapure water at pH 3 (HCl). Subsequently, the samples, also adjusted to pH 3 (HCl), were passed through the cartridges at a flow rate of 5 mL min^{-1}. The cartridges were then washed with 5 mL of MeOH. Finally, the analytes were eluted from the cartridges using 3 mL of 5% NH_4OH in MeOH. Prior to the LC injection, the extracts were evaporated to dryness under a gentle stream of N_2 and redissolved in 1 mL of ultrapure water adjusted at pH 3 (HCOOH).

Wastewater samples (influent and effluent) were collected in pre-cleaned amber glass bottles from a wastewater treatment plant (WWTP) located in Tarragona (Spain). Subsequently, the samples were filtered using a 0.22 µm nylon membrane (Supelco, Bellefonte, PA, USA), acidified to pH 3 (HCl) and stored at 4 ºC until analysis.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterisation of SCX sorbents

The easy preparation of polymeric materials by traditional polymerisation and the versatility offered by the availability of many monomers with different functionalities have led to this approach being extensively applied for obtaining sorbents for SPE, such as molecularly imprinted polymers (MIPs) [24]. Thus, the choice of monomers and the development of polymerisation were essential factors to be controlled and evaluated. The most common stra-

tegies to synthesise polymers by traditional polymerisation with SCX character involve introducing a sulphonic acid group into their structure by post-modification [25] and copolymerisation, with the latter being the most commonly used technique due to its simplicity and the fact that it only requires a single step [26, 27]. In this study, two SCX polymers were synthesised by traditional polymerisation using both strategies.

The first synthesis was performed by the copolymerisation of three monomers: AMPSA and HEMA, as functional monomers, and PETRA as the cross-linker. With respect to the structure of AMPSA, this monomer allowed hydrophilic and ionic interactions to be established thanks to its amide group and sulphonic acid, respectively. In contrast, the hydroxyl and ester groups present in the structure of HEMA and PETRA provided more hydrophilicity to the whole material, as can be seen in Fig. 1 (a). The principal parameter to take into account was the ratio between the monomers. In these syntheses, three ratios of AMPSA/HEMA/PETRA were tested: 5/75/20, 10/70/20 and 20/60/20 (%, w/w). It was decided to keep the content of PETRA constant (20%) in order to obtain polymers with the same mechanical stability. In contrast, the AMPSA content was varied from 5% to 20% in order to synthesise three polymers with different IECs. After polymerisation, the resulting materials were translucent with high swelling capacity. With respect to characterisation, when the IEC of each material was evaluated using the titration method with NaOH, the IEC values obtained were 0.17, 0.42 and 0.84 mmol g^{-1} for 5%, 10% and 20% AMPSA polymers, respectively. The AMPSA/HEMA/PETRA polymer with a ratio of 20/60/20 presented the highest IEC by far, and was thus selected for further characterisation. The results obtained from FTIR spectroscopy for the first SCX polymer are shown in Table 2, in which the presence of the sulphonic group in the polymeric structure was confirmed. The low surface

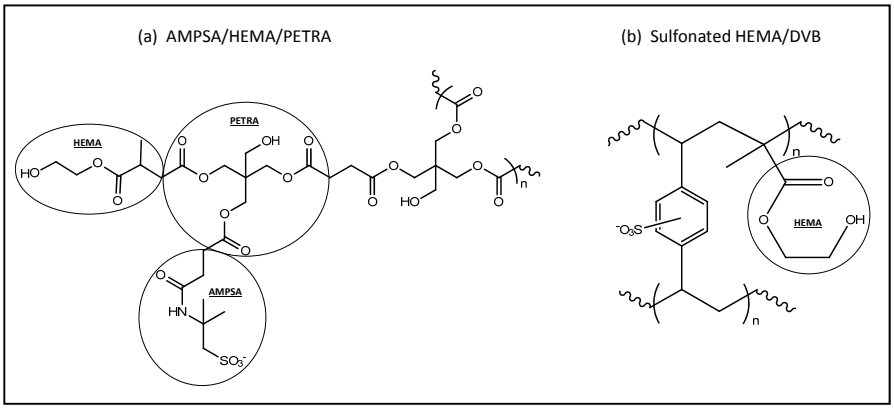


Fig. 1. Chemical structure of the polymers synthesised with strong cation-exchange (SCX) behaviour: a) AMPSA/HEMA/PETRA and b) Sulphonated HEMA/DVB.

Table 2. Results obtained from the characterisation of the two SCX polymers.

	Ion-exchange capacity (IEC) (mmol g^{-1})	Characteristic bands from IR spectrum	Surface Area (m^2 g^{-1})
AMPSA/HEMA/PETRA (20/60/20)	0.84	▪ S=O stretching vibration (1148 cm^{-1}) ▪ N-H stretching vibration of amide group (1625 cm^{-1})	<5
AMPSA/HEMA/PETRA (10/70/20)	0.42		<5
AMPSA/HEMA/PETRA (5/75/20)	0.17		<5
Sulphonated HEMA/DVB (50/50)	2.05	▪ S=O stretching vibration (1090-1160 cm^{-1}) ▪ C=C stretching vibration of DVB (1450 cm^{-1})	~150

area of this polymer (< 5 m^2 g^{-1}) confirmed that, in the dry state, the pores present in its structure are completely closed, while in the wet state, they open to provide a high swelling capacity.

The second SCX polymeric material was obtained by the post-modification of a HEMA/DVB 50/50 (%, w/w) copolymer, previously synthesised by traditional polymerisation. These monomers were selected in order to allow hydrophilic interactions, thanks to the ester and hydroxyl groups in the HEMA structure and also π-π interactions, due to the aromatic rings in the DVB structure, as shown Fig. 1 (b). In our previous study [23], several monolithic coatings were synthesised for stir bar sorptive extraction (SBSE), one of which being a copolymer of HEMA/DVB with a ratio between monomers of 50/50 (% w/w) which provided acceptable recovery values (40-80%) for a group of moderately polar PPCPs. For this reason, it was decided to post-modify this monolithic material with H_2SO_4 to obtain a SCX polymer for SPE. Once the copolymerisation of HEMA/DVB was completed and the monolith was crushed, concentrated H_2SO_4 was added to the polymer and heated for 4 h at 70 ºC. The reaction time of 4 h was selected according to the results from the study of the sulphonation degree of a styrene-DVB copolymer [28], in which higher reaction times led to a polymer with higher ion-exchange capacities. Table 2 shows that the sulphonated HEMA/DVB copolymer displayed higher IEC and surface area values (2.05 mmol g^{-1} and ~150 m^2 g^{-1}, respectively) than the AMPSA/HEMA/PETRA (20/60/20) polymer (0.84 mmol g^{-1} and < 5 m^2 g^{-1}, respectively), with more ion-exchange sites and surface area to keep the analytes strongly retained. Thus, from the polymer syntheses, we obtained two resins with SCX character by two different approaches, which were further evaluated as SCX SPE sorbents.

3.2. LC-(ESI)MS/MS conditions

Prior to chromatographic separation, the optimisation of MS/MS conditions

was required in order to achieve the highest possible sensitivity for all of the compounds studied. Therefore, each parameter was studied by injecting each of the compounds at 500 μg L^{-1} in ultrapure water at pH 3 (HCOOH) in flow injection analysis (FIA). Since the target analytes presented acidic or basic functional groups in their structures, they were divided individually or in groups into eight windows applying negative or positive ESI ionisation. Moreover, identification and confirmation MRM transitions, together with their cone voltage and collision energy, were optimised for each analyte. All of the optimised MS/MS conditions for each compound are detailed in Table 1.

With respect to the chromatographic separation, 17 compounds were successfully separated in just 16 minutes, thanks to the efficiency and speed of the Ascentis® Express C_{18} column with Fused-Core technology. Since most of the target compounds have basic properties, an acidic aqueous phase with HCOOH at pH 3 was used as solvent A. Higher signal responses were observed for the basic compounds when the mobile phase was acidified with HCOOH rather than with acetic acid. This fact could be explained by the lower pK_a value of HCOOH, which promotes better ionisation of basic functional groups.

In order to evaluate the performance of the LC-MS/MS method, linearity, instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) were evaluated for each compound. All of the target analytes showed a good linear range ($r^2 \geq 0.996$) between 0.50 and 250 μg L^{-1}, except for salicylic acid and ibuprofen (5-500 μg L^{-1}), with the lowest point in the calibration curve being considered as the IQLs. The instrumental detection limits (IDLs), calculated as a signal-to-noise ratio (S/N) of 3, ranged from 0.05 to 1.50 μg L^{-1} for all of the compounds.

3.3. Solid-phase extraction optimisation

As well as the selection of the suitable analytes, the SPE conditions, such as sample pH, elution solvent and volume, washing solvent and volume, were optimised to promote and enhance the retention of the compounds on the sorbents and, thus, demonstrate the SCX behaviour of the polymeric materials.

As expected with a SCX sorbent, the sample pH in the loading step plays an important role since ionic interactions need to be promoted. Therefore, the effect of the sample pH was evaluated by loading 10 mL of ultrapure water through the cartridges spiked at 10 μg L^{-1} with the analyte mixture and adjusted at different pH values (3, 6 and 11). Due to the permanent negative charge of the sulphonic groups in the structure of the sorbents, as well as the high pK_a values of the basic compounds ($pK_a > 7$), all of the basic compounds were completely retained onto the sorbents when the sample pH was 3, while the acidic analytes were not or only slightly retained onto the AMPSA/HEMA/PETRA and the sulphonated HEMA/DVB polymers, respecttively. This fact could be attributed to the aromatic rings present in the sulphonated HEMA/DVB, which might enhance the reversed-phase interactions (mainly π-π), even in the case of the acidic analytes. In contrast, it was

demonstrated that the sample pH values of 6 and 11 were not suitable to completely retain the analytes *via* ion-exchange mechanisms. Therefore, the optimal sample pH was 3 for all of the compounds studied with both SCX polymers.

The elution step was the following parameter to be optimised in order to break the ionic interactions between the analytes and the sorbents using basic and organic media. Basic elution solutions were tested to release the basic compounds from the sorbent, such as 5% NH_4OH solution in MeOH or ACN (5 mL). When basic MeOH and basic ACN were compared, 5% NH_4OH solution in MeOH provided better results (~30% more) than basic ACN solution and, thus, it was selected as the elution solvent for further analysis. Moreover, Fig. 2 shows the recovery values (%) obtained in the elution step for a representative group of compounds when several elution volumes, from 1 to 10 mL of 5% NH_4OH solution in MeOH, were tested using the sorbent based on AMPSA/HEMA/PETRA. As can be observed, no signify-cant differences where obtained between 3 mL and 5 mL used. Therefore, we selected 3 mL as the optimal elution volume to provide a complete removal of all of the basic compounds from the sorbent and because higher elution volumes would lead to longer evaporation step. Similar results regarding the elution step were obtained for the sulphonated HEMA/DVB sorbent.

Finally, the evaluation of the washing step is crucial when SCX sorbents are used. The washing step needs to be selective in order to remove interferences (including acidic and neutral compounds), retained on the sorbent by reversed-phase interactions, while the basic compounds are still bound to the sorbent by cation-exchange interactions. Fig. 3 shows the recovery values (%) obtained in the elution step for a representative group of compounds when the washing step was included using different washing solvents and volumes. Two washing solvents were tested: MeOH and ACN (2 mL). No

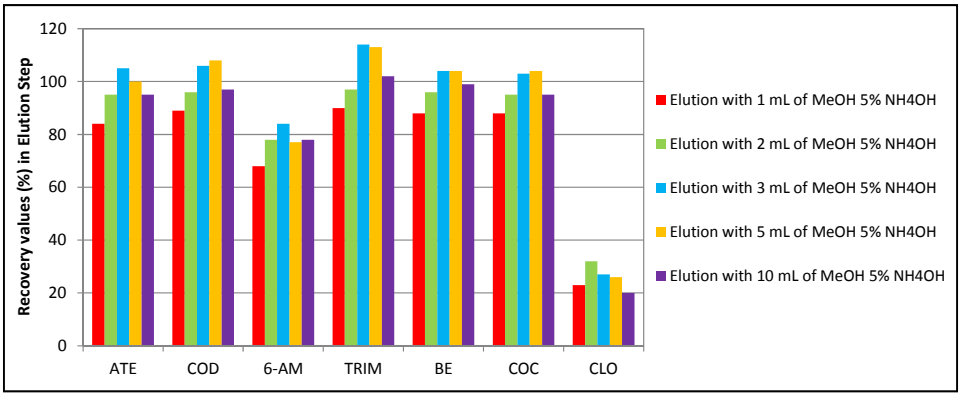


Fig. 2. Recovery values (%) obtained in the elution step for a representative group of compounds in ultrapure water after applying different elution volumes using the sorbent based on AMPSA/HEMA/PETRA.

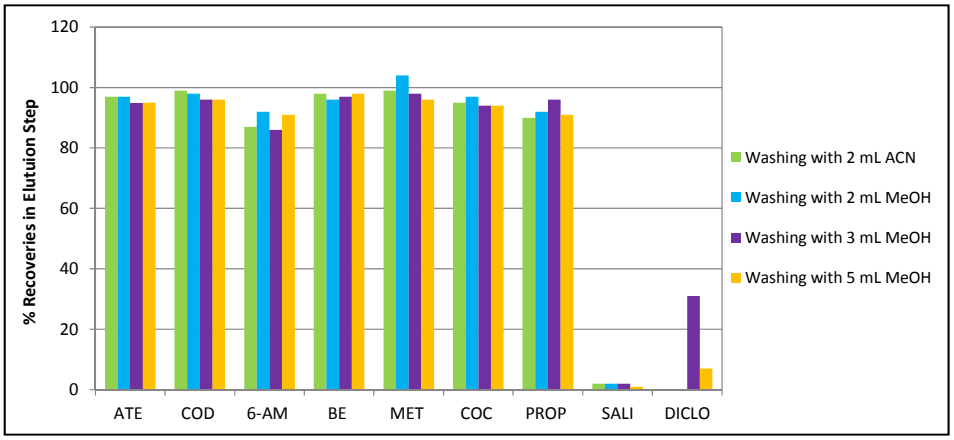


Fig. 3. Recovery values (%) obtained in the elution step for a representative group of compounds in ultrapure water after applying different washing solvents and volumes.

significant losses were observed during the washing step using either organic solvents. However, some compounds, such as MET or 6-AM, were less recovered after a washing step using ACN than using MeOH. Therefore, MeOH was chosen as the best washing solvent. With respect to the volume of MeOH used, 2, 3 and 5 mL were evaluated. Since no important losses were observed when higher washing solvent volumes were applied, 5 mL of MeOH was considered to be the best volume for the washing step. It should be mentioned that, in this step, acidic compounds were entirely removed from both sorbents since they were simply retained through reversed-phase interactions.

Under the optimised SPE protocol with a sample volume of 10 mL, the recovery values of the basic compounds were between 66% and 97% using the AMPSA/HEMA/PETRA (20/60/20) sorbent, and between 90% and 111% using the sulphonated HEMA/DVB (50/50). When the sample volume was increased from 10 mL to 500 mL, the recovery values decreased slightly in both sorbents (10% to 20% less), especially in the case of RANI and BE, with recoveries between 45% and 60%.

3.4. Application to environmental samples

Once the applicability of the two SCX materials as SPE sorbents was demonstrated for the extraction of pharmaceuticals and illicit drugs in ultrapure water, obtaining promising results, the two extracting materials were also applied to wastewater samples to evaluate the effectiveness of the washing step in removing interferences while retaining the basic compounds. Therefore, the ion suppression/enhancement effect and recovery values were evaluated in effluent and influent wastewaters, percolating 50 mL and 25 mL of sample, respectively, through both cartridges. Lower sample volumes were used than in ultrapure water due to the complexity of the matrices. In addition, these real sample volumes are commonly usual in the literature [16, 29].

In this study, special attention was paid to the sample extraction procedure in order to gain sensitivity through a suitable preconcentration step and, at the same time, to reduce matrix interferences as much as possible and lower the ion suppression/enhancement effect. Fig. 4 shows the results obtained after evaluating this effect, which was calculated as the percentage decrease in the signal response of the analytes spiked at 100 µg L^{-1} after the SPE extraction versus the intensity of the same concentration of the analyte in ultrapure water. In general, less ion suppression/enhancement was observed when the wastewater samples were loaded through the AMPSA/HEMA/PETRA (20/60/20) sorbent rather than the sulphonated HEMA/DVB (50/50), with low values ranging from -12% to 21% being recorded in both cases. Moreover, when a commercial SCX sorbent was used under the same SPE conditions (Oasis MCX), the ion suppression/enhancement values were similar to the values obtained using the in-house sorbents. It should be mentioned that, using Oasis MCX, MOR presented a higher ion suppression (17 and 20% in both effluent and influent waste-waters), while this sorbent was suitable to reduce the ion suppression/enhancement for EDDP and META (from -1 to 3%). Therefore, using a simple washing step, it was possible to obtain low values of ion suppression/enhancement rather than using the isotope labelled internal standard, which is one of the most common approaches for compensating this effect. For instance, Bijlsma *et al.* [16] found similar values of ion suppression/enhancement (between 20% and -15%) when 50 mL of surface waters (a cleaner matrix than waste-

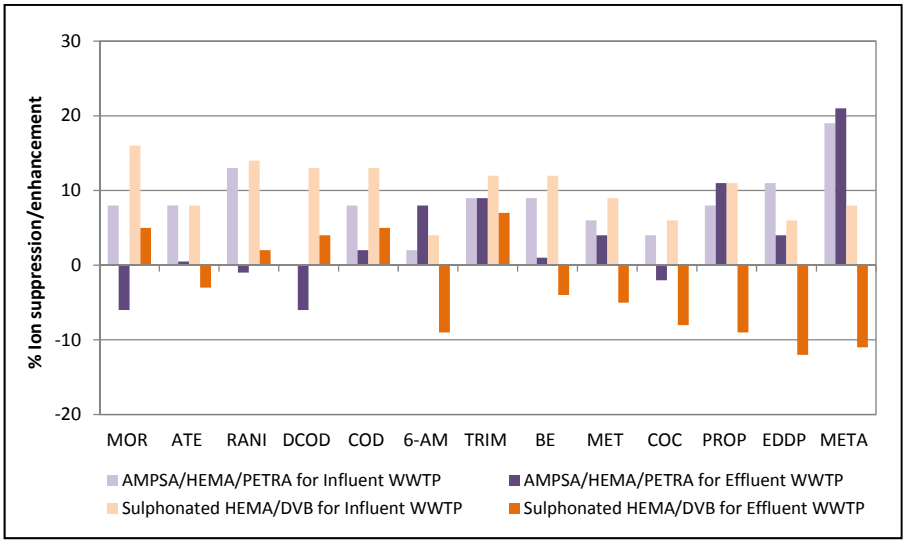


Fig. 4. Ion suppression/enhancement (%) for basic compounds when in 25 mL of influent WWTP and 50 mL of effluent WWTP were extracted using the AMPSA/HEMA/PETRA (20/60/20) and the sulphonated HEMA/DVB (50/50) sorbents.

waters) were loaded through an Oasis MCX cartridge, even when internal standards were added. Because of the low ion suppression/enhancement effects provided by the two SCX polymers, the recovery values for all of the basic compounds in the wastewater samples were then evaluated.

With respect to recovery values in wastewater samples, Table 3 shows that the results obtained using the AMPSA/HEMA/PETRA (20/60/20) sorbent were much lower than in ultrapure water, especially in the case of ATE, RANI, DCOD, BE and MET, with recovery values ranging from 31% to 46% when 25 mL of influent wastewater sample was loaded. Moreover, after percolating 50 mL of effluent wastewater sample, the recovery values were still lower than in influent wastewaters, despite being a less complicated matrix. This fact could be attributed to the higher sample volume (50 mL of effluent wastewaters) loaded through the cartridge and, as a result, a higher amount of interferences present in the matrix, which compete with the analytes to bind to the sorbent. Moreover, the low specific surface area of this polymer (< 5 m^2 g^{-1}) could strongly influence on the retention of the analytes, reducing its binding capacity. It should be pointed out that the hydroxyl and ester functionalities present in the structure of the AMPSA/HEMA/PETRA (20/60/20) sorbent might not be strong enough to keep the analytes bound to the sorbent in the presence of matrix components after loading wastewater samples. In the case of the sulphonated HEMA/DVB (50/50) sorbent, the recovery values obtained in both effluent and influent wastewaters (70-97%)

Table 3. Recovery values (%) of the studied compounds when ultrapure water, effluent and influent WWTP samples were loaded onto the AMPSA/HEMA/PETRA (20/60/20) and the sulphonated HEMA/DVB (50/50) sorbents.

Analytes	Recovery values (%)					
	AMPSA/HEMA/PETRA (20/60/20)			Sulphonated HEMA/DVB (50/50)		
	50 mL of ultrapure water [a]	50 mL of effluent WWTP [a]	25 mL of influent WWTP [b]	50 mL of ultrapure water [a]	50 mL of effluent WWTP [a]	25 mL of influent WWTP [b]
MOR	83	38	44	108	70	73
ATE	85	19	31	112	89	83
RANI	77	19	46	72	39	24
DCOD	87	21	35	113	84	84
COD	93	31	51	111	81	80
6-AM	84	38	75	93	89	79
TRIM	94	67	81	113	87	85
BE	59	5	31	86	76	89
MET	91	27	42	108	97	97
COC	92	57	92	102	98	93
PROP	88	106	107	93	95	86
EDDP	81	68	71	66	43	41
META	89	89	104	82	85	79

[a] 50 mL of ultrapure water/effluent wastewater spiked with the analytes' mixture at 0.5 μg L^{-1}.
[b] 25 mL of influent wastewater spiked with the analytes' mixture at 1 μg L^{-1}.
% RSDs (*n*=3) were lower than 16%.

were similar to those in ultrapure water, except for RANI and EDDP, ranging from 24% to 43%, due to its higher specific surface area and IEC value. The low retention of RANI on the sorbent might be attributed to its high polarity, while the poor recovery values for EDDP might be explained by the lack of polar functionalities in its structure. Finally, in order to obtain a high enough preconcentration factor and acceptable recovery values, 50 mL and 25 mL were the selected sample volumes of effluent and influent wastewaters, respectively.

Although in-house SCX sorbents allowed the ion suppression/enhancement to be overcome when effluent and influent wastewaters were applied, the AMPSA/HEMA/PETRA (20/60/20) sorbent failed to keep retaining the studied analytes when wastewater samples were loaded. The SPE performance of the two in-house sorbents was also compared with Oasis MCX sorbent in terms of recovery values. Most of the compounds were highly recovered from both effluent and influent wastewaters (>70%) using the commercial sorbent. However, Oasis MCX failed to recover BE, whose recovery values were between 23-32% in wastewater samples, while the in-house sulphonated HEMA/DVB (50/50) sorbent was able to extract this compound perfectly (76-89%). Therefore, since the sulphonated HEMA/DVB (50/50) provided better SPE performance in wastewater samples due to its good physical and chemical properties rather than the AMPSA/HEMA/PETRA (20/60/20) sorbent, the former was chosen as the SCX sorbent for the validation of the proposed analytical method. Moreover, it should be pointed out that, from the best of our knowledge to date, both AMPSA/HEMA/PETRA and sulphonated HEMA/DVB polymers have been the only in-house SCX materials synthesised and applied as SPE sorbents in environmental waters.

3.5. Method validation

Prior to the application of the sulphonated HEMA/DVB (50/50) sorbent for the determination of pharmaceuticals and illicit drugs in environmental waters by LC-MS/MS, the whole analytical procedure was validated for 50 mL of effluent and 25 mL of influent wastewaters by a matrix-matched calibration procedure. Linear range, method detection limits (MDLs), method quantification limits (MQLs), repeatability and reproducibility between days were evaluated for each type of matrix. Firstly, a blank sample of effluent and influent wastewaters was analysed and most of the basic compounds were detected at low levels of ng L^{-1}. Thus, the calibration curves were performed by subtracting their signals present in the blank. In both matrices, MDLs and MQLs were estimated as three or ten times the standard deviation of the analyte signal in the blank (n=5), respectively. In the case of effluent wastewaters, all of the basic compounds displayed good linearity in a range between 30 and 5000 ng L^{-1} with regression coefficients (r^2) greater than 0.998. The MDLs in effluent wastewaters ranged from 5 to 10 ng L^{-1}, except for TRIM and COC (2 ng L^{-1}), and the MQLs in this matrix were between 10 and 30 ng L^{-1} for all of the compounds. When influent wastewaters were analysed, satisfactory linearity was obtained for all of the compounds,

ranging from 60 to 8000 ng L^{-1}, except for RANI (200-8000 ng L^{-1}), with regression coefficients ($r^2 > 0.995$). The MDLs and MQLs in influent wastewaters ranged from 10 to 20 ng L^{-1} and from 40 to 60 ng L^{-1}, respectively, with the exception of RANI (MDL = 40 ng L^{-1} and MQL = 200 ng L^{-1}). The repeatability and reproducibility between days of five samples spiked at 1000 ng L^{-1} in both types of matrix, expressed as % relative standard deviation (%RSD), were lower than 16% and 23%, respectively. To sum up, the validation data obtained by the proposed method provided promising results in terms of the determination of the studied analytes at low levels of ng L^{-1} in wastewater samples.

3.6. Analysis of environmental water samples

The developed method for the determination of pharmaceuticals and illicit drugs from environmental water samples was applied to monitor their fate in effluent and influent wastewaters from a WWTP and, to this end, several wastewater samples were collected on different weekdays and at weekends. The presence of the analytes found was confirmed according to Commission Decision 2002/657/EC [30].

In the case of untreated waters, both pharmaceuticals and illicit drugs were detected in influent wastewaters, except for the metabolite of morphine, 6-AM, which was not detected in some samples. As shown in Table 4, higher concentrations were found for pharmaceuticals than for illicit drugs, since their consumption is higher and more frequent. For pharmaceuticals, ATE and RANI, an agent for treating hypertension and a stomach protector, respecttively, showed the highest levels with concentrations ranging from 333 to 3293 ng L^{-1}, while COC and its metabolite, BE, presented the highest concentrations (163-1294 ng L^{-1}) among

Table 4. Concentrations found of analytes in different effluent and influent wastewater samples analysed by SCX-SPE/LC-MS/MS.

	Concentrations (ng L^{-1})	
Analytes	**Influent WWTP**	**Effluent WWTP**
MOR	58-309	n.d.
ATE	333-1269	620-843
RANI	1028-3293	<MQL-865
DCOD	n.d.	43-91
COD	<MQL-474	491-748
6-AM	n.d.-715	n.d.
TRIM	116-1857	<MQL-103
BE	334-1294	135-4003
MET	97-155	113-166
COC	163-500	<MQL-2925
PROP	49-100	73-112
EDDP	<MQL	<MQL-97
META	54-74	12-81

n.d., non detected %RSD (n = 6) < 22%.

the illicit drugs studied. In contrast, the least detected illicit drugs were DCOD and EDDP with values below their MQLs. The high concentration found of BE might be attributed that this compound is the major metabolite of COC excreted from human body, matching the findings reported by other authors [16, 31].

Regarding effluent wastewaters, the concentrations found in this matrix were generally lower than in influent wastewaters, except for COD, BE, COC and EDDP. A possible explanation may be that, after a secondary treatment, a possible conversion of their conjugated metabolites to the original substances took place after the treatment processes, as well as the sampling period which was not the same [32]. The secondary treatment was completely effective for MOR, 6-AM and RANI, the concentrations of which decreased significantly after this treatment. However, BE, COC and ATE were still the most abundantly found compounds in effluent wastewaters. The range of concentrations of pharmaceuticals and illicit drugs found in wastewater samples is in line with several studies reported previously [33, 34].

4. Conclusions

Two novel materials with SCX behaviour were successfully synthesised by two different synthetic strategies: copolymerisation between three monomers (AMPSA/HEMA/PETRA) and the post-modification with H_2SO_4 of a copolymer (HEMA/DVB). The characterisation of the synthesised materials allowed the confirmation of the SCX behaviour with the presence of the sulphonic group.

These materials were applied as sorbents for the SPE of pharmaceuticals and illicit drugs from wastewater samples. Following optimisation of the SPE protocol, both SCX sorbents provided low ion suppression/enhancement values when effluent and influent wastewaters were analysed. Moreover, the sulphonated HEMA/DVB sorbent allowed better recovery values in wastewater samples than the AMPSA/HEMA/PETRA sorbent, due to its ability to establish ionic, hydrophilic and hydrophobic interactions with the analytes.

Therefore, the sulphonated HEMA/DVB sorbent was selected for further method validation and quantification of the analytes in effluent and influent wastewaters. Low MDLs were obtained by the developed analytical method ranging from 2 to 40 ng L^{-1} in both type of wastewaters. Most of the pharmaceuticals and illicit drugs were detected in all wastewater samples and the highest concentrations were attributed to BE (334-1294 ng L^{-1}), ATE (333-1269 ng L^{-1}) and RANI (1028-3293 ng L^{-1}).

Acknowledgements

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

References

[1] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Analytical methods for personal-care products in environmental waters, TrAC, Trends Anal. Chem. 30 (2011) 749-760.

[2] N. Fontanals, P.A.G. Cormack, R.M. Marcé, F. Borrull, Mixed-mode ion-exchange polymeric sorbents: dual-phase materials that improve selectivity and capacity, TrAC, Trends Anal. Chem. 29 (2010) 765-779.

[3] N. Masqué, R.M. Marcé, F. Borrull, New polymeric and other types of sorbents for solid-phase extraction of polar organic micropollutants from environmental water, TrAC, Trends Anal. Chem. 17 (1998) 384-394.

[4] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, Presence of pharmaceuticals and hormones in waters from sewage treatment plants, Water Air Soil Pollut. 217 (2011) 267-281.

[5] P. Vázquez-Roig, V. Andreu, C. Blasco, Y. Picó, SPE and LC-MS/MS determination of 14 illicit drugs in surface waters from the Natural Park of L'Albufera (ValSncia, Spain), Anal. Bioanal. Chem. 397 (2010) 2851-2864.

[6] E.Y. Ordóñez, J.B. Quintana, R. Rodil, R. Cela, Determination of artificial sweeteners in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1256 (2012) 197-205.

[7] N.A. Stoilova, A.R. Surleva, G. Stoev, Simultaneous determination of nine quinolones in food by liquid chromatography with fluorescence detection, Food Anal. Meth. 6 (2013) 803-813.

[8] G. Font, A. Juan-García, Y. Picó, Pressurized liquid extraction combined with capillary electrophoresis-mass spectrometry as an improved methodology for the determination of sulfonamide residues in meat, J. Chromatogr. A 1159 (2007) 233-241.

[9] T. Ogawa, H. Hattori, R. Kaneko, K. Ito, M. Iwai, Y. Mizutani, T. Arinobu, A. Ishii, H. Seno, High-throughput and simultaneous analysis of eight central-acting muscle relaxants in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry in the positive and negative ionization modes, Anal. Bioanal. Chem. 400 (2011) 1959-1965.

[10] A. Gheorghe, A. van Nuijs, B. Pecceu, L. Bervoets, P.G. Jorens, R. Blust, H. Neels, A. Covaci, Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry, Anal. Bioanal. Chem. 391 (2008) 1309-1319.

[11] J. Haginaka, Molecularly imprinted polymers as affinity-based separation media for sample preparation, J. Sep. Sci. 32 (2009) 1548-1565.

[12] P.A.G. Cormack, A.Z. Elorza, Molecularly imprinted polymers: synthesis and characterisation, J. Chromatogr. B 804 (2004) 173-182.

[13] W.W. Buchberger, Current approaches to trace analysis of pharmaceuticals and personal care products in the environment, J. Chromatogr. A 1218 (2011) 603-618.

[14] I. Tarcomnicu, A.L.N. van Nuijs, W. Simons, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent waste-water by reversed-phase liquid chromatography coupled to tandem mass spectrometry, Talanta 83 (2010) 795-803.

[15] H. Zheng, L.G. Deng, X.A. Lu, S.C. Zhao, C.Y. Guo, J.S. Mao, Y.T. Wang, G.S. Yang, H.Y. Aboul-Enein, UPLC-ESI-MS-MS determination of three beta(2)-agonists in pork, Chromatographia 72 (2010) 79-84.

[16] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater, J. Chromatogr. A 1216 (2009) 3078-3089.

[17] O. Quintela, E. Lendoiro, A. Cruz, A. de Castro, A. Quevedo, C. Jurado, M. López-Rivadulla, Hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) determination of cocaine and its metabolites benzoylecgonine, ecgonine methyl ester, and cocaethylene in hair samples, Anal. Bioanal. Chem. 396 (2010) 1703-1712.

[18] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C. Sherrington, R.M. Marcé, Hypercrosslinked strong anion-exchange resin for extraction of acidic pharmaceuticals from environmental water, J. Sep. Sci. 35 (2012) 2621-2628.

[19] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, Hypercross-linked polymer microspheres with weak anion-exchange character Preparation of the microspheres and their applications in pH-tuneable, selective extractions of analytes from complex environmental samples, J. Chromatogr. A 1215 (2008) 21-29.

[20] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N. Fontanals, Synthesis and application of hypercross-linked polymers with weak cation-exchange character for the selective extraction of basic pharmaceuticals from complex environmental water samples, J. Chromatogr. A 1217 (2010) 1575-1582.

[21] P.A.G. Cormack, A. Davies, N. Fontanals, Synthesis and characterization of microporous polymer microspheres with strong cation-exchange character, React. Funct. Polym. 72 (2012) 939-946.

[22] Y. Xu, L.G. Chen, H. Wang, X.P. Zhang, Q.L. Zeng, H.Y. Xu, L. Sun, Q. Zhao, L. Ding, Preparation of magnetic strong cation exchange resin for the extraction of melamine from egg samples followed by liquid chromategraphy-tandem mass spectrome-

try, Anal. Chim. Acta 661 (2010) 35-41.

[23] N. Gilart, R.M. Marcé, P.A.G. Cormack, N. Fontanals, F. Borrull, Synthesis and characterisation of new polar monolithic coatings for stir bar sorptive extraction, Talanta (*Submitted*) (2014).

[24] A. Beltran, F. Borrull, P.A.G. Cormack, R.M. Marcé, Molecularly-imprinted polymers: useful sorbents for selective extractions, TrAC, Trends Anal. Chem. 29 (2010) 1363-1375.

[25] M.Y. Ding, Z.H. Wang, R. Zheng, Rapid separation of proteins by capillary HPLC on a short polymethacrylate-based strong cation-exchange monolithic column, Chin. J. Chem. 28 (2010) 567-572.

[26] X. Chen, H.D. Tolley, M.L. Lee, Polymeric strong cation-exchange monolithic column for capillary liquid chromatography of peptides and proteins, J. Sep. Sci. 32 (2009) 2565-2573.

[27] B. Gu, Y. Li, M.L. Lee, Polymer monoliths with low Hydrophobicity for strong cation-exchange capillary liquid chromatography of peptides and proteins, Anal. Chem. 79 (2007) 5848-5855.

[28] P. Akkaramongkolporn, T. Ngawhirunpat, P. Opanasopit, Preparation and evaluation of differently sulfonated styrene-divinylbenzene cross-linked copolymer cationic exchange resins as novel carriers for drug delivery, AAPS PharmSciTech 10 (2009) 641-648.

[29] A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Analysis of drugs of abuse in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 395 (2009) 819-828.

[30] E.C.D. 2002/657/EC, European Commission Decision 2002/657/EC, Off. J. Eur. Commun. 221 (2002) 8-36.

[31] I. González-Mariño, J.B. Quintana, I. Rodríguez, M. González-Díez, R. Cela, Screening and selective quantification of illicit drugs in wastewater by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry, Anal. Chem. 84 (2012) 1708-1717.

[32] T. Henriksen, R.K. Juhler, B. Svensmark, N.B. Cech, The relative influences of acidity and polarity on responsiveness of small organic molecules to analysis with negative ion electrospray ionization mass spectrometry (ESI-MS), J. Am. Soc. Mass. Spectrom. 16 (2005) 446-455.

[33] K.H. Langford, M. Reid, K.V. Thomas, Multi-residue screening of prioritised human pharmaceuticals, illicit drugs and bactericides in sediments and sludge, J. Environ. Monit. 13 (2011) 2284-2291.

[34] M. Gros, M. Petrović, D. Barceló, Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters, Talanta 70 (2006) 678-690.

3.1.4. Discussion of results

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

The results of the experimental research included in this section have been discussed individually in the previously cited scientific papers. However, in this section, the most relevant aspects of these will be presented. As can be seen from these studies, the selectivity towards a specific group of compounds with acidic or basic functionalities was successfully achieved using selective sorbents for solid-phase extraction (SPE), such as a commercial molecularly imprinted polymer (MIP) and two in-house strong cation-exchange (SCX) sorbents.

In these studies, special attention was paid to the effectiveness of including a washing step to remove interferences and, as a result, reduce the matrix effect (ME) (ion suppression or enhancement) as much as possible. As observed in the results, both the commercial MIP (Affinilute MIP-NSAIDs from Biotage) and the in-house SCX polymeric materials (the 2-acrylamido-2-methylpropane sulphonic acid (AMPSA)/2-hydroxyethyl methacrylate (HEMA)/pentaerythritol triacrylate (PETRA) and sulphonated HEMA/divinylbenzene (DVB) polymers) worked satisfactorily as SPE sorbents since they were able to retain the analytes of interest completely, as well as cleaning up the matrix without analyte losses and removing many interfering compounds. For instance, the first study presented the great performance of the molecularly imprinted solid-phase extraction (MISPE) of five acidic pharmaceuticals from effluent wastewater samples. The MISPE results were compared to the results obtained with other commercial SPE sorbents, such as the hydrophilic copolymeric sorbent Oasis HLB, and two mixed-mode sorbents, Oasis WAX and Oasis MAX, with weak anion-exchange (WAX) and strong anion-exchange (SAX) properties, respectively. The most important feature was that the MISPE extracts were much cleaner after percolating 250 mL of effluent wastewater than those obtained by the other sorbents. As a consequence, when the analysis was performed by liquid chromatography-ultraviolet-visible detection (LC-UV), the MISPE chromatograms were practically free from interferences, showing the best SPE performance. With respect to both in-house SCX sorbents, they were also capable of extracting the basic analytes selectively (pharmaceuticals and illicit drugs) and providing highly clean extracts after loading 25 or 50 mL of wastewater samples. It should be noted that the clean-up of the samples using the MIP sorbent was more evident and effective than the washing step of the SCX SPE protocol, as 250 mL of effluent wastewater samples were percolated and many interferences were present in the sample.

The selectivity offered by the MIP and SCX sorbents is due to their ability to establish specific interactions with the analytes of interest. While the MIP presented specific cavities with high affinities towards a group of pharmaceuticals belonging to the group of non-steroidal and anti-inflammatory drugs (NSAIDs), the SCX sorbents with sulphonic

(SO_3^-) groups in their structure interacted specifically with the basic analytes through ion-exchange interactions. Thanks to the specific interactions of these types of sorbents, the clean-up step could be performed using organic solvents, disrupting the non-specific interferences and eluting the interfering compounds. Thus, the washing solvent employed in the MISPE and SCX SPE protocols was 5 mL of acetonitrile/water (40/60) and 5 mL of pure methanol, respectively. A relevant fact was observed when both in-house SCX sorbents were applied in effluent wastewater samples. The AMPSA/HEMA/PETRA polymer was used in real samples, which underwent a significant decrease in recovery values when 50 and 25 mL of effluent and influent wastewaters were loaded, respectively. Its failure was attributed to the low specific surface area and the presence of many interfering compounds that compete with the analytes for retention onto the active sites of this polymer. In contrast, the sulphonated HEMA/DVB sorbent successfully retained all of the basic compounds when real samples were analysed, due to its numerous sulphonic groups and its higher specific surface area. Therefore, the sulphonated HEMA/DVB was selected for the method validation. Under the optimised SPE conditions for the MIP and SCX SPE sorbents, the recovery values of the studied analytes for 250 and 50 mL of effluent wastewater samples were satisfactory, with values ranging from 45% to 85% and from 39% to 97%, respectively. Although both sorbents provided good results in terms of recovery values, it should be noted that the enrichment factor achieved using the MISPE protocol was five times higher than using the SCX SPE protocol, resulting in lower method detection limits (MDLs, 0.5-2 ng L^{-1} using the MISPE and 2-10 ng L^{-1} using the SCX SPE for effluent wastewaters).

As mentioned previously, including a clean-up step in the SPE protocol is essential for eliminating interferences, particularly when working with electrospray ionisation (ESI) in liquid chromatography-tandem mass spectrometry (LC-MS/MS). In these studies, LC-(ESI)MS/MS was the instrumental technique of choice and, thus, the ME was studied and it was checked whether the sample preparation proposed was effective enough to reduce this undesirable effect. From the results included in these studies, it can be concluded that both MIP and SCX SPE sorbents helped to remove many interfering compounds, but they were not able to eliminate the ME at all. The type of solvent used in the clean-up step plays an important role in the ME, but the sample volume is also essential, since it determines the amount of interferences that can be retained onto the sorbent. As expected, with its ability to preconcentrate 250 mL of wastewater sample, the MIP led to higher ion suppression/enhancement (from -15% to 75%), even when the clean-up step allowed a great level of removal of interferences. Currently, in the literature, another study reports the use of a commercial MIP specifically for NSAIDs (SupelMIP NSAIDs from Supelco) for the extraction of a number of pharmaceuticals,

including ibuprofen, diclofenac, naproxen and clofibric acid, from sewage water samples [1]. The analytical method developed in this study also showed the great SPE performance of this MIP in terms of ME. However, it is important to note that the sample volume percolated in this study was only 25 mL, which resulted in less matrix interferences. With respect to SCX SPE sorbents, they provided less ion suppression/enhancement (from -12% to 21%) for the same type of wastewater because the clean-up step was performed with a pure organic solvent and the sample volume was lower (25 or 50 mL, depending on the type of wastewater). In this study, the sample volume was lowered, in comparison to the MISPE study, because it was conducive to a lower ME, easier sample handling and a less time-consuming process, while maintaining an acceptable enrichment factor. In order to compensate the ion suppression/enhancement, the most suitable option was the use of matrix-matched calibration for the validation of MISPE and SCX SPE and LC-MS/MS methods.

With regard to the SCX study, the two in-house SCX polymers were synthesised using traditional polymerisation, in which the final product was a monolithic material. The most outstanding feature is that these in-house SCX sorbents are the first in-house polymeric materials with polar and ion-exchange functionalities. Thus, the AMPSA and the sulphonated DVB monomers contained the SO_3^- group, while the HEMA and PETRA monomers offered polarity to the whole polymeric material due to the hydroxyl and ester functional groups. This characteristic enhances the retention of both polar and basic organic contaminants, with this being the main difference from the previous in-house hypercrosslinked mixed-mode sorbents synthesised by our research group, which were based on a hypercrosslinked vinylbenzyl chloride (VBC)-DVB network [2-5]. However, these mixed-mode hypercrosslinked polymers had microporous structures with much higher specific surface areas (1000-1470 $m^2 g^{-1}$) than the in-house polar monolithic polymers (<5-150 $m^2 g^{-1}$), resulting in lower binding capacities. In addition, the types of interactions that the in-house SCX monolithic sorbents established with the analytes were different. While the AMPSA/HEMA/PETRA polymer was able to interact through hydrogen bonding, the sulphonated HEMA/DVB could establish both hydrogen bonding and π-π interactions, with the latter being essential, as the analytes' structures contained aromatic rings. Once the syntheses were optimised, these SCX polymers were applied as SPE sorbents in ultrapure water and wastewater samples. Recently, our research group has published the synthesis of novel hypercrosslinked microporous polymeric microspheres with SCX character, showing excellent values for specific surface area (1070-1370 $m^2 g^{-1}$) and IECs (1.7-2.8 mmol g^{-1}) [6]. Currently, these novel materials are being tested as SPE sorbents for a further application in environmental analysis but they have not been published yet. Another recent study has reported the synthesis of magnetic SCX resins, using magnetite (Fe_3O_4) as the core, poly(styrene-DVB)

(PS-DVB) as the polymer matrix and acetyl sulphonate as sulphonation agent for extracting melamine from egg samples [7]. In this study, the presence of the sulphonic group in the resin and the clean-up of the matrix led to the selective extraction of melamine with negligible ion suppression. Apart from the publications mentioned above, currently, there is no publication in which an SCX in-house resin is used in sample preparation. Moreover, with respect to the determination of pharmaceuticals and illicit drugs, the two in-house SCX polymeric sorbents developed in this Doctoral Thesis are the first to be applied for extracting these types of compounds from environmental waters. In the literature, most of the analytical studies, including the determination of these contaminants in the same matrices, have used the commercial SCX sorbent Oasis MCX [8,9] or the hydrophilic Oasis HLB [10,11].

The second study, included in this section, presented a novel approach, consisting of the direct coupling between the commercial MIP used previously as an SPE sorbent and MS/MS, without the need to include chromatographic separation. This study was the first to report the MISPE-MS/MS coupling for the selective extraction and rapid determination of six acidic pharmaceuticals (four NSAIDs and two lipid regulators) from wastewater samples. Due to the absence of chromatographic separation, all of the analytes, together with the interfering compounds of the matrix, entered the ionisation source at the same time. Therefore, lower sample volumes were selected (50 and 10 mL for effluent and influent wastewater samples, respectively). Moreover, special attention was paid to the carrier liquid that transports the analytes into the ionisation source, since it could enhance ionisation of the analytes. Despite the low sample volumes used and the best carrier liquid for enhancing the signal responses of the analytes, high values of ion suppression were obtained (up to 70%, except for clofibric acid and fenoprofen (84%)). These values were slightly higher than those reported in the first study, in which the MISPE extract was injected into the LC-MS/MS, thereby helping chromatographic separation to separate the analytes from the interferences. Another explanation for these results may be that not only did the interfering compounds lead to a decrease in the analyte signal, but also the analytes themselves may have caused ion suppression among them. To overcome this effect, a matrix-matched calibration curve with 50 mL of effluent wastewater samples was the preferred option. In this study, an internal standard (gemfibrozil-d_6) was included prior to the injection in order to correct possible variabilities in LC injection and ionisation. As expected, higher MDLs were obtained for the developed MISPE-MS/MS methodology, ranging from 0.05 to 0.10 μg L^{-1}. The developed analytical method enabled the quantification of the six acidic pharmaceuticals in effluent and influent wastewaters, with the highest concentrations being shown for gemfibrozil (<0.05-4.06 μg L^{-1}), naproxen (<0.05-6.40 μg L^{-1}) and ibuprofen (0.54-20.17 μg L^{-1}), with the latter being tentatively quantified, as only a

multiple reaction monitoring (MRM) transition was obtained. In the literature, there are only three studies reporting the direct coupling of a MIP as SPE sorbent and MS for determining ciprofloxacin and phenothiazines in urine samples [12,13] and benzodiazepines in human plasma [14]. Although the proposed direct MISPE-MS/MS coupling could be applied for determining acidic pharmaceuticals in wastewater samples providing rapid and simple analytical performance, more improvements should be made in order to reduce the ME and obtain higher sensitivity. Therefore, to develop fast and simple analytical methodologies, the direct coupling between selective extraction and sensitive detection techniques may prove to be a good option in different research fields. In this way, the excellent performance of the in-house SCX sorbents for SPE in terms of recovery values and ME may be a promising choice for direct coupling with MS techniques.

From the results derived from these studies, it can be concluded that selectivity is becoming essential in the analytical chemistry and environmental research field. To this end, selectivity can be achieved thanks to powerful MS techniques, but also by developing selective and specific materials to be used in sample preparation in order to promote the extraction the analytes of interests, as well as reducing the undesirable ME. Both MIPs and mixed-mode polymers as SPE sorbents are good options for providing selectivity to the whole analytical method. Despite the fact that the mixed-mode sorbents are not as selective as MIPs, these sorbents have become the optimal choice when analytes with acidic or basic functional groups need to be extracted. The commercialisation of mixed-mode sorbents is more extensive than MIPs and many manufacturers and different functionalised sorbents are available. Therefore, it is important to pay attention to the numerous commercially available materials for different extraction techniques in order to obtain selective extractions free from interferences and facilitate chromatographic separation and MS detection.

References

[1] S. Zorita, B. Boyd, S. Jönsson, E. Yilmaz, C. Svensson, L. Mathiasson, S. Bergström, Anal. Chim. Acta 626 (2008) 147.

[2] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N. Fontanals, J. Chromatogr. A 1217 (2010) 1575.

[3] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, F. Borrull, J. Chromatogr. A 1217 (2010) 2855.

[4] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. A 1215 (2008) 21.

[5] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C. Sherrington, R.M. Marcé, J. Sep. Sci. 35 (2012) 2621.

[6] P.A.G. Cormack, A. Davies, N. Fontanals, React. Funct. Polym. 72 (2012) 939.
[7] Y. Xu, L.G. Chen, H. Wang, X.P. Zhang, Q.L. Zeng, H.Y. Xu, L. Sun, Q. Zhao, L. Ding, Anal. Chim. Acta 661 (2010) 35.
[8] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 34 (2011) 1091.
[9] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, J. Chromatogr. A 1216 (2009) 3078.
[10] M.R. Boleda, M. Huerta-Fontela, F. Ventura, M.T. Galceran, Chemosphere 84 (2011) 1601.
[11] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Anal. Chem. 79 (2007) 3821.
[12] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, J. Sep. Sci. 29 (2006) 1230.
[13] E.C. Figueiredo, G.B. Sanvido, M.A.Z. Arruda, M.N. Eberlin, Analyst 135 (2010) 726.
[14] E.C. Figueiredo, R. Sparrapan, G.B. Sanvido, M.G. Santos, M.A.Z. Arruda, M.N. Eberlin, Analyst 136 (2011) 3753.

3.2. Determination of pharmaceuticals and personal care products by stir bar sorptive extraction followed by liquid chromatography-tandem mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

So far in this Doctoral Thesis, the development and evaluation of novel and selective materials for solid-phase extraction (SPE) have been presented for extracting pharmaceuticals and illicit drugs from environmental waters. Although SPE is considered to be the extraction technique of choice when liquid samples are analysed, solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) have gained popularity due to their ability to extract volatile and non-volatile compounds, as well as the possibility of desorbing the analytes without the consumption of organic solvents, meaning that they are environmentally-friendly extraction techniques [1,2]. However, the main drawback of these techniques has been the lack of commercial extracting phases, especially for SBSE, in which polydimethylsiloxane (PDMS) was the only commercial extracting phase for this technique until few years ago. This PDMS SBSE coating is commercialised by Gerstel, under the name of Twister. The availability of the only apolar PDMS coating for SBSE has resulted in the extraction of mainly apolar compounds (e.g. polycyclic aromatic hydrocarbons (PAHs), pesticides, volatile organic compounds (VOCs), among others) from different environmental, food and biological matrices [2,3].

In particular, in environmental analysis, the control of polar compounds is becoming a scientific concern due to their tendency to persist in the aquatic environment, causing adverse effects to animal and human health. For these reasons, in the last few years, interest has grown in developing novel commercial and in-house extracting phases for SBSE with a more polar character. In 2011, Gerstel introduced two new commercial extracting phases for SBSE: EG Silicone Twister and Acrylate Twister, with the latter about to be marketed. The polarity of these commercial coatings was achieved thanks to the poly(ethyleneglycol) (PEG)-modified silicone of EG Silicone Twister and the polyacrylate (PA) and PEG of the Acrylate Twister, as shown Figure 1. Since their introduction to the market, a few applications have been reported, which have been included in the review paper discussed in the Section 1.2.2.1. The first study presented in the present Section includes the evaluation of these two new commercial coatings for SBSE (EG Silicone Twister and Acrylate Twister) and their comparison to the classic PDMS-coated stir bar (Twister) for extracting a group of pharmaceuticals and personal care products (PPCPs) from environmental waters. All parameters affecting the SBSE extraction of the studied compounds were optimised. Due to the polarity and low volatility of the analysed compounds, the analytes were further desorbed by liquid desorption and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Since the publication of the review paper on SBSE, other studies have reported the use of the EG Silicone Twister. For instance, it has been used for extracting chlorophenols and chloroanisoles from wine samples [4], bisphenols from PCPs [5] and

odour compounds from water samples [6], all of which were analysed by SBSE (thermal desorption (TD)) followed by gas chromatography-mass spectrometry (GC-MS).

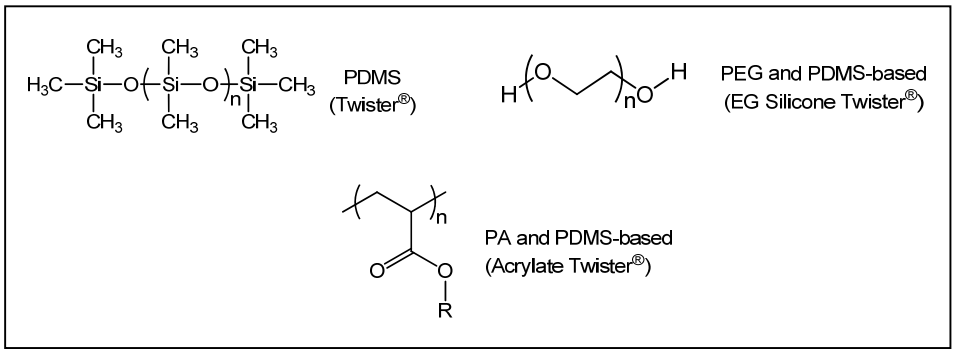


Figure 1. Structures of the monomers on which the commercial coated stir bars are based.

Prior to the emergence of these two new commercial phases for SBSE, different synthetic approaches had been proposed to obtain more polar SBSE coatings and tackle the principal limitation of SBSE, such as sol-gel technology, monolithic materials and polyurethane foams. In Section 1.2.2.1, these strategies to synthesise novel SBSE coatings are extensively discussed in the review included. Of these, many examples of SBSE coatings obtained by sol-gel technology can be found in the literature. This strategy enables the control of the morphology and structure of the coating during synthesis and provides high mechanical and chemical stability, thanks to the chemical attachment of the coating to the glass bar. With the monolithic approach, the presence of PDMS is completely eliminated, using suitable monomers (functional monomer and crosslinker), depending on the analytes to be extracted. This strategy has been widely used for its simplicity and the availability of a large variety of monomers with different functionalities. The monolithic coating can be attached chemically (as in sol-gel technology) or physically to the glass bar. In the former case, the chemical attachment of the monolithic coating has been extensively exploited by Huang and co-workers, showing great mechanical stability. Some of the functional monomers used to provide polarity are vinylimidazole (VI), vinylpyrrolidone (VPD), vinylpyridine (VP) or vinylphthalimide (VPH), among others [7-10]. Meanwhile, our research group has developed novel polar coatings for SBSE through the physical attachment of a monolithic coating to a glass bar, providing mechanical resistance, using a spring that covers the glass bar. Thus, two polar monolithic coatings were synthesised, based on poly(VPD-*co*-DVB) and poly(methacrylic acid (MAA)-*co*-DVB) for the SBSE of group of PPCPs from environmental waters, analysed by LC-MS/MS [11,12]. Both monolithic coatings provided successful SBSE performance in comparison to the commercial

PDMS-coated stir bar, with it being capable of extracting even the more polar compounds with acceptable recovery values.

Along the same lines, our research group continued working on the development of more polar coatings for SBSE and, as a result, improving the extraction of polar contaminants from environmental waters. Taking into account the promising SPE performance of the polar mixed-mode in-house sorbents for extracting both polar and basic compounds due to the use of polar monomers, such as 2-hydroxyethyl methacrylate (HEMA) and pentaerythritol triacrylate (PETRA), these monomers, together with divinylbenzene (DVB) and poly(poly(ethylene glycol) methacrylate (PEGMA), have been used for synthesising three novel polar coatings for SBSE. Thus, this section includes a study in which the synthesis and characterisation of three polar monolithic coatings were developed for further SBSE application. The in-house coatings were based on poly(HEMA-*co*-DVB), poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA). The polarity offered by these new polar in-house coatings for SBSE is shown in Figure 2, in which the structures of the monomers used are detailed. The optimisation of the most relevant factors affecting the mechanical stability of the final coating were evaluated and discussed, such as the composition of the functional monomers and crosslinkers. Subsequently, all three in-house monolithic coatings were evaluated for the SBSE of a group of PPCPs.

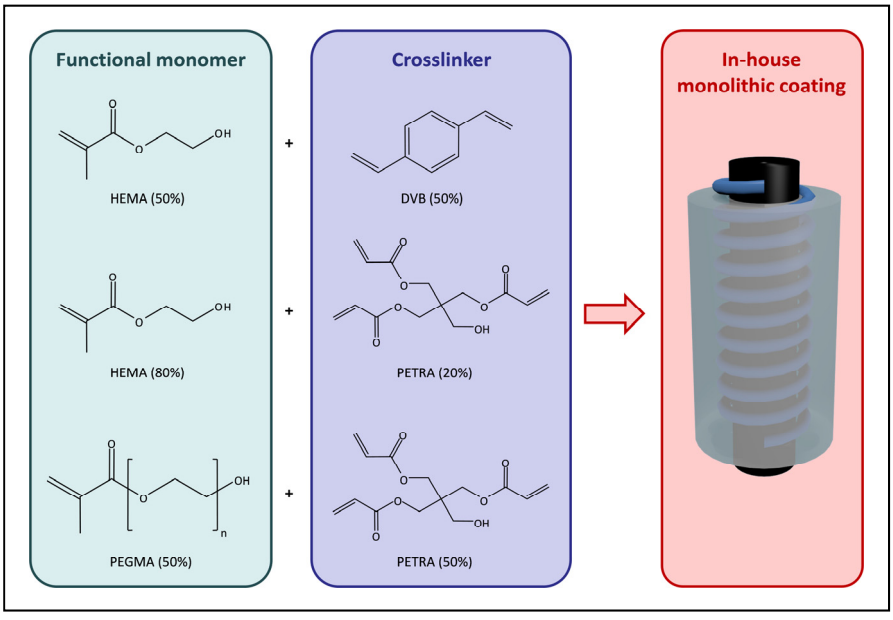


Figure 2. Structures of the functional monomers and crosslinkers used for the synthesis of in-house monolithic SBSE coatings.

From the results obtained in the previous study, the development and application of an analytical method using the best monolithic coating for SBSE followed by LC-MS/MS was performed for extracting and determining PPCPs in environmental water samples. In both analytical studies, the same group of compounds were selected in order to compare the SBSE performance provided by the commercial and in-house coatings.

The results obtained from the first study, in which novel commercial coatings for SBSE were presented, were published in the *Analitica Chimica Acta* 774 (2013) 51-60. Moreover, the following two articles include the results from the synthesis of several in-house SBSE coatings, which have been submitted for publication in the *Talanta*, and the results from the analytical application of the in-house monolithic coating for SBSE, which are published in *Journal of Chromatography A* 1295 (2013) 42-47.

References

[1] L. Ramos, J. Chromatogr. A 1221 (2012) 84.
[2] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642.
[3] M. Kawaguchi, R. Ito, H. Nakazawa, A. Takatsu, in J. Pawliszyn (Editor), Comprehensive Sampling and Sample Preparation, Elsevier Inc., USA, 2012, p. 797.
[4] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, Talanta 118 (2014) 30.
[5] J.I. Cacho, N. Campillo, P. Viñas, M. Hernández-Córdoba, J. Pharm. Biomed. Anal. 78-79 (2013) 255.
[6] N. Ochiai, K. Sasamoto, T. Ieda, F. David, P. Sandra, J. Chromatogr. A 1315 (2013) 70.
[7] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1216 (2009) 4354.
[8] X.J. Huang, N.N. Qiu, D.X. Yuan, J. Sep. Sci. 32 (2009) 1407.
[9] X.J. Huang, J.B. Lin, D.X. Yuan, R.Z. Hu, J. Chromatogr. A 1216 (2009) 3508.
[10] X. Huang, L. Chen, D. Yuan, Anal. Bioanal. Chem. 405 (2013) 6885.
[11] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, Anal. Chim. Acta 706 (2011) 135.
[12] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, J. Chromatogr. A 1225 (2012) 1.

3.2.1. Novel coatings for stir bar sorptive extraction to determine pharmaceuticals and personal care products in environmental waters by liquid chromatography and tandem mass spectrometry

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

NOVEL COATINGS FOR STIR BAR SORPTIVE EXTRACTION TO DETERMINE PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN ENVIRONMENTAL WATERS BY LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

Núria Gilart, Núria Miralles, Rosa Maria Marcé, Francesc Borrull, Núria Fontanals

Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo s/n, 4.007 Tarragona, Spain

Abstract

Two new commercially available polar coatings for stir bar sorptive extraction (SBSE), consisting of polyacrylate (PA) with a proportion of polyethyleneglycol (PEG) (Acrylate Twister®) and PEG modified silicone (EG Silicone Twister®), were evaluated and compared with the classic coating based on polydimethylsiloxane (PDMS Twister®) for the extraction of a group of pharmaceuticals and personal care products (PPCPs) from wastewater samples.

The SBSE parameters, such as sample pH, agitation speed, extraction temperature, extraction time, desorption solvent and time, were optimised in order to achieve suitable sorption of the target analytes. The EG Silicone coating enabled more efficient extraction of some polar compounds as well as improving the sorption of apolar compounds, in comparison with the other two coatings.

Finally, the method of SBSE followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using the EG Silicone coating was validated achieving good linearity (r^2>0.994, except for CBZ (r^2>0.989)), precision (%RSD < 17%) and low limits of quantification (LOQs) (20-40 ng L^{-1}). The SBSE/LC-MS/MS methodology was applied for the determination of PPCPs in wastewater samples.

Keywords: *commercial polar coatings; stir bar sorptive extraction; tandem mass spectrometry; pharmaceuticals and personal care products; wastewaters*

1. INTRODUCTION

Over recent decades, many efforts have been made in order to simplify sample enrichment techniques, minimise the use of organic solvents and achieve sensitive and cost-effective analytical methods [1-3]. To this end, extraction techniques, such as solid-phase extraction (SPE), stir bar sorptive extraction (SBSE) or solid-phase microextraction (SPME), have been widely applied in environmental applications over the last few years [4]. Even though SPE is the most commonly used sample preparation technique for liquid samples

[5], SBSE and SPME are considered as good alternatives to SPE due to their simple, clean and environmentally friendly procedures [6,7].

SBSE, first introduced by Baltussen *et al.* in 1999 [8], consists of the use of a magnetic stirring bar coated with a small amount of sorptive material immobilised on it. During the extraction, the analytes are retained onto the coating depending on the sample pH, the agitation speed and the ionic strength, among other factors. They can then be desorbed by thermal desorption (TD) or liquid desorption (LD) [2,4,9]. When SBSE is combined with gas chromatography (GC), TD is the most common technique since the stir bar is introduced into a TD unit and the analytes are directly desorbed to the column for further analysis. Although TD provides high sensitivity, it requires the use of a TD unit and its availability in the laboratory. By contrast, LD has been widely applied for the determination of labile and polar compounds followed by liquid chromatography (LC). LD is performed by the immersion of the stir bar in a small volume of an organic solvent. Thus, the choice of organic solvent, its volume and desorption time are important factors to bear in mind during SBSE optimisation [10,11].

Until recently, the only commercially available coating was based on polydimethylsiloxane (PDMS) and is known by the name PDMS Twister®. It has mostly been used in GC applications, and to a lesser extent LC, for the extraction of apolar or semi-polar analytes, such as pharmaceuticals [7,12], personal care products (PCPs) [13-16] and pesticides [17,18], in different matrices. However, the results with respect to the extraction of the most polar compounds were not satisfactory due to the apolar nature of the PDMS coating. Therefore, the challenge in SBSE has been the development of polar coatings in order to increase the versatility of this technique.

To this end, many researchers have focused their attention on the preparation of polar coatings using different strategies, such as monolithic synthesis based on poly(N-vinylpyrrolidone-co-divinylbenzene) [19] or poly(vinylpyridine-ethylene dimethacrylate) [20]; sol-gel technology based on PDMS and polypyrrole [21] or PDMS and poly(vinylalcohol) [22]; or solvent polymerisation based on poly(methyl methacrylate–ethylene glycol dimethacrylate–acrylic acid) [23]. Moreover, other authors have also reported a new format of stir bar consisted of a PDMS tube packed with different types of carbon combining both sorption and adsorption principles in order to improve the PDMS performance [24]. Simplicity of preparation, high permeability, high capacity and mechanical stability were the main advantages of these novel in-house extracting phases in comparison to the classic PDMS [4,25,26].

Very recently, two new polar coatings for SBSE have been trademarked by Gerstel which are based on polyacrylate (PA) with a proportion of poly(ethylene) glycol (PEG), known as Acrylate Twister®, and PEG modified silicone, known as EG Silicone Twister®. To date, only two studies have reported the use of these new coatings to determine benzothiazole in wastewater samples [27] and volatile organic com-

pounds (VOCs) in peppermint essential oil, perfume and coffee samples [28].

To the best of our knowledge, this is the first time that these two new commercial polar coatings for SBSE (Acrylate Twister® and EG Silicone Twister®) have been evaluated and compared with the PDMS coating for the extraction of pharmaceuticals and personal care products (PPCPs). Furthermore, the best coating performance was used to determine the studied compounds in environmental waters by SBSE/LC-MS/MS.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Paracetamol (PARA), caffeine (CAFF), antipyrine (APy), benzotriazole (BZT), propranolol hydrochloride (PROP), pridinol methanesulfonate salt (PRID), methylparaben (MPB), carbamazepine (CBZ), propylparaben (PrPB), 2,4-dihydroxybenzophenone (DHB), benzylparaben (BzPB), 2,2-dihydroxy-4-methoxybenzophenone (DHMB), diclofenac (DICLO), benzophenone-3 (BP-3), triclocarban (TCC) and triclosan (TCS) were purchased from Sigma-Aldrich (Steinheim, Germany). All PPCPs standards used were of high purity grade (>97%).

Stock solutions of individual standards were prepared by dissolving each compound in methanol (MeOH) at a concentration of 1000 mg L^{-1}. A mixture of all compounds in MeOH at a concentration of 10 mg L^{-1} was prepared weekly. Working solutions were prepared daily by diluting the previous solution with water. These solutions were stored at 4 ºC. The structures and log $K_{O/W}$ values of these analytes are presented in Table 1.

Dichloromethane (DCM) for pesticide residue analysis, HPLC grade MeOH and acetonitrile (ACN) were supplied by Prolabo (Llinars del Vallès, Spain). Formic acid (HCOOH) (≥95%) from Sigma-Aldrich (Steinheim, Germany) and sodium hydroxide (NaOH) (≥98%) from Prolabo (Llinars del Vallès, Spain) were used to adjust the pH of the mobile phase and the sample. Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain).

2.2. Sample collection

Wastewater samples were collected from the influent, secondary effluent and tertiary effluent of a domestic sewage treatment plant (STP), which is located in a city with a population of 22,000 inhabitants, by using pre-cleaned amber glass bottles. The tertiary treatment was performed by reverse osmosis. All the samples were filtered using a 0.45 µm nylon membrane (Supelco, Bellefonte, PA, USA), acidified to pH 5 (HCOOH) and stored at 4 ºC until analysis.

2.3. LC-(ESI)MS/MS analysis

The chromatographic experiments were performed with an Agilent 1200 series LC, equipped with an automatic injector (volume injected was 50 µL), a degasser, a quaternary pump and a column oven, and a 6410 series triple quadrupole mass spectrometer using an electrospray ionisation (ESI) interface from Agilent Technologies (Waldbronn, Germany).

Table 1. Structures and log $K_{o/w}$ of the studied analytes and LC-(ESI)MS/MS conditions for the analysis of PPCPs by MRM mode.

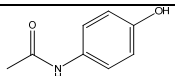
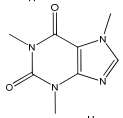
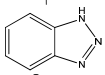
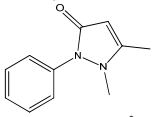
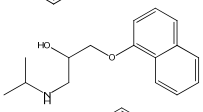
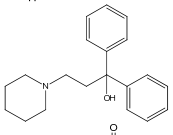
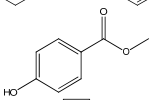
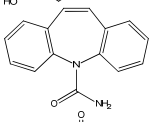
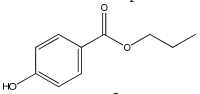
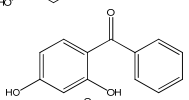
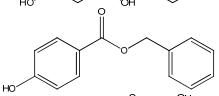
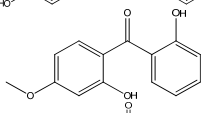
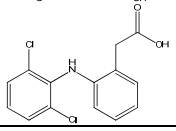
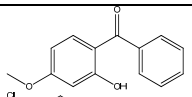
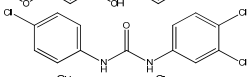
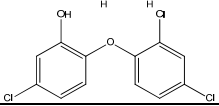
Analyte	Structure	Log $K_{O/W}$ [a)]	t_R (min)	MRM trans.	Ion. mode	Cone volt. (V)	Colli. ener.(V)
PARA		0.5	4.5	**152>110** 152>93	+	100	15 25
CAFF		-0.6	5.6	**195>138** 195>110	+	125	15 25
BZT		0.4	7.3	**120>65** 120>92	+	100	25 15
APy		1.4	7.9	**189>145** 189>115	+	100	30 30
PROP		2.9	9.1	**260>116** 260>183	+	125	15 15
PRID		3.4	9.5	**296>115** 296>193	+	125	30 30
MPB		1.9	10.8	**151>92** 151>136	-	80	15 5
CBZ		1.9	12.9	**237>193** 237>179	+	150	35 35
PrPB		2.9	15.3	**179>92** 179>136	-	100	15 5
DHB		3.2	16.2	**213>135** 213>169	-	130	15 5
BzPB		3.6	17.6	**227>92** 227>136	-	100	10 20
DHMB		4.3	18.7	**243>93** 243>123	-	80	15 15
DICLO		4.5	21.3	**294>250** 294>214	-	75	5 15

Table 1. Continued.

Analyte	Structure	Log $K_{O/W}$ [a)]	t_R (min)	MRM trans.	Ion. mode	Cone volt. (V)	Colli. ener.(V)
BP-3		4.0	23.6	**229>151** 229>105	+	130	15 15
TCC		6.1	25.2	**313>160** 313>126	-	130	5 15
TCS		5.3	25.6	**287>35** 289>35	-	18 18	8 8

[a)]Log $K_{o/w}$ calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2012 ACD/Labs). The quantification MRM transitions are shown in bold.

The analytical column was a Kromasil 100 C_{18} (150 x 4.6 mm i.d.) with 5 µm particle size from Teknokroma (Barcelona, Spain) and the mobile phase was ACN and ultrapure water adjusted to pH 3 with HCOOH. The flow rate was 0.6 mL min^{-1} and the temperature of the column oven was set at 45 ºC. The gradient applied was as follows: 15% to 55% ACN in 12 min (held for 5 min), then to 95% ACN in 8 min and kept constant for 3 min and then decreased back to the initial conditions in 2 min. Chromatographic separation was achieved in 30 min.

The (ESI)MS/MS parameters were optimised by direct injection of each analyte. N_2 was used as the collision gas and its flow rate was set at 12 L min^{-1}. A source temperature of 350 ºC, a nebuliser pressure of 45 psi (N_2) and a capillary potential of 4000 V were applied. The analyses were performed in multiple reaction monitoring (MRM) mode. The MRM transitions, the cone voltage, the collision energy as well as the ionisation mode are summarised in Table 1.

2.4. Stir bar sorptive extraction

Stir bars for sorptive extraction were obtained from Gerstel (Mulheim and der Ruhr, Germany). They consisted of a 10 mm long glass-encapsulated magnetic stir bar with three different external coatings: 24 µL of PDMS (PDMS Twister®, 0.5 mm thickness), 32 µL of EG Silicone (EG Silicone Twister®) and 25 µL of PA (Acrylate Twister®).

A similar SBSE procedure was optimised and applied to each stir bar. The stir bar was inserted into a flask with 50 mL of sample adjusted to pH 5. Samples were stirred for 4 hours at room temperature (25 ºC). After extraction, the stir bar was removed from the sample using magnetic tweezers and dried with a lint-free tissue. For LD, the stir bar was introduced into a 2 mL vial containing 1 mL of MeOH and placed in an ultrasonic bath for 15 min (except for the PA coating, which had a desorption time of 60 min). Subsequently, the extracts were diluted in ultrapure water (1:1, v/v) and analysed in LC-(ESI)MS/MS. As the supplier recommended, the stir bar was cleaned with

1 mL MeOH/DCM (1:1, v/v) after use for 30 min in the ultrasonic bath, dried with a lint-free tissue and kept in a vial for the next analysis. Each stir bar was reused at least 40-50 times for environmental samples.

3. RESULTS AND DISCUSSION

3.1. LC-(ESI)-MS/MS conditions

Under optimised chromatographic separation, MS/MS parameters were evaluated by injecting each compound at 250 μg L^{-1} individually in flow injection analysis (FIA). All of the analytes studied were divided individually or in groups into six windows depending on their ESI ionisation (positive or negative) related to their acidic or basic properties. Moreover, two MRM transitions were selected for each analyte for their identification and confirmation. Optimised cone voltage and collision energy for each MRM transition are summarised in Table 1.
All selected compounds showed good linearity (r^2>0.997) by direct injection with a linear range of 0.5-200 μg L^{-1}, except for PARA, APy, DHMB and TCS (2-200 μg L^{-1}) and for PRID (25-200 μg L^{-1}). The limits of detection (LODs), calculated as signal-to-noise ratio (S/N) of 3, ranged from 0.15 to 0.5 μg L^{-1}, except for PRID (5 μg L^{-1}).

3.2. Optimisation of the SBSE procedure

In order to improve the extraction of polar compounds, which had been limited by the PDMS coating, two new commercial coatings for SBSE have been studied and compared with it. These novel coatings were expected to display polar behaviour due to their chemical structures, which are composed of PA and PEG, as stated in the information provided by the manufacturer. Therefore, a group of PPCPs with a wide range of polarities were selected to evaluate these three SBSE coatings. It was expected that the polar functionalities present in these new coatings would extract polar and semi-polar compounds more effectively than the PDMS coating, which has an apolar structure.
Both the extraction and desorption steps were then optimised. It should be pointed out that, as the target analytes were afterwards separated by LC, the desorption step was performed by LD mode.

3.2.1. Extraction conditions

During the optimisation of the extraction conditions, several parameters were evaluated such as agitation speed, extraction temperature, sample pH and extraction time. Starting with the agitation speed, this parameter may influence the mass transfer of the analytes towards the coating. However, several studies have demonstrated that the differences observed stirring at different agitation speeds were negligible [29,30]. Finally, it was decided to set the stirring rate at 1000 rpm in order to maintain the integrity of the stir bar.
Another variable in SBSE is the extraction temperature. The extraction equilibrium is achieved faster at elevated temperatures, but the $K_{O/W}$ partition coefficient of the analytes and, thus, the extraction efficiencies are lower [4]. Moreover, the lifetime of a PDMS coating can be decreased by working at

temperatures higher than 40 ºC [31]. Therefore, all of the SBSE experiments were performed at room temperature.

Thus, the following parameters were optimised under these experimental conditions: 50 mL ultrapure water spiked at 4 μg L^{-1} with the analyte mixture stirring at 1000 rpm for 1 hour at room temperature, and the LD was performed using 1 mL MeOH placed in an ultrasonic bath for 15 min.

With respect to sample pH, the analyte retention can be influenced by this factor. This effect was investigated at different pH values (3, 5, 7 and 9). In the case of the PDMS coating, there were no significant differences between the different pH values. However, it was observed that all those analytes with log $K_{O/W}$ below 3.5 were not retained onto the PDMS coating due its hydrophilicity (see Table 1 for log $K_{O/W}$ information). In contrast, the PA and EG Silicone coatings displayed the same profile and the analyte response increased from pH 3 to 5, while no differences were observed between pH 5 and 7. However, adjusting the pH to 9, the signal of some analytes decreased, such as DHB, DHMB and DICLO. Therefore, as the pH did not have a significant influence in terms of extraction efficiency, it was decided to set the sample pH at 5 for all of the stir bars because, as a compromise, high signal responses for all of the compounds were obtained.

The extraction time is also a very important parameter and it was studied from 1 to 5 h for each stir bar. Fig. 1 shows the extraction time for a representative group of analytes. For the PDMS-coated stir bar, no relevant differences were observed through the extraction time, except in the case of BP-3 which displayed an increased response when the extraction time was 3 h, after which point it decreased. In the case of the PA coating, the signal of the target analytes was constant at longer extraction times, apart from TCC which reached equilibrium in 4 h. Finally, when the EG Silicone coating was used, BzPB, BP-3, TCC and TCS showed their highest response at 5 h. In line with previous studies [14,19,32], 4 h was selected as the extraction time for further studies as a compromise between the analysis time and the extraction efficiencies.

3.2.2. Liquid desorption conditions

Once the extraction conditions had been optimised, the desorption solvent and time were studied. It should be mentioned that the LD was performed by sonication because it accelerates an efficient desorption of the analytes from the stir bar [17]. Under the optimised extraction conditions (50 mL of ultrapure water at pH 5 spiked at 4 μg L^{-1} with the analyte mixture being stirred for 4 h at 1000 rpm), MeOH and ACN were tested as the desorption solvent for all three stir bars, For the LD, 1 mL of organic solvent was placed in an ultrasonic bath for 15 min. It was observed that MeOH showed a slightly higher signal response for the target analytes than ACN. Therefore, MeOH was chosen as the desorption solvent for each stir bar. As an example, Fig. 2 shows the signal response of a representative group of analytes when they were desorbed using MeOH or ACN with the PA coating. As can be seen, in the case of this coating, significant differences were observed and higher peak areas were obtained

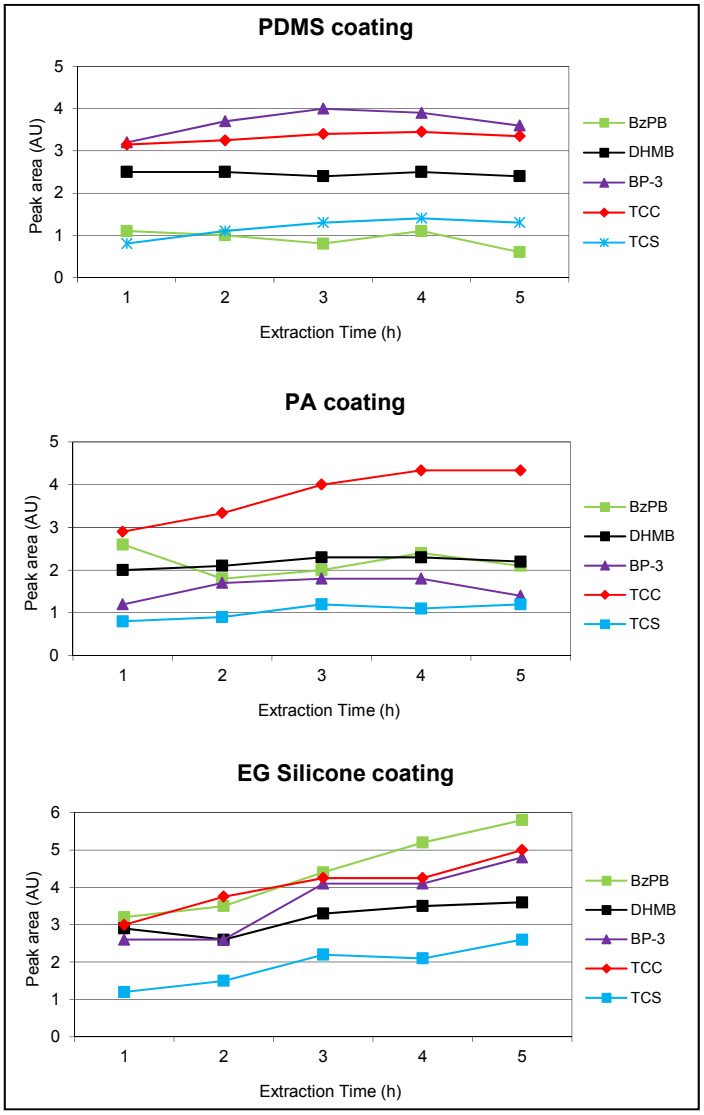


Fig. 1. The effect of the extraction time on the analyte response for a representative group of compounds depending on the stir bar.

for all of these analytes using MeOH as desorption solvent rather than ACN.

In the next step, the desorption time was evaluated (15 and 60 min) using MeOH as the desorption solvent. For almost all of the analytes, the desorption slightly increased from 15 to 60 min with the PDMS and EG Silicone coatings. However, the PA coating required nearly 60 min in order to desorb the same amount of analytes as the other stir bars. This fact could be explained because the carboxyl group in the PA coating might establish stron-

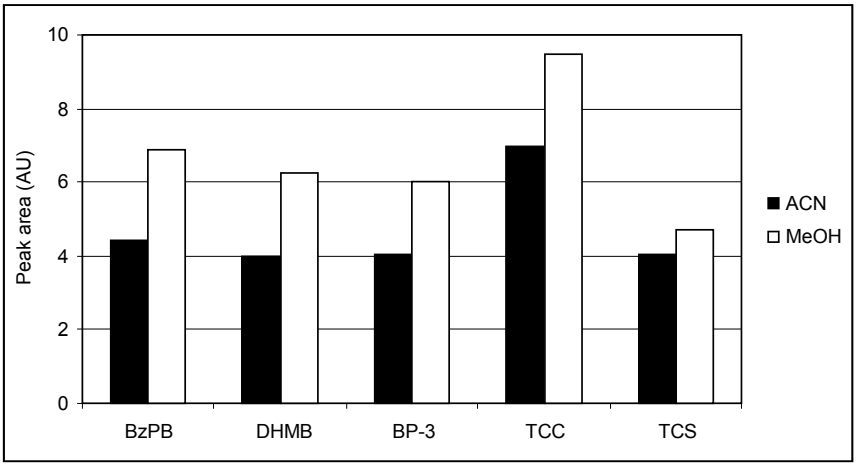


Fig. 2. The effect of the desorption solvent on the analyte response for a representative group of compounds using the PA coating.

ger interaction with those analytes with basic functionalities and so a longer desorption time was necessary. Therefore, 15 min was chosen as the optimal desorption time with all coatings, except for the PA coating, which had a desorption time of 60 min. Finally, the MeOH volume was evaluated, testing 1, 5 and 10 mL. It was observed that increasing the desorption solvent volume did not improve the analyte response significantly and 1 mL of MeOH was selected as the optimal volume for the desorption process, as it was enough volume to cover the stir bar.

To summarise, the optimal conditions for SBSE for the PDMS and EG Silicone coatings were as follows: 50 mL of sample at pH 5 extracted at room temperature by agitating at 1000 rpm for 4 h of extraction time; and 1 mL of MeOH in an ultrasonic bath for 15 min for the LD. Meanwhile, the same SBSE conditions were used for the PA coating, except for the desorption time which was increased to 60 min. The recovery values (%) in ultrapure water for each stir bar under the optimal conditions for SBSE are detailed in Table 2. All three coatings still seemed to be limited with respect to the extraction of polar compounds, taking into account that both PA and EG Silicone stir bars are PDMS-based. Nevertheless, there was a great improvement of extraction efficiencies for apolar compounds when the EG Silicone coating was used. For example, DHB and BzPB were extracted with recovery values of about 1% with the classical PDMS-coated stir bar, while their recoveries increased to 24 and 39%, respectively, with the new commercial EG Silicone coating, thanks to their polar functionalities. In addition, although TCC has a very high log $K_{o/w}$ (6.1), it was better recovered using the EG Silicone and PA stir bars rather than using the PDMS one. This fact could be explained by the interactions through hydrogen bonds between the diamide group present in TCC structure and the coatings. However, the PA-coated stir

Table 2. Recovery values (%) obtained when 50 mL of ultrapure water spiked at 4 μg L^{-1} with the analyte mixture were extracted with PDMS, PA and EG Silicone coatings.

Analyte	Recovery values (%)		
	PDMS coating	PA coating	EG Silicone coating
PARA	-	<1	-
CAFF	<1	-	-
BZT	<1	<1	<1
APy	-	1	<1
PROP	<1	2	2
PRID	2	2	3
MPB	<1	2	1
CBZ	<1	<1	<1
PrPB	<1	2	10
DHB	<1	9	24
BzPB	1	14	39
DHMB	8	9	26
DICLO	<1	<1	<1
BP-3	34	10	45
TCC	16	43	59
TCS	40	42	80

% RSD (n=3) were lower than 15% for %R > 16%.

bar was not as promising as the EG Silicone coating for extracting either polar or apolar compounds, since its recovery values were close to those obtained with the PDMS coating. It is important to point out that during the whole SBSE/LC-MS/MS method, no losses of the target analytes were observed.

3.3. Method Validation

Once these commercial polar coatings had been evaluated for the extraction of PPCPs from ultrapure water, their applicability was demonstrated in environmental waters, such as influent and secondary effluent samples from a wastewater treatment plant (WWTP). Since the most polar compounds were hardly recovered in ultrapure water using all three stir bars, only a screening method could be developed. However, the evaluation of the ion suppression/enhancement effect and the following method validation were only developed for those analytes which were better recovered by SBSE, such as PrPB, DHB, BzPB, DHMB, BP-3, TCC and TCS.

Ion suppression/enhancement is a common issue in LC-MS/MS with an ESI interface and it can cause a variation of the analyte response during the quantification of real samples. This effect has been evaluated for influent and secondary effluent WWTP samples using all three of the stir bars for SBSE. Table 3 shows the ion suppression/enhancement values (%) obtained only for those compounds which were extracted with %R ≥ 10%. This effect was

Table 3. Ion suppression/enhancement (%) when 50 mL of influent and secondary effluent WWTP were extracted with PDMS, PA and EG Silicone coatings.

Analyte	Ion suppression/enhancement (%)					
	PDMS coating		PA coating		EG Silicone coating	
	Influent	Secondary effluent	Influent	Secondary effluent	Influent	Secondary effluent
PrPB	44	0	8	0	17	1
DHB	-4	1	-3	-1	-4	-2
BzPB	-5	1	-3	-2	-5	-1
DHMB	-3	1	1	-1	1	-3
BP-3	25	11	17	4	17	12
TCC	23	9	9	54	9	32
TCS	-1	1	25	-9	25	16

% RSD (n=3) < 11%.

calculated as the percentage decrease in the signal obtained by the analyte spiked after the extraction at 50 μg L^{-1} versus the intensity of the same amount of analyte in a standard solution [33]. As expected, higher values of ion suppression/enhancement were obtained in influent rather than secondary effluent wastewaters with all three stir bars due to the complexity of the former. As for the PDMS coating, ion suppression/enhancement was not significant, with values ranging from -5% to 25%, except for PrPB in influent wastewaters (44%). However, the PA and EG Silicone coatings presented a similar profile for this effect (values from -4 to 25%), except in the case of TCC, which showed the highest ion suppression in these matrices (54 and 32%, respecttively). Bearing these results in mind, the EG Silicone coating provided the best SBSE performance in terms of recovery values and ion suppression/enhancement. Moreover, in order to provide an accurate quantification, a matrix-matched calibration was applied for the validation of SBSE/LC-MS/MS method using the EG Silicone coating for subsequent analyses of environmental samples.

Thereafter, the method was validated with 50 mL of secondary effluent wastewater for only those compounds which were recovered using the EG Silicone coating. First, a blank sample was analysed in order to subtract the possible signal of existing analytes (BzPB, DHB, PrPB and TCC appeared at low levels of concentration). PrPB, DHMB, BP-3, TCC and TCS showed good linearity in a range between 40 and 5000 ng L^{-1} and, BzPB and DHB between 20 and 5000 ng L^{-1} (r^2>0.994, except for CBZ (r^2>0.989)). The limits of quantification (LOQs) were calculated as the lowest point in the calibration curve and they ranged between 20 and 40 ng L^{-1} and the LODs between 5 and 10 ng L^{-1} for the abovementioned compounds. The repeatability and reproducibility between days of three samples spiked at 1000 ng L^{-1} , expressed as % relative standard deviation (% RSD), were lower than 10% and 17%, respectively. The analytical validation parameters of the proposed methodology using the EG

Silicone were in totally agreement with those from previous studies reported using the conventional PDMS-coated stir bar for the determination of PPCPs in environmental waters (LODs ranged from 2.5 to 10 ng L^{-1}) [14,15].

3.4. Analysis of real samples

The applicability of the SBSE/LC-MS/MS method was demonstrated by analysing different environmental samples. Table 4 shows the concentrations found in influent, secondary effluent and tertiary effluent WWTP samples, collected in different seasons and analysed in triplicate. All those analytes extracted with the EG Silicone coating for SBSE were detected in influent and effluent wastewaters and some of them were also observed in tertiary effluent wastewaters. The presence of these compounds was confirmed according to Commission Decision 2002/657/EC [34]. As an example, Fig. 3 shows MRM chromatograms obtained from an influent WWTP sample in which PrPB was found at high levels of ng L^{-1} (2360 ng L^{-1}). In addition, TCC and TCS were also detected at high concentrations in both influent and secondary effluent WWTP samples due

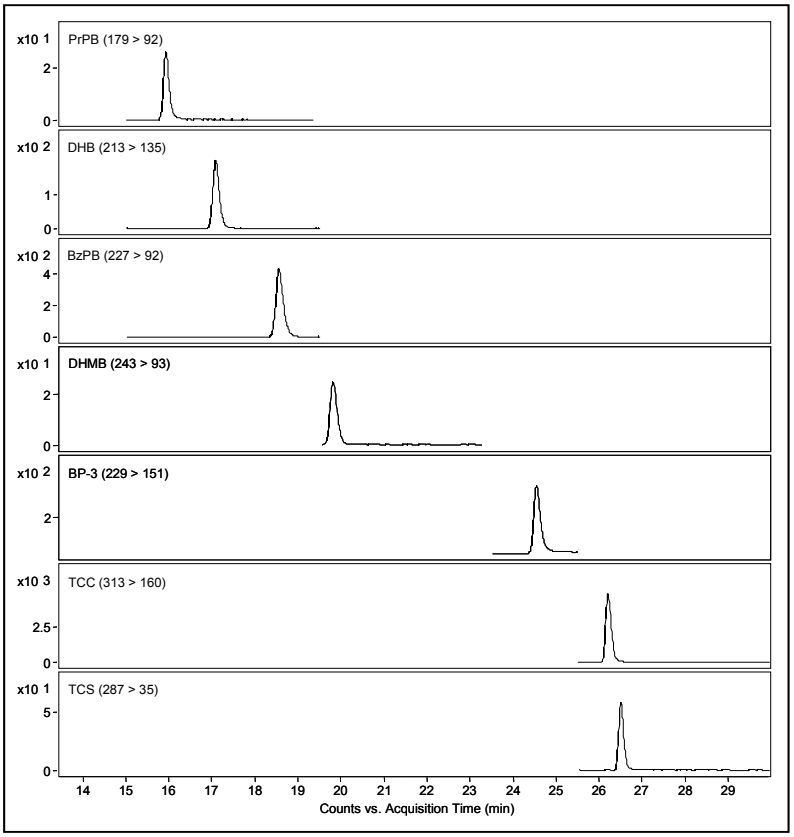


Fig. 3. MRM chromatograms obtained when analysing an influent WWTP sample by SBSE/LC-MS/MS.

Table 4. Concentrations of analytes found in influent, secondary effluent and tertiary effluent wastewater samples when they were analysed using SBSE(EG Silicone Twister®) followed by LC-MS/MS.

Analyte	Concentration found (ng L^{-1})		
	Influent	Secondary effluent	Tertiary effluent
PrPB	67 – 2360	<LOQ – 233	n.d.
DHB	118 – 767	77 – 573	20
BzPB	<LOQ – 34	58 – 681	n.d.
DHMB	<LOQ – 105	181 – 539	n.d.
BP-3	<LOQ – 328	<LOQ – 155	<LOQ
TCC	337 - 875	587 – 2086	<LOQ
TCS	<LOD - 143	100 – 1039	<LOQ

n.d. = non detected
% RSD (n=3) < 19%.

to their high usage. However, BP-3 was hardly detected, only in influent WWTP samples at low concentration.

Moreover, effluent wastewater samples from tertiary treatment, based on reverse osmosis, were analysed. Most of the analytes studied were almost completely removed, apart from DHB, BP-3, TCC and TCS, which were still detected at low levels (<LOQ - 20 ng L^{-1}). It should be mentioned that these results agreed with several studies which also reported the presence of these PPCPs in environmental samples [19,35,36].

4. CONCLUSIONS

EG Silicone coating has been demonstrated to provide the best SBSE performance for the extraction of a group of PPCPs from environmental samples, in comparison with PA and PDMS coatings.

In terms of recovery values, the EG Silicone-coated stir bar was able to improve the sorption of apolar compounds (24% to 80%), but it was still restricted in the case of more polar compounds (<1% to 3%). Moreover, the PA and PDMS coatings were only able to extract the most apolar analytes, with low recovery values (2% to 42%). Thus, the limitation of the classic PDMS coating for the extraction a group of PPCPs with different polarities has been improved with the EG Silicone-coated stir bar, especially for those semi-polar and apolar analytes. When these stir bars were applied to wastewater samples, no significant ion suppression/enhancement was observed for the majority of the compounds.

Finally, a SBSE/LC-MS/MS method validation was developed using the EG Silicone coating, achieving low LODs (5-10 ng L^{-1}). This method allowed the determination of some PPCPs in influent, secondary effluent and tertiary effluent wastewaters.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innova-

tion, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 40 (2006) 500-508.
[2] F. Sánchez-Rojas, C. Bosch-Ojeda, J. Cano-Pavón, Chromatographia 69 (2009) 79-94.
[3] J.M.F. Nogueira, Anal. Chim. Acta 757 (2012) 1-10.
[4] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642-2666.
[5] L. Ramos, J. Chromatogr. A 1221 (2012) 84-98.
[6] A. Juan-García, J. Mañes, G. Font, Y. Picó, J. Chromatogr. A 1050 (2004) 119-127.
[7] J. Martín, W. Buchberger, E. Alonso, M. Himmelsbach, I. Aparicio, Talanta 85 (2011) 607-615.
[8] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737-747.
[9] C.F. Duan, Z. Shen, D.P. Wu, Y.F. Guan, TrAC, Trends Anal. Chem. 30 (2011) 1568-1574.
[10] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54-69.
[11] P.L. Kole, G. Venkatesh, J. Kotecha, R. Sheshala, Biomed. Chromatogr. 25 (2010) 199-217.
[12] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, J. Chromatogr. A 1209 (2008) 10-16.
[13] M. Kawaguchi, R. Ito, H. Honda, N. Endo, N. Okanouchi, K. Saito, Y. Seto, H. Nakazawa, J. Chromatogr. A 1206 (2008) 196-199.
[14] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chim. 397 (2010) 2833-2839.
[15] D.R. Klein, D.F. Flannelly, M.M. Schultz, J. Chromatogr. A 1217 (2010) 1742-1747.
[16] N. Ramírez, F. Borrull, R.M. Marcé, J. Sep. Sci. 35 (2012) 580-588.
[17] A. Giordano, M. Fernández-Franzón, M.J. Ruiz, G. Font, Y. Picó, Anal. Bioanal. Chem. 393 (2009) 1733-1743.
[18] N. Ochiai, K. Sasamoto, H. Kanda, E. Pfannkoch, J. Chromatogr. A 1200 (2008) 72-79.
[19] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, Anal. Chim. Acta 706 (2011) 135-142.
[20] X.J. Huang, J.B. Lin, D.X. Yuan, R.Z. Hu, J. Chromatogr. A 1216 (2009) 3508-3511.
[21] L.R. Melo, A.M. Nogueira, F.M. Lancas, M.E.C. Queiroz, Anal. Chim. Acta 633 (2009) 57-64.
[22] C.H. Yu, B. Hu, J. Sep. Sci. 32 (2009) 147-153.
[23] Z. Talebpour, M. Safari, F. Molaabaci, A. Alizadeh, H.Y. Aboul-Enein, Chromatographia 72 (2010) 707-712.
[24] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, F. David, P. Sandra, J. Chromatogr. A 1094 (2005) 9-16.

[25] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1217 (2010) 2667-2673.
[26] X. Huang, N. Qiu, D. Yuan, B. Huang, Talanta 78 (2009) 101-106.
[27] E. Fries, Anal. Chim. Acta 689 (2011) 65-68.
[28] B. Sgorbini, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, M.R. Ruosi, C. Bicchi, J. Chromatogr. A 1265 (2012) 39-45.
[29] K. Wille, H.F. De Brabander, L. Vanhaecke, E. De Wulf, P. Van Caeter, C.R. Janssen, TrAC, Trends Anal. Chem. 35 (2012) 87-108.
[30] F.C.M. Portugal, M.L. Pinto, J.M.F. Nogueira, Talanta 77 (2008) 765-773.
[31] W.M. Liu, Y. Hu, J.H. Zhao, Y. Xu, Y.F. Guan, J. Chromatogr. A 1095 (2005) 1-7.
[32] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, J. Chromatogr. A 1225 (2012) 1-7.
[33] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019-3030.
[34] European Commission Decision 2002/657/EC, Off. J. Eur. Commun. 221 (2002) 8-36.
[35] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1216 (2009) 6994-7000.
[36] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958-2969.

3.2.2. Synthesis and characterisation of new polar monolithic coatings for stir bar sorptive extraction

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

SYNTHESIS AND CHARACTERISATION OF NEW POLAR MONOLITHIC COATINGS FOR STIR BAR SORPTIVE EXTRACTION

Núria Gilart[a], P.A.G. Cormack[b], Rosa Maria Marcé[a], Núria Fontanals[a,*], Francesc Borrull[a]

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

[b] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Abstract

Polar vinyl monomers have been used for the synthesis of several polymer monoliths, to serve as novel coatings for stir bar sorptive extraction (SBSE); the monovinyl monomers 2-hydroxyethyl methacrylate (HEMA) and poly(ethylene glycol) monomethacrylate) (PEGMA) were copolymerised with (apolar) divinylbenzene (DVB) and/or pentaerythritol triacrylate (PETRA), both of which are crosslinking agents. After the optimisation of the most important synthesis parameters, which included the ratio between total monomers and porogen, the nature of the porogen, and the monomer ratios, *inter alia*, three mechanically stable, polar monolithic coatings for SBSE were obtained which were based on poly(HEMA-*co*-DVB), poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA).
Thereafter, and in order to evaluate the hydrophilicity of the resulting monoliths, they were applied as sorbents in the SBSE of a group of emerging pollutants with a wide range of polarities. The results showed that both the poly(HEMA-*co*-DVB) and poly(PEGMA-*co*-PETRA) materials could be used to extract both polar and non-polar compounds by SBSE, in an effective manner. Taking into account the desired chemical and morphological properties, as well as the extraction efficiencies, the poly(PEGMA-*co*-PETRA) material seemed to be a particularly promising monolith for application as a novel coating in SBSE.

Keywords: *monolithic material; polar coating; traditional polymerisation; stir bar sorptive extraction*

1. INTRODUCTION

Over the last two to three decades, several significant advances in the polymer monolith field have been reported in terms of improvements to chemical properties, formats and applicability [1, 2]. The synthesis of polymer monoliths normally involves a polymerisation mixture comprising monomers, cross-

linkers, porogenic solvents and initiators, in an appropriate ratio. This mixture is placed into a mold, such as a capillary, syringe or high-performance liquid chromatography (HPLC) column, and then polymerised to deliver a monolith in the desired format [1, 3]. Such polymerisations normally yield monoliths with porous structures and available functional groups, depending on the type and composition of the monomers, the mode of initiation, the porogenic solvents and the polymerisation temperature [4, 5].

Monoliths have been exploited extensively as stationary phases for HPLC [6, 7] and capillary electrochromatography (CEC) [8, 9]. The advantages of monolithic separation media include their ease of preparation, their pH and pressure stability and their fast mass transport properties. As a result, these columns can enable high flow rates and rapid chromatographic separations with low backpressures [10, 11]. However, one potential drawback of typical monolithic materials is their higher than average mean pore size which can lead to low specific surface areas in the range of tens of $m^2\ g^{-1}$. This limitation has been tackled mainly by increasing the content of crosslinker in the polymerisation reaction [12, 13].

Polymer monoliths became even more popular when they were employed as new sample preparation materials for solid-phase extraction (SPE) [10], solid-phase microextraction (SPME) [14, 15] and stir bar sorptive extraction (SBSE) [16, 17]. Recently, many studies have focused on the development of new coatings for SBSE in order to improve the extraction of polar compounds [18]. Polydimethylsiloxane (PDMS) was the first commercially available extracting phase for SBSE, and has therefore been the most widely studied, however PDMS is a hydrophobic material thus modified versions of PDMS, in monolithic form mainly, have been developed and evaluated as promising sorptive materials for this sample preparation technique. In recent years, monoliths for SBSE have gained in popularity as novel sorbents with controllable functionality and porosity have become available through straight-forward synthetic protocols [1, 18]. For instance, monolithic materials with ion-exchange interactions between the analytes and the adsorbent have been described, using methacrylic acid stearyl ester (MASE) and ethylene glycol dimethacrylate (EGDMA) for the extraction of steroid sex hormones in urine [19] or methacrylic acid (MAA) and divinylbenzene (DVB) for the extraction of pharmaceuticals in wastewater samples [17]. In addition, hydrophilic interaction potential was installed through a poly(vinylpyridine (VP)-*co*-EGDMA) material to determine phenols in lake and sea waters [20] or a poly(vinylimidazole (VI)-*co*-DVB) material to determine veterinary residues in milk [21]. These various reports emphasised the higher extraction capacities and shorter extraction times enabled by these polar monolithic coatings compared to the classical PDMS coating. For these reasons, the development of new polar coatings for SBSE continues to be an interesting area of research activity.

In this paper we report upon the synthesis and characterisation of three novel, polar monolithic coatings for SBSE using 2-hydroxyethyl methacrylate (HEMA), pentaerythritol triacrylate (PETRA), poly(ethylene glycol) methacrylate) (PEGMA) and DVB as comono-

mers, coatings which were designed to extract polar compounds from water matrices. The effect of varying the monomer concentrations and the ratio between monomers was evaluated in order to obtain monolithic stir bars with appropriate chemical and morphological properties, as well as good mechanical stability.

2. EXPERIMENTAL

2.1. Materials

DVB (80% grade), HEMA (97% grade), PETRA (technical grade) and PEGMA ($M_n \sim 526$ Da) were supplied by Sigma-Aldrich (Steinheim, Germany) and purified by passing them through short columns packed with neutral alumina. Cyclohexanol (99% grade), 1-dodecanol (98% grade) and methanol (MeOH) were supplied by Sigma-Aldrich (Steinheim, Germany) and were used as porogens. Monoliths were synthesised *via* free radical polymerisation using 2-2'-azo*bis*(isobutyronitrile) (AIBN) from BDH (Poole, UK) as initiator. AIBN was recrystallised from methanol at low temperature.

For the evaluation of the monolithic materials as SBSE coatings, a group of pharmaceuticals and personal care products (PPCPs) with different polarities were purchased from Sigma-Aldrich (Steinheim, Germany): paracetamol (PARA), caffeine (CAFF), antipyrine (APy), benzotriazole (BZT), propranolol hydrochloride (PROP), pridinol methanesulfonate salt (PRID), methylparaben (MPB), carbamazepine (CBZ), propylparaben (PrPB), 2,4-dihydroxybenzophenone (DHB), benzylparaben (BzPB), 2,2-dihydroxy-4-methoxybenzophenone (DHMB), diclofenac (DICLO), benzophenone-3 (BP-3), triclocarban (TCC) and triclosan (TCS), of a high purity grade (>97%).

Stock solutions of individual standards were prepared by dissolving each compound in MeOH at a concentration of 1000 mg L^{-1}. A mix of all compounds in MeOH at a concentration of 10 mg L^{-1} was prepared weekly. Working solutions were prepared daily by diluting the previous solution with water. These solutions were stored at 4 ºC. Acetonitrile (ACN) from Prolabo (Llinars del Vallès, Spain) and formic acid (HCOOH) (≥95%) from Sigma-Aldrich (Steinheim, Germany) were used to adjust the pH of the mobile phase and the sample. Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain).

2.2. Preparation and characterisation of monolithic coatings for SBSE

During the preparation of monolithic materials, the aim was to develop bespoke polar coatings for SBSE applications. In a typical synthesis of a monolith, the monomers (HEMA, DVB, PETRA or PEGMA), the initiator (AIBN, 1 mol% relative to polymerisable double bonds) and the porogens (MeOH, cyclohexanol or 1-dodecanol) were mixed ultrasonically into a homogenous solution. The ratios of monomers and the compositions of porogens were optimised and are detailed in Table 1. After ultrasonication, the polymerisation mixture was poured into a 25 mL thick-walled glass Kimax culture tube, and then the stir bar set-up (as shown Fig. 1) immersed vertically into the tube. Subsequently, the polymerisation solution was placed on an ice-bath and purged with oxygen-free nitrogen for 5

Table 1. Composition of the monomer solutions used in the preparation of different monolithic coatings for SBSE.

Monolithic stir bar	Monomers (% w/w)	M-to-P ratio (%w/w)[a]	Porogen
MSB1	HEMA/DVB (15/85)	38:62	1-dodecanol
MSB2	HEMA/DVB (50/50)	38:62	1-dodecanol
MSB3	HEMA/DVB (80/20)	38:62	1-dodecanol
MSB4	HEMA/PETRA (80/20)	40:60	1-dodecanol/cyclohexanol (70/30, v/v)
MSB5	HEMA/PETRA (90/10)	40:60	1-dodecanol/cyclohexanol (70/30, v/v)
MSB6	HEMA/PETRA (95/5)	40:60	1-dodecanol/cyclohexanol (70/30, v/v)
MSB7	PEGMA/PETRA (20/80)	40:60	MeOH/cyclohexanol (50/50, v/v)
MSB8	PEGMA/PETRA (50/50)	40:60	MeOH/cyclohexanol (50/50, v/v)

[a] Monomer-to-porogen ratio.
AIBN was used as initiator (1 mol% relative to polymerisable double bonds).

minutes before being sealed under N_2. Polymerisation was carried out by placing the sealed glass tube in a water bath set at 60 ºC for 24 h. Once the polymerisation was complete, the culture tube was crushed carefully and the stir bar set-up removed from the rest of the monolith. The stir bar was pushed out from the glass tube and it was washed by stirring overnight in MeOH. Finally, the surplus monolithic material was washed by Soxhlet extraction with MeOH for 24 h to remove unreacted monomers, initiator and porogen and dried *in vacuo* at 40 ºC for 24 h.

The monolithic materials for SBSE were characterised further, and the surplus material was used for this purpose. Their specific surface areas were determined using a Brunauer-Emmett-Teller (BET) treatment of N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter (Norcross, GA). Prior to analysis, the samples were dried overnight *in vacuo* at 40 ºC. Fourier-Transform Infrared (FTIR) spectroscopic analyses were performed on a Perkin-Elmer Spectrum One FT-IR spectrometer (Massachusetts, USA).

2.3. SBSE/LC-UV performance

After synthesis optimisation, the evaluation of the monolithic coatings was performed for the SBSE of a group of PPCPs. The SBSE conditions were adapted from a previous study developed in our research group, [22] and were as follows: 50 mL of ultrapure water spiked at 0.2 mg L^{-1} at pH 5 and stirred at 500 rpm for 6 hours at room temperature. Then, the analytes were desorbed by liquid desorption using 5 mL of MeOH stirring at 500 rpm for 15 min. Finally, the extracts were evaporated to dryness under a gentle stream of N_2 and they were redissolved in 1 mL of MeOH/H_2O (1/1, v/v).

Next, the extracts obtained after the SBSE of the studied analytes using three selected monolithic SBSE coatings were analysed by liquid chromatography-ultraviolet detection (LC-UV). The chromatographic system was an Agilent

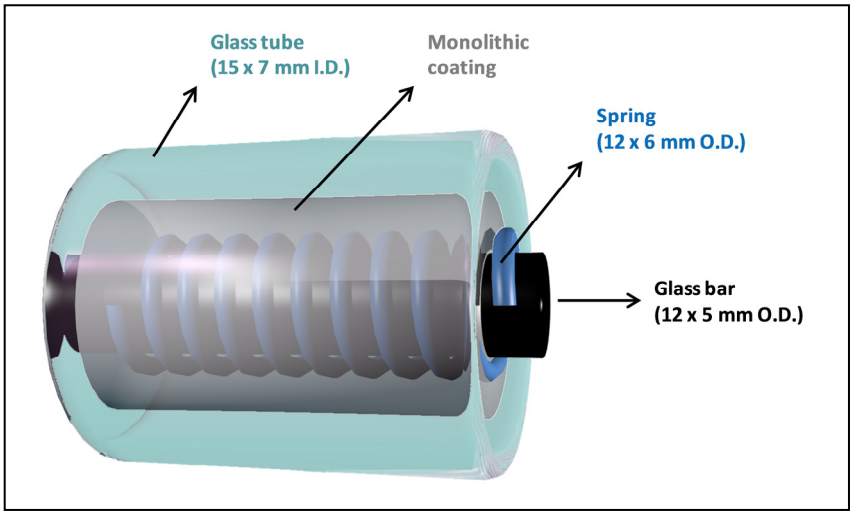


Fig. 1. The stir bar set-up used to produce the novel stir bars.

1100 liquid chromatograph equipped with an injection valve with a 20 µL loop and a UV spectrophotometric detector (Agilent, Waldbronn, Germany). The chromatographic column was a Kromasil 100 C_{18} (150 x 4.6 mm i.d.) with 5 µm particle size from Teknokroma (Barcelona, Spain). The mobile phase was ACN and ultrapure water adjusted to pH 3 with HCOOH, at a flow rate of 0.6 mL min^{-1}. The temperature of the column oven was set at 45 ºC. The gradient profile was initially from 15% to 55% ACN in 12 min (held for 5 min), then to 95% ACN in 8 min and kept constant for 3 min and then decreased back to the initial conditions in 2 min. The UV detection was achieved at 254 nm. The chromatographic separation was achieved in 30 min.

3. RESULTS AND DISCUSSION

3.1. Optimisation of the monolith syntheses for SBSE

For a monolithic synthesis, several parameters play an important role in realising the desired rigidity, porosity and polarity of the resulting monolith. With this in mind, special attention was paid to the following factors: polymerisation type and temperature, the choice of the monomers and porogens, the ratio between total monomers and porogen and, finally, the ratio between monomers.

First of all, all the syntheses were performed by thermally-initiated free radical copolymerisation, a methodology which has been used extensively in the monolith field in order to provide a simple and easy way to copolymerise two or more vinyl monomers within the same reaction vessel to give a copolymer with the desired properties [1, 4, 23]. Thus, the polymerisation tem-

perature is an important parameter which influences the polymerisation kinetics but which also controls the porous properties. At higher temperatures, the polymerisation reaction is faster and a larger number of growing nuclei are formed; as a consequence, the surface area increases [4, 5, 24]. Moreover, in a previous study [22] it was observed that the higher the polymerisation temperature, the softer the monolithic coating that was obtained. Therefore, the developed monolithic syntheses were performed at 60 ºC for 24h.

Another parameter which was evaluated was the choice of the monomers, a choice which was guided by the functionality desired for the sorptive materials. With the aim being the synthesis of monolithic coatings with polar character, three hydrophilic monomers, namely HEMA, PEGMA and PETRA, were selected. Although DVB is non-polar, it was selected for use because it is one of the most commonly used crosslinkers, but also because it can potentially establish π-π interactions with certain analytes. Thus, HEMA and PEGMA acted as polar functional monomers, whereas PETRA and DVB served as polar and apolar crosslinkers, respectively. Different monomer combinations were evaluated (Table 1).

Regarding the ratio of total monomers to porogenic solvent, this parameter was set at 38/62 (% w/w) for the HEMA/DVB combinations, and at 40/60 (% w/w) for the other monomer combinations, because in preceding works it was demonstrated that higher monomer concentrations led to softer monolithic coatings which were broken easily during SBSE experiments [17, 22].

Finally, appropriate combinations of pairs of monomers, the nature of the porogen and the ratio between monomers were evaluated for each synthesis in order to obtain a mechanically stable monolith with polar functionalities. The different synthesis conditions used for the synthesis of each monolithic coating are detailed in the following section, and are also shown in Table 1.

3.1.1. Poly(HEMA-*co*-DVB) monolithic coating

The first copolymerisation attempted involved HEMA as functional monomer and DVB as crosslinker. This combination was selected because the ester and hydroxyl groups present in HEMA and the aromatic ring present in DVB could potentially enhance the sorption of the studied analytes.

The choice of the porogen was essential to bring all the components in the polymerisation mixture into solution and to create pores in the monolith. Then, the nature and the level of the porogen can be used to control the morphology and the total pore volume [25]. For syntheses MSB1 through to MSB3, even although 1-dodecanol is a thermodynamically poor porogen for styrenic polymers, it was selected as porogen because it is a good solvent for HEMA.

The ratio between the functional monomer (HEMA) and crosslinker (DVB) was of utmost importance to control the morphology of the monolithic coating. In our study, we focused on this parameter in order to develop mechanically stable and polar monolithic coatings for SBSE. The first type of monolith based on poly(HEMA-*co*-DVB) was adapted from a previous study

where a monolithic column for the separation of small molecules was developed [26]. Thus, different HEMA/DVB compositions were tested: 15/85, 50/50 and 80/20 (% w/w). It was observed that the higher the DVB content the harder but more brittle was the coating obtained, resulting in unstable coatings for SBSE. Moreover, high levels of DVB made it difficult to remove the stir bar from the glass tube, due to the rigidity of the polymer. Thus, the MSB1 (15/85 (% w/w)) coating was too hard and brittle for use. By contrast, the MSB3 (80/20 (% w/w)) coating was considered to be too soft. Fortunately, the poly(HEMA-*co*-DVB) monolithic coating with a 50/50 ratio of HEMA to DVB had high mechanical stability and could be used for SBSE.

Bearing in mind all the polymerisation parameters, the poly(HEMA-*co*-DVB) monolith synthesised as a SBSE coating (MSB2) was obtained under the following optimised conditions: HEMA/DVB content of 50/50 (% w/w), 1-dodecanol as porogen and the ratio between total monomers and porogen of 40/60 (% w/w), polymerised for 24 h at 60 °C.

Afterwards, the surplus monolithic material obtained from the production of the poly(HEMA-*co*-DVB) (50/50, % w/w) stir bar was characterised using the BET method. It had a specific surface area (SSA) of 160 $m^2 g^{-1}$, which was moderate, thus a respectable extraction capacity was anticipated. In addition, and as shown in Fig. 2a, FTIR spectroscopy confirmed the successful

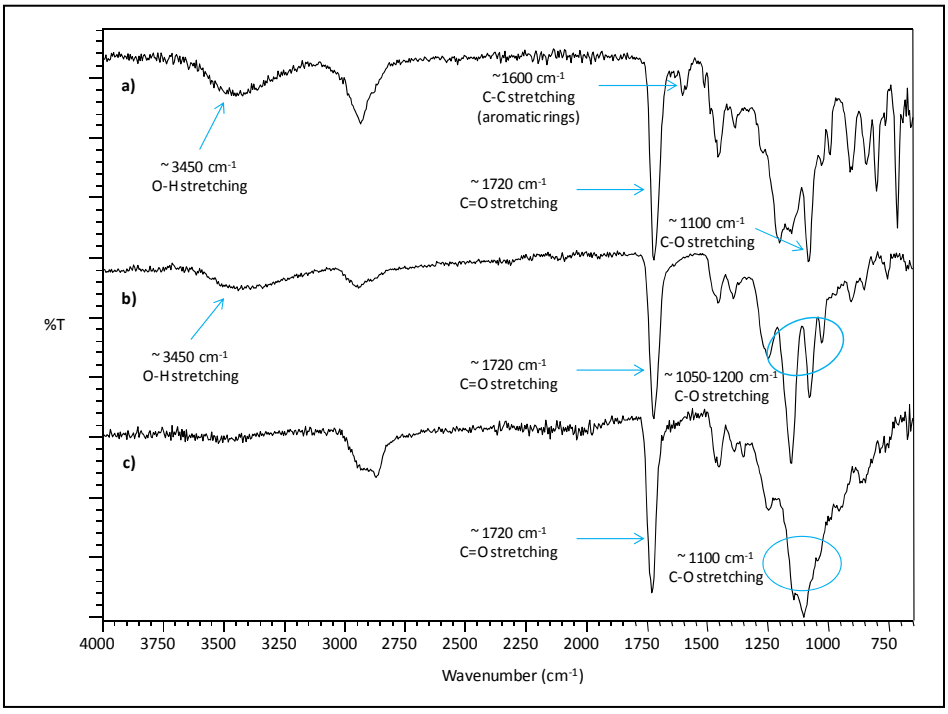


Fig. 2. FTIR spectra obtained for each monolith: a) poly(HEMA-*co*-DVB), b) poly(HEMA-*co*-PETRA) and c) poly(PEGMA-*co*-PETRA).

copolymerisation of HEMA and DVB. The spectrum showed the characteristics bands ascribed to C-C stretching in the aromatic rings (~1600 cm^{-1}) of DVB. The presence of HEMA in this monolith was confirmed by an intense band ascribed to C=O stretching (1720 cm^{-1}) and C-O stretching (~1100 cm^{-1}) of an ester and O-H stretching (broad band at around 3450 cm^{-1}) of the hydroxyl group in HEMA.

3.1.2. Poly(HEMA-*co*-PETRA) monolithic coating

Regarding the poly(HEMA-*co*-PETRA) monolith, we followed the synthesis conditions of a monolithic column for CEC cited in a previous report [9]. The porogenic solvent was a binary mixture comprising of 1-dodecanol and cyclohexanol. In recent years, several reports have cited the use of these porogens in polymerisations [17, 22, 27, 28], having in common the use of a polar functional monomer together with DVB as crosslinker. Moreover, in these studies, high contents of cyclohexanol were used in order to deliver monoliths with small pores, and correspondingly high SSAs of 500 $m^2 g^{-1}$ [17] and 600 $m^2 g^{-1}$ [22] were obtained. However, in our work, the composition of the porogenic mixture was 70/30 (1-dodecanol/cyclo-hexanol, % w/w), which delivered lar-ger pores and conferred higher per-meability.
When the ratio between the functional monomer (HEMA) and crosslinker (PETRA) was varied, different compositions using low levels of PETRA (5-20% w/w crosslinker) were tested. As expected, MSB6 with the lowest content of PETRA was found to be soft and to crumble easily. When increasing the crosslinker content up to 20%, we observed an improvement in the stability of the coating. Therefore, for MSB4 the composition of monomer feed for the poly(HEMA-*co*-PETRA) monolithic coating was set at 80/20 (% w/w).
Once conditions for the synthesis of the poly(HEMA-*co*-PETRA) monolith were selected (MSB4), the surplus MSB4 monolith was characterised. Firstly, its FTIR spectrum (Fig. 2b) showed two characteristic bands between 1050 and 1200 cm^{-1}, which can be attributed to the C-O stretching of the ester moieties present in HEMA and PETRA. Moreover, O-H stretching (broad band at around 3450 cm^{-1}) was observed in the poly(HEMA-*co*-PETRA) FTIR spectrum. The SSA of this monolithic material was 5 $m^2 g^{-1}$, which was much lower than that obtained for the poly(HEMA-*co*-DVB) monolith, but this is because MSB4 is a gel-type polymer. To confirm this, a known mass of MSB4 copolymer was swollen in MeOH for 24h, and after this time we observed an increase in the volume of the poly(HEMA-*co*-PETRA) monolith, equivalent to 1.2 mL of MeOH per gram of polymer. Therefore, this copolymer could be considered to be useful as a monolithic coating for SBSE provided that it is used in a swollen state.

3.1.3. Poly(PEGMA-*co*-PETRA) monolithic coating

A novel combination between the monomers PEGMA and PETRA was developed, taking into account the hydrophilic character of both monomers. As for the porogen used in this synthesis, a binary porogenic solvent was selected once again, namely MeOH/cyclohexanol. MeOH offers

good solubility to PEG materials, but it could not be used alone as a porogen because it is considered a poor pore forming solvent [29]. For this reason, a mixture of MeOH and cyclohexanol at a ratio of 50/50 (% w/w) was applied in order to dissolve all the polymerisation reagents and also to control the porous properties of the monoliths.

The composition of this copolymer was also evaluated; PEGMA/PETRA ratios of 50/50 (% w/w) and 20/80 (% w/w) were tested. Under the former synthesis conditions (MSB8), we obtained a translucent, jelly-like monolith with good mechanical stability and high swelling behaviour. These properties are attributed to the fact that PEGMA is a macromonomer which can swell and deswell irrespective of the crosslink density of the polymer network as a whole. Therefore, this type of material shrinks when dried, of course. When we increased the crosslinker content (up to 80%) to obtain a less swellable monolith, we observed that the MSB7 composition was not good enough in terms of mechanical stability, because the monolithic coating obtained was too brittle. Thus, the best monomer composition for the poly(PEGMA-*co*-PETRA) monolithic coating was 50/50 (% w/w), and this yielded a suitable monolithic coating for further SBSE experiments.

Using the preferred conditions for the synthesis of poly(PEGMA-*co*-PETRA) monoliths (MSB8), MSB8 was characterised by FTIR spectroscopy (Fig. 2c); similar bands as appeared in the two previous FTIR spectra (Figs. 2a and 2b) were observed. Bearing in mind the gel-type polymer obtained in this synthesis, we performed a swelling capacity test, by the immersion of the polymer in MeOH for 24h. As expected, an impressive increase in the volume of the copolymer was observed, resulting in a swelling capacity of 2.9 mL of MeOH per gram of polymer. This copolymer presented the highest stability and swelling capacity. Analytes can penetrate easily into the interior of the monolithic structure when it is in a swollen state.

To summarise, the optimum conditions for each of the three types of copolymers were: MSB2 for poly(HEMA-*co*-DVB), MSB4 for poly(HEMA-*co*-PETRA) and MSB8 poly(PEGMA-*co*-PETRA).

3.2. SBSE application

Once the syntheses had been optimised and the monoliths were characterised, the three monolithic coatings (MSB2, MSB4 and MSB8) were applied in SBSE for the extraction of a group of PPCPs with different polarities from water matrices. The SBSE conditions are detailed in section 2.3, which were adapted from a previous study developed in our research group [22]. The choice of the studied compounds was performed depending on their polarity. Thereby, several compounds with log $K_{O/W}$ values ranging from -0.6 to 6.1 were selected, resulting in a great variety of polarities.

Several reviews [30-32] related to SBSE have correlated the log $K_{O/W}$ values of the analytes with their partition coefficient between the PDMS phase and the aqueous phase, giving a good indication if and how well a certain analyte can be extracted with a PDMS-coated stir bar. Thus, the analytes with log $K_{O/W}$ values above 3 are expected to be successfully extracted with recovery values higher than 70% in this apolar coating. In this way, all three in-house

polar monolithic coatings for SBSE are expected to improve the PDMS phase performance due to the numerous polar functionalities in their structures.

Fig. 3 shows the recovery values of each analyte using all the monolithic coating for SBSE depending on their log $K_{O/W}$ values. Firstly, all the in-house SBSE coatings showed some degree of uptake for the selected analytes. As can be seen, the monolith based on poly(HEMA-*co*-PETRA (80/20, % w/w) showed the lowest analyte uptake. This is likely to be because this monolith is essentially non-porous in the dry state and does not swell significantly in water.

However, when the poly(HEMA-*co*-DVB) (50/50, % w/w) monolithic coating was used, significant increases in the uptake of analytes was observed in comparison to the poly(HEMA-*co*-PETRA) monolith. In particular, the poly(HEMA-*co*-DVB) monolith was able to improve the retention of those analytes with moderate polarity, such as BzPB, DHMB, DICLO, BP-3, TCC and TCS. This improvement in performance could be attributed to the presence of the aromatic rings derived from DVB residues and the polar functionalities of HEMA that enabled π-π and hydrogen bonding interactions, respectively, with the studied analytes. Moreover, the substantially higher SSA (160 m^2 g^{-1}) that poly(HEMA-*co*-DVB) presented in comparison to the poly(HEMA-*co*-PETRA) monolith (5 m^2 g^{-1}) could also promote and increase the retention of the studied analytes.

Finally, the final copolymer tested as a polar coating for SBSE was the poly(PEGMA-*co*-PETRA) monolith (50/50, % w/w). Although this copolymer is non-porous in the dry state, when used in the wet state analytes can gain ready access to the interior of the monolith and this led to better recovery values for most of the

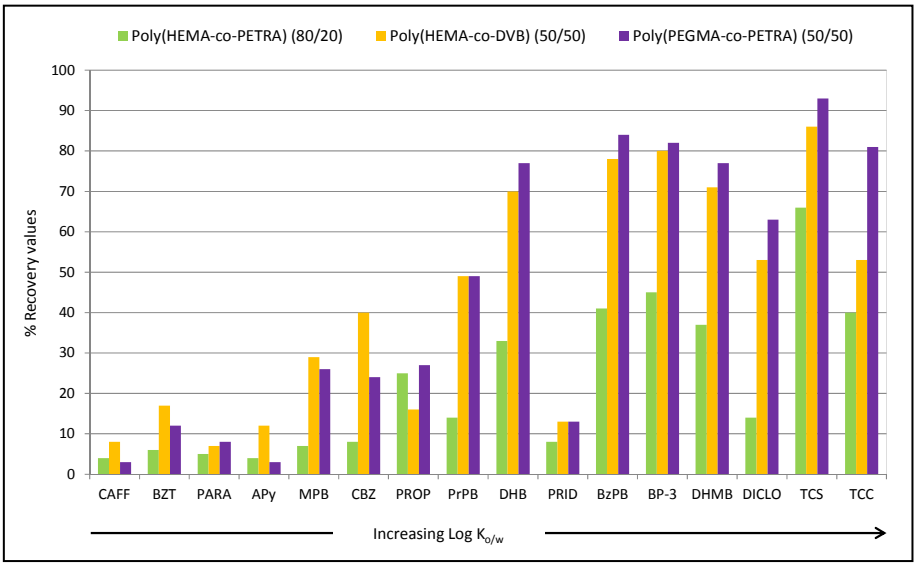


Fig. 3. Recovery values of the studied analytes when using poly(HEMA-*co*-DVB), poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA) as SBSE monolithic coatings.

Talanta (submitted)

compounds, except for APy and CBZ, whose recovery values were better when using the poly(HEMA-*co*-DVB) monolith.

It should be highlighted that these recovery values are superior to those found so far in previous studies, in which PDMS coated-stir bar was used for extracting PPCPs from aqueous samples [17, 22, 33, 34]. For instance, Quintana et al. [33] reported the extraction of several acidic and polar organic contaminants from water samples using SBSE(PDMS) followed by gas chromatography-mass spectrometry (GC-MS). While TCS and CBZ showed a 50% and 7% of recovery values, respectively, using the PDMS-coated stir bar under optimised conditions, these compounds could be better extracted using the in-house monolithic SBSE coatings (above 60% and 20%, respectively). Other studies about the SBSE of PPCPs were reported by Bratkowska et al. [17, 22], who synthesised two monolithic coatings for the SBSE of PPCPs and their performances were compared to the classical PDMS-coated stir bar. In these studies, it was demonstrated that the PDMS-coated stir bar was only able to extract the most apolar compounds, such as TCC and BP-3 (95% and 85%, respectively). In contrast, both monolithic coatings, based on poly(MAA-*co*-DVB) and poly(N-vinylpyrrolidone (VPD)-*co*-DVB), offered a much better SBSE performance than the classical PDMS-coated stir bar and the results were comparable to those obtained using the in-house polar monolithic coatings of the present study. While the recovery values of DHB using the poly(HEMA-*co*-DVB) and poly(PEGMA-*co*-PETRA) were 70% and 77%, respectively, the poly(VPD-*co*-DVB) was able to extract only 50% of DHB. However, CBZ was better extracted using the poly(VPD-*co*-DVB) (83%) than using the in-house polar monolithic coatings, with recovery values ranging from 8% to 40%.

Considering the results of all the in-house monolithic coatings, a particularly promising performance in SBSE was achieved when the novel monolithic coating poly(PEGMA-*co*-PETRA) was applied for the extraction of polar and apolar compounds. Further application to complex real samples is therefore fully justified.

4. Conclusions

In this study, three polar monomers (HEMA, PEGMA and PETRA), and DVB, were copolymerised in varying ratios, with control of the nature and amount of porogen, among other polymerisation parameters, to deliver several monolithic coatings for SBSE that had variable mechanical stability and polarity.

After synthesis optimisation and morphological characterisation, poly(HEMA-*co*-DVB), poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA) monoliths, with ratios of monomers of 50/50, 80/20 and 50/50 (% w/w), respectively, were selected as the best monolithic coatings with the desired mechanical stability for SBSE.

When these copolymers were applied as coatings for the SBSE of a group of polar and apolar PPCPs, the best SBSE performance was achieved by the poly(PEGMA-*co*-PETRA) coating, due to its high number of polar sites provided by its hydroxyl and ethylene oxide.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] L. Xu, Z.G. Shi, Y.Q. Feng, Porous monoliths: sorbents for miniaturized extraction in biological analysis, Anal. Bioanal. Chem. 399 (2011) 3345-3357.

[2] O.G. Potter, E.F. Hilder, Porous polymer monoliths for extraction: Diverse applications and platforms, J. Sep. Sci. 31 (2008) 1881-1906.

[3] A. Nordborg, E.F. Hilder, Recent advances in polymer monoliths for ion-exchange chromatography, Anal. Bioanal. Chem. 394 (2009) 71-84.

[4] F. Svec, Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation, J. Chromatogr. A 1217 (2010) 902-924.

[5] M.R. Buchmeiser, Polymeric monolithic materials: Syntheses, properties, functionalization and applications, Polymer 48 (2007) 2187-2198.

[6] G. Yang, C. Yan, L. Bai, J. Li, Y. Duan, A simple and efficient method for preparation of a strong cation exchange monolith and its application to separation of HSA, Anal. Methods 4 (2012) 1098-1104.

[7] G. Guiochon, Monolithic columns in high-performance liquid chromatography, J. Chromatogr. A 1168 (2007) 101-168.

[8] J. Lin, X.P. Wu, X.C. Lin, Z.H. Xie, Preparation of polymethacrylate monolithic stationary phases having bonded octadecyl ligands and sulfonate groups: Electrochromatographic characterization and application to the separation of polar solutes for pressurized capillary electrochromatography, J. Chromatogr. A 1169 (2007) 220-227.

[9] X.C. Wang, X.C. Lin, Z.H. Xie, J.P. Giesy, Preparation and evaluation of a neutral methacrylate-based monolithic column for hydrophilic interaction stationary phase by pressurized capillary electrochromatography, J. Chromatogr. A 1216 (2009) 4611-4617.

[10] A. Namera, A. Nakamoto, T. Saito, S. Miyazaki, Monolith as a new sample preparation material: Recent devices and applications, J. Sep. Sci. 34 (2011) 901-924.

[11] F. Svec, C.G. Huber, Monolithic materials - Promises, challenges, achievements, Anal. Chem. 78 (2006) 2100-2107.

[12] I. Nischang, I. Teasdale, O. Bruggemann, Porous polymer monoliths for small molecule separations: advancements and limitations, Anal. Bioanal. Chem. 400 (2011) 2289-2304.

[13] E.G. Vlakh, T.B. Tennikova, Preparation of methacrylate monoliths, J. Sep. Sci. 30 (2007) 2801-2813.

[14] D. Djozan, M.A. Farajzadeh, S.M. Sorouraddin, T. Baheri, Synthesis and application of high selective monolithic fibers based on molecularly imprinted polymer for SPME of trace methamphetamine, Chromatographia 73 (2011) 975-983.

[15] F. Wu, J.H. Chen, W.P. Lu, Z. Lin, L. Zhang, Solid-phase microextraction using poly (1-hexadecene-co-trimethylolpropane trimethacrylate) coating for analysis of pesticides in tea samples by high-performance liquid chromatography, Curr. Anal. Chem. 7 (2011) 341-348.

[16] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, Preparation of a mixed stir bar for sorptive extraction based on monolithic material for the extraction of quinolones from wastewater, J. Chromatogr. A 1217 (2010) 2667-2673.

[17] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, Preparation of a polar monolithic stir bar based on methacrylic acid and divinylbenzene for the sorptive extraction of polar pharmaceuticals from complex water samples, J. Chromatogr. A 1225 (2012) 1-7.

[18] R. Lucena, Extraction and stirring integrated techniques: examples and recent advances, Anal. Bioanal. Chem. 403 (2012) 2213-2223.

[19] X.J. Huang, D.X. Yuan, B.L. Huang, Determination of steroid sex hormones in urine matrix by stir bar sorptive extraction based on monolithic material and liquid chromatography with diode array detection, Talanta 75 (2008) 172-177.

[20] X.J. Huang, N.N. Qiu, D.X. Yuan, Direct enrichment of phenols in lake and sea water by stir bar sorptive extraction based on poly (vinylpyridine-ethylene dimethacrylate) monolithic material and liquid chromatographic analysis, J. Chromatogr. A 1194 (2008) 134-138.

[21] X.J. Huang, N.N. Qiu, D.X. Yuan, Simple and sensitive monitoring of sulfonamide veterinary residues in milk by stir bar sorptive extraction based on monolithic material and high performance liquid chromatography analysis, J. Chromatogr. A 1216 (2009) 8240-8245.

[22] 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, Anal. Chim. Acta 706 (2011) 135-142.

[23] W. Guan, C. Han, X. Wang, X. Zou, J. Pan, P. Huo, C. Li, Molecularly imprinted polymer surfaces as solid-phase extraction sorbents for the extraction of 2-nitrophenol and isomers from environmental water, J. Sep. Sci. 35 (2012) 490-497.

[24] S. Xie, F. Svec, J.M.J. Fréchet, Preparation of porous hydrophilic monoliths: Effect of the polymerization conditions on the porous properties of poly (acrylamide-co-N,N′-methylenebisacrylamide) monolithic rods, J. Polym. Sci., Part A: Polym. Chem. 35 (1997) 1013-1021.

[25] D.C. Sherrington, Preparation, structure and morphology of polymer supports, Chem. Commun. (1998) 2275-2286.

[26] K.N. Smirnov, I.A. Dyatchkov, M.V. Telnov, A.V. Pirogov, O.A. Shpigun, Effect of monomer mixture composition on structure and chromatographic properties of poly(divinylbenzene-co-ethylvinylbenzene-co-2-hydroxyethyl methacrylate) monolithic rod columns for separation of small molecules, J. Chromatogr. A 1218 (2011) 5010-5019.

[27] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, Sensitive determination of strongly polar aromatic amines in water samples by stir bar sorptive extraction based on poly(vinylimidazole-divinylbenzene) monolithic material and liquid chromatographic analysis, J. Chromatogr. A 1216 (2009) 4354-4360.

[28] X. Huang, N. Qiu, D. Yuan, B. Huang, A novel stir bar sorptive extraction coating based on monolithic material for apolar, polar organic compounds and heavy metal ions, Talanta 78 (2009) 101-106.

[29] X. Chen, H.D. Tolley, M.L. Lee, Polymeric cation-exchange monolithic columns containing phosphoric acid functional groups for capillary liquid chromatography of peptides and proteins, J. Chromatogr. A 1217 (2010) 3844-3854.

[30] M. Kawaguchi, R. Ito, H. Nakazawa, A. Takatsu, Environmental and biological applications of stir bar sorptive extraction, in: J. Pawliszyn (Editor), Comprehensive Sampling and Sample Preparation, Elsevier Inc., USA, 2012, pp. 797-818.

[31] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, Novel stir bar sorptive extraction methods for environmental and biomedical analysis, J. Pharm. Biomed. Anal. 40 (2006) 500-508.

[32] F. David, P. Sandra, Stir bar sorptive extraction for trace analysis, J. Chromatogr. A 1152 (2007) 54-69.

[33] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Multiresidue analysis of acidic and polar organic contaminants in water samples by stir-bar sorptive extraction–liquid desorption–gas chromatography–mass spectrometry, J. Chromatogr. A 1174 (2007) 27-39.

[34] N. Gilart, N. Miralles, R.M. Marcé, F. Borrull, N. Fontanals, Novel coatings for stir bar sorptive extraction to determine pharmaceuticals and personal care products in environmental waters by liquid chromatography and tandem mass spectrometry, Anal. Chim. Acta 774 (2013) 51-60.

3.2.3. Preparation of a polar monolithic coating for stir bar sorptive extraction of emerging contaminants from wastewaters

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

PREPARATION OF A POLAR MONOLITHIC COATING FOR STIR BAR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM WASTEWATERS

Núria Gilart[a], P.A.G. Cormack[b], Rosa Maria Marcé[a], Francesc Borrull[a,*], Núria Fontanals[a]

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel·lí Domingo, s/n, 43007 Tarragona, Spain

[b] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Abstract

A new polar monolith based on poly(poly(ethylene glycol) methacrylate-*co*-pentaerythritol triacrylate) (poly(PEGMA-*co*-PETRA)) was first synthesised, after the optimisation of the polymerisation conditions, and applied as a coating for the stir bar sorptive extraction (SBSE) of a group of pharmaceuticals and personal care products (PPCPs) from environmental water samples.

Several parameters affecting extraction and liquid desorption in SBSE were investigated to achieve the optimal sorption efficiencies for the studied analytes. Under the optimised experimental conditions, a rapid, simple and sensitive SBSE performance was provided by the in-house monolithic stir bar. Moreover, the in-house coating was able to extract and desorb most of the studied analytes more effectively and quickly, due to its polar behaviour and suitable mechanical and physical properties, in comparison with the recently commercialised polar stir bars (EG Silicone Twister® and Acrylate Twister®).

The analytical methodology, including SBSE followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), was validated and successfully applied for the determination of a group of PPCPs in wastewater samples.

Keywords: *monolithic polar material; stir bar sorptive extraction; polar emerging contaminants; wastewater samples*

1. INTRODUCTION

Over the last few years, stir bar sorptive extraction (SBSE) has gained popularity as an extraction technique for the determination of emerging organic contaminants (EOCs) in complex matrices due to its environmentally friendly performance, simplicity and sensitivity [1-3].

Although SBSE has successfully been applied for the determination of organic contaminants in environmental, biological and food matrices at trace levels

prior to a chromatographic technique [4-7], its main drawback is the availability of commercial coatings. Until very recently, polydimethylsiloxane (PDMS) was the only commercially available coating for SBSE, known by the name PDMS Twister® from Gerstel, being suitable for the extraction of those analytes with more apolar characteristics (i.e. log $K_{O/W}$ > 3) [3,8]. In order to overcome the limitations of PDMS for the extraction of more polar compounds, very recently, two new commercial coatings have been introduced by Gerstel, known as EG Silicone Twister® and Acrylate Twister®. Both are PDMS-based, the former modified with poly(ethylene) glycol (PEG), and the latter with polyacrylate (PA). Few studies have reported the use of these new coatings for SBSE to determine volatile organic compounds (VOCs) in cosmetic samples [9], benzothiazole [10] and pharmaceuticals and personal care products (PPCPs) [11] in wastewaters. All of these studies demonstrated a certain improvement on the extraction efficiencies of the most polar analytes in comparison with the classical PDMS coating. Nevertheless, new approaches in the development of SBSE coatings with polar functionalities are required to improve the extraction of more polar analytes and to extend the range of SBSE applications.

Up to now, many efforts have focused on the preparation of novel polar coatings for SBSE and several synthetic strategies have been developed. The first of these was sol-gel technology, which allowed novel materials to be obtained based on PDMS modified with β-cyclodextrin to determine estrogens and bisphenol A in water samples [12] or based on PDMS modified with poly(vinylalcohol) to extract organophosphorus pesticides in honey [13], among others. All of these coatings, chemically attached to a glass bar, were shown to be more thermally and chemically stable as well as more effective in terms of extraction efficiencies of both polar and apolar target analytes than with PDMS coating [1]. However, the sol-gel process involved more laborious steps during the polymerisation than other strategies, such as the preparation of monoliths.

Along the same lines, the second approach was the development of monolithic materials, which have became popular in recent years due to their high permeability and simplicity in preparation. For these reasons, a number of monolithic coatings with different chemical behaviours have been synthesised for SBSE applications. Some examples of this approach consisted of vinylphthalimide (VPA) and *N,N'*-methylenebisacrylamide (MBAA) to determine benzimidazoles in milk and honey [14] or, alternatively, vinylpyrrolidone (VPD) and divinylbenzene (DVB) to extract PPCPs from environmental waters [15], which enabled the effective extraction of polar compounds that were not retained onto the PDMS coating. In addition, this simple strategy has enabled a porous structure with high surface areas to be developed which results in an enhancement of extraction efficiencies [1,16].

Monolithic materials are highly versatile in comparison with the classic PDMS stir bar because different interactions can be achieved depending on the monomers used during their syntheses. To the best of our knowledge, there is no report that has previously used

poly(ethylene glycol) methacrylate (PEGMA) as a functional monomer and pentaerythritol triacrylate (PETRA) as a cross-linker for the preparation of a monolith. The polar nature of both monomers might significantly increase the polarity of the final monolith. Therefore, the aim of this paper is the development of a polar monolithic coating for SBSE by the copolymerisation of PEGMA and PETRA monomers. Furthermore, its SBSE performance, followed by liquid chromatography-mass spectrometry in tandem (LC-MS/MS), was applied to extract PPCPs from wastewater samples.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

For the synthesis of the monolithic coating, PETRA (technical grade) and PEGMA (M_n~526) as monomers, and cyclohexanol (99%) and methanol (MeOH) (99.7%) as porogens were purchased from Sigma-Aldrich (Steinheim, Germany); 2-2'-azobis(isobutylronitrile) (AIBN) as initiator was supplied by BDH (Poole, UK). The monomers were purified by passing them through a short column filled with neutral alumina and the initiator was recrystallised at a low temperature from MeOH prior to use.

For the evaluation of the polar sorptive material, paracetamol, caffeine, antipyrine, benzotriazole, propranolol hydrochloride, pridinol methanesulfonate salt, methylparaben, carbamazepine, propylparaben, 2,4-dihydroxybenzophenone (DHB), benzylparaben, 2,2-dihydroxy-4-methoxybenzophenone (DHMB), diclofenac, benzophenone-3 (BP-3), triclocarban and triclosan were purchased from Sigma-Aldrich (Steinheim, Germany) (>97%). The structures, pK_a and log $K_{O/W}$ values of these analytes are presented in Table 1. Individual standard solutions of 1000 mg L^{-1} of each compound were prepared in MeOH and a standard mixture solution of 10 mg L^{-1} was prepared weekly in MeOH. The standard mixture solution was diluted daily with ultrapure water to give the required concentration and stored at 4 ºC.

For LC analyses, HPLC grade MeOH and acetonitrile (ACN) were supplied by Prolabo (Llinars del Vallès, Spain); formic acid (HCOOH) (≥95%), sodium chloride (NaCl) (99%) and sodium hydroxide (NaOH) (≥98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain).

2.2. Stir bar preparation

The detailed preparation of the poly(PEGMA-*co*-PETRA) monolithic coating is described in our previous study [17]. The ratios of the reagents and the polymerisation conditions were as follows: a ratio between monomers of 50/50 (% w/w), a ratio between total monomers and porogen of 40/60 (% w/w), a porogenic solvent consisted of 50% (w/w) cyclohexanol and 50% (w/w) MeOH, AIBN (1 mol% relative to polymerisable double bonds), polymerised in a water bath at 60 ºC for 24 h. After the polymerisation, the monolithic stir bar was washed with MeOH, being stirred overnight. The final poly(PEGMA-*co*-PETRA) had the following dimensions: 12 mm in length and a

polymer thickness of 1 mm, which corresponds to a polymer volume of 225 µL.

2.3. LC-(ESI)-MS/MS analysis

The extracts were analysed with an Agilent 1200 series LC coupled to a 6410 series triple quadrupole mass spectrometer with an electrospray ionisation (ESI) interface, an automatic injector (volume injected was 50 µL), a degasser, a quaternary pump and a column oven from Agilent Technologies (Waldbronn, Germany).

The chromatographic column was a Kromasil 100 C_{18} (150 x 4.6 mm i.d.) with 5 µm particle size from Tekno-kroma (Barcelona, Spain). ACN and ultrapure water adjusted to pH 3 with HCOOH were used as the mobile phase. The gradient started increasing from 15% to 55% ACN in 12 min (held for 5 min), then to 95% ACN in 8 min and kept constant for 3 min. Finally, it decreased back to the initial conditions in 2 min. The chromatographic analyses were performed at 45 ºC with a flow rate of 0.6 mL min^{-1}.

A flow injection of a standard solution of each compound was used to find the optimal conditions in the ESI source. These conditions were as follows: nebuliser pressure of 45 psi, drying gas (N_2), flow rate of 12 L min^{-1}, source temperature of 350 ºC and a capillary potential of 4000 V. Ionisation mode, cone voltage and collision energies were optimised for each analyte in order to obtain two multiple reaction monitoring (MRM) transitions. The conditions are described in Table 1.

2.4. Stir bar sorptive extraction

The SBSE procedure was as follows: the stir bar was inserted into a 100-mL flask with 50 mL of sample adjusted to pH 7 with NaOH, containing 15% of NaCl. Samples were stirred at 500 rpm for 1 hour at room temperature (25 ºC). After extraction, the stir bar was removed from the sample using magnetic tweezers and dried with a lint-free tissue. In terms of the liquid desorption (LD), the stir bar was immersed in a vial containing 5 mL of a mixture of MeOH/ACN (1/1, v/v), stirring at the same speed for 10 min. The extracts were then evaporated to dryness under a gentle stream of N_2. Prior to the LC injection, the extracts were redissolved in 1 mL of MeOH/H_2O (1/1, v/v). After each use, the stir bar was cleaned 3 times with 5 mL MeOH/ACN (1/1, v/v), stirring for 10 min. Each stir bar was reused at least 20-30 times with environmental samples.

All of the environmental water samples (from an influent, secondary effluent and tertiary effluent of a domestic sewage treatment plant (STP)) were collected in pre-cleaned amber glass bottles, filtered using a 0.45 µm nylon membrane (Supelco, Bellefonte, PA, USA), acidified to pH 3 (HCOOH) and stored at 4 ºC until analysis.

3. RESULTS AND DISCUSSION

3.1. LC-(ESI)-MS/MS conditions

MS/MS parameters were also optimised by injecting each compound at 250 µg L^{-1} in MeOH/H_2O (1/1, v/v) individually in flow injection analysis (FIA). Depending on their acidic or basic

Table 1. Retention time, pK_a, log $K_{o/w}$ and LC-(ESI)MS/MS acquisition parameters in MRM mode for the analysis of the target analytes.

Analyte	t_R (min)	pK_a	Log $K_{o/w}$	Precursor ion (m/z)	Cone volt. (V)	Product ion (m/z) (Collision energy (V))		Ionis. mode (ESI)
						Identif.	Confirm.	
Paracetamol	4.1	9.2	0.5	152	100	110 (15)	93 (25)	+
Caffeine	5.4	13.4	-0.6	195	125	138 (15)	110 (25)	+
Benzotriazole	7.6	8.5	0.4	120	100	65 (25)	92 (15)	+
Antipyrine	8.3	13.3	1.4	189	100	145 (30)	115 (30)	+
Propranolol	9.9	9.5	2.9	260	125	116 (15)	183 (15)	+
Pridinol	10.3	9.7	3.4	296	125	115 (30)	193 (30)	+
Methylparaben	11.4	8.3	1.9	151	80	92 (15)	136 (5)	-
Carbamazepine	13.6	13.7	1.9	237	150	193 (35)	179 (35)	+
Propylparaben	15.9	8.2	2.9	179	100	92 (15)	136 (5)	-
DHB	17.0	7.7	3.2	213	130	135 (15)	169 (5)	-
Benzylparaben	18.4	8.2	3.6	227	100	92 (10)	136 (20)	-
DHMB	19.6	7.1	4.3	243	80	93 (15)	123 (15)	-
Diclofenac	22.2	4.2	4.5	294	75	250 (5)	214 (15)	-
BP-3	24.3	7.6	4.0	229	130	151(15)	105 (15)	+
Triclocarban	25.9	12.7	6.1	313	130	160 (5)	126 (15)	-
Triclosan	26.3	7.9	5.3	287 / 289	8	35 (18)	35 (18)	-

properties, the studied compounds were divided, individually or in groups, into six windows applying negative or positive ESI ionisation. In addition, identification and confirmation MRM transitions were selected for each analyte. The optimised cone voltage and collision energy for each MRM transition are summarised in Table 1.

All of the selected compounds showed good linearity ($r^2 \geq 0.997$) by direct injection with a linear range of 0.50-200 μg L^{-1}, except for paracetamol, antipyrine, DHMB, triclosan (2-200 μg L^{-1}) and pridinol (25-200 μg L^{-1}). The instrumental detection limits (IDLs), calculated as signal-to-noise ratio (S/N) of 3, ranged from 0.15 to 0.50 μg L^{-1}, except for pridinol (5 μg L^{-1}). The instrumental quantification limits (IQLs), as the concentration of the lowest point of the calibration curve, were between 0.50 and 25 μg L^{-1}.

3.2. Optimisation of the SBSE procedure

To overcome the lack of availability of polar commercial stir bars, over the last few years, several monomers with different polarities have been combined in order to synthesise polar monoliths [1,16]. There are several monomers whose polarity properties might be very beneficial. However, they have not been used in the preparation of a monolith yet. In our previous research [17], a monolith coating for SBSE based on poly(PEGMA-*co*-PETRA) was first synthesised. The choice of these monomers was dependent on the final chemical and physical properties of the desired monolithic material.

Therefore, thanks to the hydroxyl and ester functional groups of both PEGMA and PETRA, the resulting monolith was expected to contribute to the sorption of more polar compounds onto the coating. In addition, the use of PEGMA resulted in a gelatinous polymer, providing mechanical stability and swelling capacity.

Subsequently, the evaluation of this polar monolithic material as SBSE coating was performed. Bearing in mind the polar structure of the resulting monolith, a group of PPCPs with different polarities was selected to test the extraction efficiency of our in-house coating and compare it with the commercial stir bars [11,15,18]. Then, in order to obtain high extraction efficiencies, several main parameters involved in the extraction and desorption steps were optimised. In this study, desorption was performed by LD because the analytes were then separated by LC.

3.2.1. Extraction conditions

In terms of the optimisation of the extraction conditions, several factors, including sample pH, ionic strength, extraction time, agitation speed and temperature, have been taken into account for SBSE performance.

The SBSE procedure was optimised under these initial conditions: 50 mL ultrapure water spiked at 2 µg L^{-1} with the analytes' mixture stirring at 500 rpm for 1 hour at room temperature, and the LD was performed using 5 mL of MeOH stirring at the same speed for 10 min.

The effect of the sample pH on the analytes' response was evaluated at different pH values (3, 5, 7 and 9) because it may influence the analytes' sorption onto the coating. As expected, all of the analytes behaved according to their pK_a values (Table 1). For instance, diclofenac (pK_a = 4.2) showed a lower response when the pH was different from 3 because it was turned into its anionic form. In contrast, those compounds with pK_a around 9 presented higher signal response when higher pH values were applied (from 3 to 7) because they were uncharged, promoting the hydrophilic interactions with the coating, and their sorption onto it decreased to pH 9 because these analytes were almost in their anionic state. In addition, carbamazepine and caffeine showed an increase in their response when the sample pH was higher (from 3 to 9) due to their high pK_a values (> 13). The above trend can be seen in Fig. 1 where the effect of the sample pH for a representative group of analytes is shown. Since most of the target analytes (10 analytes out of 16), such as propranolol, methylparaben, DHMB, propylparaben, BP-3, among others, showed better responses at pH 7, this pH value was selected as the optimal sample pH for further research.

The addition of salts, such as NaCl, has been demonstrated to modify the ionic strength of the sample solution and, as a consequence, increase the response of more polar analytes [1]. Therefore, the ionic strength of the sample was adjusted by the addition of NaCl from 0% to 30% w/v. In the range from 0 to 15%, it was observed that almost all the analytes presented higher sorption onto the coating at higher percentages of NaCl. However, with the highest percentage (i.e., 30%) of NaCl, their signal responses decreased. These results could be explained by the salting-

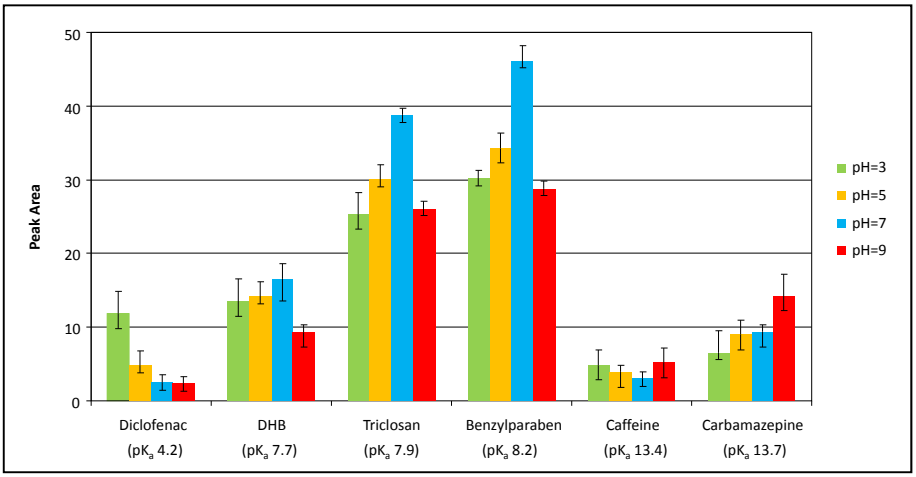


Fig. 1. The effect of sample pH on the analyte response for a representative group of compounds, whose pK$_a$ values are detailed in brackets.

out effect, which provided higher responses for the analytes, and then, when 30% of NaCl was added, the electrostatic interactions between the analytes and the salt ions caused a decrease in their signal responses [19]. Therefore, the addition of 15% of NaCl was chosen to provide better results for most of the studied analytes.

Regarding extraction time, the SBSE time was varied between 1 and 8 h. Fig. 2 details the effect of the extraction time on the analytes' response for a representative group of compounds. It was observed that the analytes almost reached the equilibrium in just 1 hour, except triclocarban, whose sorption onto the coating was lower than the rest of the compounds due to its apolar behaviour. When longer extraction times were applied, no significant improvement on the analytes' response was observed. As a compromise between the sensitivity and the time consumed, 1 h was selected as the optimal extraction time. This extraction step was performed in a short period of time in comparison with those provided by other in-house or commercial stir bars reported previously, which needed between 2 and 6 h to guarantee the achievement of the equilibrium at similar stirring rates or even higher [10,11,15,18,20,21]. As mentioned previously, the presence of PEGMA provides high permeability and swelling capacity to the monolith, which enables the analytes to go more easily into the in-house coating and interact quickly with the polar functionalities, providing a rapid SBSE performance.

The agitation speed was also studied and two agitation rates (500 and 1000 rpm) were tested. It was observed that similar results were obtained at both agitation speeds. However, it is expected that at a higher agitation speed, the monolithic coating would be damaged. Thus, to keep the integrity of our in-house stir bar, the agitation speed was set at 500 rpm for further analyses.

Finally, the extraction temperature was maintained constant at 25 °C during

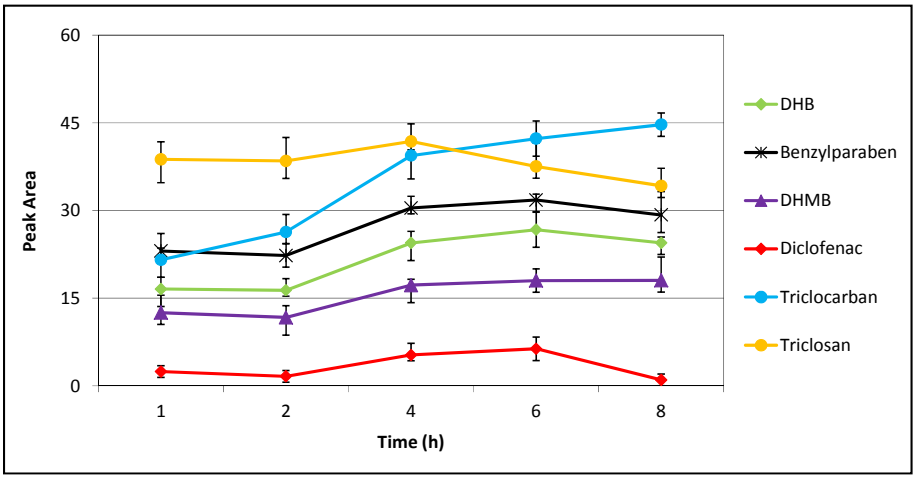


Fig. 2. The effect of extraction time on the analyte response for a representative group of compounds.

SBSE experiments, since no marked improvement in the analytes' response was observed at higher temperatures.

3.2.2. Liquid desorption conditions

To ensure the complete desorption of the analytes, different solvents were tested under the optimised extraction conditions: MeOH, ACN and a mixture of MeOH/ACN (1/1, v/v). When 5 mL of these desorption solvents were evaluated, it was observed that ACN provided a higher signal response than MeOH for all of the compounds. Moreover, stirring with 5 mL of a mixture of MeOH/ACN (1/1, v/v), slightly higher analyte responses were obtained in comparison with ACN. Therefore, a mixture of MeOH/ACN (1/1, v/v) was chosen as the desorption solvent for the subsequent experiments. When the solvent volume was increased up to 10 mL, the LD results did not improve. Finally, 5 mL of a mixture of MeOH/ACN (1/1, v/v) was selected to provide the best results and 5 mL was the minimum volume necessary to cover the in-house stir bar sufficiently, immersed in the desorption solvent.

In order to ensure the complete desorption of the target analytes from the polar coating, the desorption time was also investigated between 5 and 30 min.It was observed that stirring for 10 min was long enough to desorb all of the analytes from the in-house stir bar and it was selected as the optimal desorption time.

After the optimisation procedure, the SBSE conditions used for a further application in wastewaters were as follows: 50 mL of sample at pH 7, containing 15% of NaCl, extracted at room temperature by stirring at 500 rpm for 1h for the extraction and 5 mL of a mixture of MeOH/ACN (1/1, v/v), stirring at the same speed for 10 min for the LD. Under the optimal conditions, the recovery values (%) for each analyte in ultrapure water are detailed in Table 2. The in-house stir bar was able to extract most of the compounds with recovery values between 13% and

Table 2. Recovery values (%) obtained when different coatings were applied in SBSE of ultrapure water.

Analyte	Recovery values (%)		
	Poly(PEGMA-*co*-PETRA) [a]	EG Silicone Twister® [b]	Acrylate Twister® [b]
Paracetamol	5	-	<1
Caffeine	2	-	-
Benzotriazole	9	<1	<1
Antipyrine	2	<1	1
Propranolol	19	2	2
Pridinol	13	3	2
Methylparaben	20	1	2
Carbamazepine	25	<1	<1
Propylparaben	38	10	2
DHB	55	24	9
Benzylparaben	64	39	14
DHMB	56	26	9
Diclofenac	33	<1	<1
BP-3	55	45	10
Triclocarban	51	59	43
Triclosan	55	80	42

[a] 50 mL of sample spiked at 2 μg L^{-1}, %RSD (n = 3) were lower than 10% for %R > 20%.
[b] 50 mL of sample spiked at 4 μg L^{-1}, %RSD (n=3) were lower than 15% for %R > 10% [11].

64%, except for paracetamol, caffeine, benzotriazole and antipyrine (2% to 9%).

3.4. Comparison to commercial stir bars

The SBSE performance of the poly(PEGMA-*co*-PETRA) monolithic coating was compared with two comercially available polar stir bars (EG Silicone Twister® and Acrylate Twister®), which have recently been commercialised by Gerstel. These commercial stir bars have been applied for the extraction of EOCs from environmental waters [11]. From that study, it was observed that, in fact, the polar commercial coatings were not as effective as expected under their optimised SBSE conditions. Table 2 shows the low recovery values obtained when both commercially available coatings were applied in SBSE of ultrapure water. While the Acrylate Twister® was not able to recover even the most apolar compounds, such as DHMB, BP-3 or triclocarban, the EG Silicone Twister® provided slightly better SBSE performance for those compounds with more apolar behaviour. However, both commercial coatings were barely capable of extracting the most polar analytes (%R < 3% for those with log $K_{o/w}$ < 3.4) even after a 4 hours extraction. A possible explanation could be that their coatings are PDMS-based, which is apolar. In contrast, the SBSE using the in-house coating provided higher recovery values for more polar compounds, extracting

for only 1 h, especially for those analytes with log $K_{o/w}$ between 1.9 and 3.4, achieving recovery values from 13% to 55%. This notable improvement could be attributed to the fact that a high number of hydroxyl and ester functional groups are present in the monolithic structure.

Most of the in-house coatings synthesised in the past have shown progress in terms of extraction efficiencies and sensitivity. However, their use still involved long extraction times (< 2 h) [14,15,18,19]. In this paper, the use of the in-house monolith as the SBSE coating enabled better and less time-consuming extraction of the studied analytes than those using the commercial or in-house stir bars, due to its suitable physical properties and high swelling capacity, promoting the diffusion of the analyte through the monolithic material. Moreover, the high permeability and hydrophilicity of PEG-acrylate or PEG-diacrylate monomers have recently reported when they were used to synthesise monolithic columns applied in normal phase and capillary LC for the separation of small molecules, such as peptides and proteins [22-25].

3.5. Application to environmental water samples

After SBSE optimisation, the in-house stir bar was applied for the extraction of PPCPs in wastewaters, such as influent and secondary effluent samples from a wastewater treatment plant (WWTP).

Taking into account the application of SBSE in complex matrices and the use of a ESI interface in LC-MS/MS, the effect of the ion suppression/enhancement was evaluated for influent and effluent wastewaters and calculated as the percentage decrease in the signal intensity obtained by the target analytes spiked at 100 µg/L after the extraction of 50 mL of real sample versus the intensity of the same amount of the analytes in ultrapure water [26]. As expected, higher values of ion suppression/enhancement were obtained in influent than in secondary effluent wastewaters due to the complexity of the matrix. For secondary effluent wastewater samples, this effect ranged from -6% to 27%, except for paracetamol and caffeine, whose ion suppression values were the highest ones, 31% and 43%, respectively. In contrast, the ion suppression/enhancement values for influent wastewaters increased and ranged between -22% and 24%, except for paracetamol, caffeine, methylparaben, propylparaben and triclocarban (29% to 48%). Moreover, similar results with respect to this effect were obtained when these analytes were extracted using commercial stir bars, providing ion suppression/enhancement values between -5% and 25%, except for propylparaben (44%) and triclocarban (54%) [11]. Although the resulting effect of ion suppression/enhancement was slightly significant for some compounds, especially the most polar ones, higher matrix effects (from -40% to 65%) were found when using other extraction techniques, such as solid-phase extraction (SPE), in comparison to SBSE, as reported previously [27-29].

The recovery values of the analytes when the in-house stir bar was used to extract PPCPs from wastewater samples are listed in Table 3. The recoveries obtained for both secondary effluent

Table 3. Recovery values (%) obtained for the studied analytes when the poly(PEGMA-co-PETRA) coating was applied in SBSE followed by LC-MS/MS of 50 mL of water samples spiked at 2 μg L^{-1} with the analyte mixture.

Analyte	Recovery values (%)	
	Secondary effluent WWTP[a)]	Inffluent WWTP[b)]
Paracetamol	3	4
Caffeine	1	2
Benzotriazole	8	9
Antipyrine	2	2
Propranolol	15	17
Pridinol	-	-
Methylparaben	12	14
Carbamazepine	19	24
Propylparaben	24	27
DHB	28	37
Benzylparaben	45	50
DHMB	36	42
Diclofenac	25	31
BP-3	44	54
Triclocarban	49	31
Triclosan	43	43

[a)] % RSD (n=3) < 15% for %R > 12%
[b)] % RSD (n=3) < 18% for %R > 14%

and influent wastewaters were slightly lower than those achieved in ultrapure water (15% to 54% for the most apolar compounds). It should be pointed out that, taking into account the poor sorption of pridinol onto the coating, it was expected that this compound would not be recovered from real samples.

Once the applicability of the in-house stir bar was demonstrated in real samples and in order to compensate the ion suppression/enhancement, the method was validated using a matrix-matched calibration with 50 mL of secondary effluent wastewater. First of all, a blank sample was analysed and a number of compounds, such as propranolol, carbamazepine, benzylparaben, BP-3, triclocarban and benzotriazole, were detected. The calibration was then achieved by subtracting the signal of existing analytes in the blank. All of the compounds showed good linearity in a range between 50 and 5000 ng L^{-1}, except for DHMB (100-5000 ng L^{-1}), diclofenac (500-5000 ng L^{-1}) and triclocarban (100-5000 ng L^{-1}), with regression coefficients (r^2) greater than 0.997 (except for propranolol (r^2 > 0.991)). The method detection limits (MLDs) and method quantification limits (MQLs) were calculated as signal-to-noise ratio (S/N) of 3 or 10, respectively. MDLs were between 15 to 20 ng L^{-1} for all of the compounds, except for diclofenac (50 ng L^{-1}) and MQLs ranged

from 50 and 500 ng L^{-1}. Particularly, in the case of benzotriazole, which registered a high signal in the blank, the MDL and MQL were tentatively calculated as three or ten times the standard deviation of the analyte signal in the blank (*n*=3), respectively. The repeatability and reproducibility between days of three samples spiked at 2000 ng L^{-1}, expressed as % relative standard deviation (% RSD), were lower than 16% and 20%, respectively. In the present work, apart from the development of a rapid SBSE, this analytical methodology enabled a suitable method validation for all of the studied compounds, including those with polar behaviour, which were hardly recovered at all using the commercially available stir bars and for which, consequently, the validation method could not be applied [11].

The SBSE/LC-MS/MS method was then applied to the analysis of different environmental samples, such as influent, secondary effluent and tertiary effluent wastewaters. It is important to mention that the tertiary effluent wastewaters were included in the following quantification because the effective treatment plant, based on reverse osmosis, should provide clean water samples in order to reuse them again. For this reason, it was also decided to check the occurrence of the studied analytes at this point. The concentrations found for each analyte in the different wastewater samples are shown in Table 4.

Table 4. Concentrations of found analytes in influent, secondary effluent and tertiary effluent wastewater samples when they were analysed using the in-house stir bar for SBSE followed by LC-MS/MS

Analyte	Concentration (ng L^{-1})		
	Influent WWTP	**Secondary effluent WWTP**	**Tertiary effluent WWTP**
***Paracetamol**	> 5000	n.d.	n.d.
***Caffeine**	> 5000	<LOD	n.d.
***Benzotriazole**	3005	610	156
***Antipyrine**	n.d.	n.d.	n.d.
Propranolol	<LOQ	84	<LOQ
Pridinol	-	-	-
Methylparaben	> 5000	n.d.	n.d.
Carbamazepine	n.d.	246	n.d.
Propylparaben	2370	n.d.	n.d.
DHB	372.1	<LOQ	62
Benzylparaben	<LOD	<LOQ	<LOQ
DHMB	n.d.	n.d.	n.d.
Diclofenac	966	641	<LOD
BP-3	627	218	161
Triclocarban	n.d.	125	<LOQ
Triclosan	n.d.	n.d.	n.d.

* These compounds were tentatively quantified since their recovery values were lower than 10%. % RSD (*n*=3) < 23% n.d. = non detected

It should be pointed out that paracetamol, caffeine, benzotriazole and antipyrine were tentatively quantified since these compounds were slightly recovered in secondary effluent wastewaters (%R<10%). As can be seen, all of the studied analytes were detected in the analysed samples, except antipyrine, DHMB and triclosan. In influent wastewaters, the highest levels of ng L^{-1} were recorded for paracetamol, caffeine, benzotriazole, propylparaben, diclofenac and BP-3 (627 – 5000 ng L^{-1}). After the secondary treatment, it was observed that most of the studied compounds were effectively removed from the wastewaters, except benzotriazole, propranolol, carbamazepine, diclofenac, BP-3 and triclocarban. However, some analytes, such as benzotriazole, DHB and BP-3, still remained, even after the tertiary treatment (62.1, 155.9 and 160.7 ng L^{-1}, respectively), being discharged into groundwaters and surface waters to reuse them. Moreover, higher concentrations of some analytes (DHB, carbamazepine or triclocarban) were found in secondary or tertiary effluent than influent wastewaters. This fact could be attributed to a possible conversion of their conjugated metabolites to the original substances after the treatment processes as well as the sampling period which was not the same [30,31]. The presence of this group of PPCPs in similar samples was reported previously [18,32,33] with results that are strongly in line with the present study.

4. CONCLUSIONS

After an optimisation procedure including both synthesis and extraction, this was the first time that a polar monolithic coating for SBSE based on poly(PEGMA-*co*-PETRA) has successfully been applied for the extraction of a group of PPCPs from wastewaters. The presence of PEGMA provides a high degree of permeability in the polymer and promotes a high diffusion of the analytes, allowing rapid extraction in just 1 h.

Moreover, the results obtained in terms of extraction efficiencies for both polar and apolar compounds were better than those obtained using two new commercially available polar coatings (EG Silicone Twister® and Acrylate Twister®).

The combination of SBSE/LC-MS/MS was demonstrated for application in complex matrices, such as influent and effluent wastewaters, since no significant ion suppression/enhancement values were obtained for hardly any of the compounds (from -22% to 24%), except for paracetamol, caffeine, propylparaben and triclocarban.

Finally, the developed analytical methodology was evaluated in terms of linearity, LODs and precision, achieving low LODs (15-50 ng L^{-1}) for all of the selected compounds, overcoming the poor sorption of polar compounds onto the commercial stir bars. Moreover, the in-house coating for SBSE followed by LC-MS/MS allowed the detection and quantification of a broader range of analytes compared to commercial coatings in environmental water samples.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Science and Innovation (CTQ2011-24179) and the Department of Innovation, Universities and Enterprises (Project 2009 SGR 223) for financial

support. N. Gilart would also like to thank the Department of Innovation, Universities and Enterprises and the European Social Fund for a predoctoral grant (FI-DGR 2011).

REFERENCES

[1] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642.
[2] C.F. Duan, Z. Shen, D.P. Wu, Y.F. Guan, TrAC, Trends Anal. Chem. 30 (2011) 1568.
[3] J.M.F. Nogueira, Anal. Chim. Acta 757 (2012) 1.
[4] F. Sánchez-Rojas, C. Bosch-Ojeda, J. Cano-Pavón, Chromatographia 69 (2009) 79.
[5] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chim. 397 (2010) 2833.
[6] D.R. Klein, D.F. Flannelly, M.M. Schultz, J. Chromatogr. A 1217 (2010) 1742.
[7] M. Kawaguchi, R. Ito, K. Saito, H. Nakazawa, J. Pharm. Biomed. Anal. 40 (2006) 500.
[8] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.
[9] B. Sgorbini, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, M.R. Ruosi, C. Bicchi, J. Chromatogr. A 1265 (2012) 39.
[10] E. Fries, Anal. Chim. Acta 689 (2011) 65.
[11] N. Gilart, N. Miralles, R.M. Marcé, F. Borrull, N. Fontanals, Anal. Chim. Acta 774 (2013) 51.
[12] Y.L. Hu, Y.J. Zheng, F. Zhu, G.K. Li, J. Chromatogr. A 1148 (2007) 16.
[13] C.H. Yu, B. Hu, J. Sep. Sci. 32 (2009) 147.
[14] X.J. Huang, L.L. Chen, D.X. Yuan, X.B. Luo, J. Sep. Sci. 34 (2011) 3418.
[15] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, Anal. Chim. Acta 706 (2011) 135.
[16] R. Lucena, Anal. Bioanal. Chem. 403 (2012) 2213.
[17] N. Gilart, R.M. Marcé, P.A.G. Cormack, N. Fontanals, F. Borrull, Talanta (*Submitted*) (2014).
[18] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, J. Chromatogr. A 1225 (2012) 1.
[19] X.J. Huang, N.N. Qiu, D.X. Yuan, J. Sep. Sci. 32 (2009) 1407.
[20] X.J. Huang, N.N. Qiu, D.X. Yuan, J. Chromatogr. A 1194 (2008) 134.
[21] F.C.M. Portugal, M.L. Pinto, J.M.F. Nogueira, Talanta 77 (2008) 765.
[22] X. Chen, H.D. Tolley, M.L. Lee, J. Chromatogr. A 1217 (2010) 3844.
[23] Y. Li, M.L. Lee, J. Jin, J.P. Chen, Talanta 99 (2012) 91.
[24] X. Chen, H.D. Tolley, M.L. Lee, J. Sep. Sci. 32 (2009) 2565.
[25] B. Gu, Y. Li, M.L. Lee, Anal. Chem. 79 (2007) 5848.
[26] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[27] J. Martín, W. Buchberger, E. Alonso, M. Himmelsbach, I. Aparicio, Talanta 85 (2011) 607.
[28] A. Juan-García, J. Mañes, G. Font, Y. Picó, J. Chromatogr. A 1050 (2004) 119.
[29] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Talanta 74 (2008) 1299.
[30] M.A. Sousa, C. Gonçalves, E. Cunha, J. Hajslová, M.F. Alpendurada, Anal. Bioanal. Chem. 399 (2011) 807.

[31] T. Henriksen, R.K. Juhler, B. Svensmark, N.B. Cech, J. Am. Soc. Mass. Spectrom. 16 (2005) 446.

[32] M. Pedrouzo, F. Borrull, E. P Pocurull, R.M. Marcé, Water Air Soil Pollut. 217 (2011) 267.

[33] K. Yu, B. Li, T. Zhang, Anal. Chim. Acta 738 (2012) 59.

3.2.4. Discussion of results

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

In the previous sections, novel coatings for stir bar sorptive extraction (SBSE) with increased polarity, including both commercial and in-house coatings, have been presented and discussed in detail in each scientific paper. In this section, the most significant features regarding the results derived from these studies will be considered.

One of the aims of this Doctoral Thesis was the improvement of the extraction of polar organic contaminants using stir bar sorptive extraction (SBSE) through the use of new commercial and in-house coatings. As it has already been mentioned, since the only commercially available stir bar was based on polydimethylsiloxane (PDMS) until very recently, many in-house coatings were synthesised showing an enhancement of polarity and, therefore, extending the range of SBSE applications. In these studies, several commercial and in-house coatings for SBSE were presented. With respect to the commercial coatings, two new stir bar commercialised by Gerstel have recently been introduced on the market and they are based on poly(ethyleneglycol) (PEG)-modified silicone (EG Silicone Twister) and polyacrylate (PA) with a proportion of PEG (Acrylate Twister). Although these new commercial stir bars contain PEG and PA monomers with polar functionalities, they are still based on PDMS, resulting in a decrease in polarity. Moreover, they are expected to be synthesised by sol-gel methodology, and so are stable to thermal desorption (TD) as well as liquid desorption (LD).

With respect to in-house polar monolithic coatings, three in-house extracting phases for SBSE were synthesised using different polar functional monomers and crosslinkers and they were based on 2-hydroxyethyl methacrylate (HEMA) and divinylbenzene (DVB); HEMA and pentaerythritol triacrylate (PETRA); and poly(ethylene glycol) methacrylate) (PEGMA) and PETRA. Thanks to the polar functional groups present in the structures of the monomers, such as ester and hydroxyl groups, the in-house polar coatings worked better than the commercial ones for extracting polar contaminants from aqueous samples. It is important to note that not only hydrogen bondings can be established between the coatings and the analytes. In the case of the poly(HEMA-*co*-DVB) coatings, the analytes can also interact with the coating through π-π interactions, due to the presence of DVB, enhancing the sorption of those analytes with aromatic rings. Despite the fact that these in-house monolithic coatings had more polar functional groups than the commercial stir bars, these monolithic coatings were not as thermally stable as the commercial ones, and therefore, might not be suitable for TD. The main reason for this is because high temperatures can cause chemical and physical damage, such as cracks, making them incompatible with TD. Although thermal stability tests were not performed for these in-house coatings, it was observed that, for example, the synthesised coating based on poly(PEGMA-*co*-PETRA) started to break in the dry state, with it being necessary to keep it in aqueous solution all the time. Due to

the incompatibility of the monolithic coatings with TD and the low volatility and great variety of polarities of the studied analytes, in the case of most of the in-house polar monolithic coatings for SBSE reported in the literature, as well as those included in this Doctoral Thesis, desorption by LD was preferable in order to keep their integrity, before analysing the SBSE extract by liquid chromatography (LC). The thermal stability of in-house monolithic coatings is a pending issue in the research field of polymeric materials. Scientists should focus their efforts on this aspect in order to obtain suitable materials to be desorbed by TD and enhance the sensitivity of the whole method.

The mechanical stability of the coating is a crucial factor to take into account in SBSE, because the friction between the extracting phase and the bottom of the sample vessel can lead to physical damage. Moreover, the constant movement of the stir bar at high stirring rates can also cause losses of the coating. While the commercial stir bars presented a high mechanical stability at high stirring rates (1000-1500 rpm), the in-house coatings synthesised in the laboratory are sometimes characterised by a lack of mechanical stability because the coating is not attached strongly enough to the glass bar that covers the magnet. For this reason, one way to improve their stability was introducing a spring around the glass bar, while the monolithic coating would be displayed around it during the polymerisation. Our research group had previously synthesised SBSE coatings using this stir bar set-up and based on poly(vinylpyrrolidone (VPD)-*co*-DVB [1] and poly(methacrylic acid (MAA)-*co*-DVB) [2], with it being successfully applied for the SBSE of polar organic contaminants from environmental waters. The mechanical stability of the in-house coating was not only enhanced by the spring, but also by the composition of the functional monomer and crosslinker: the higher the content of crosslinker, the more rigid the coating obtained will be. Therefore, the optimisation of the ratio between the functional monomer and the crosslinker was essential to provide the optimal stability and rigidity to the SBSE coating. In general, all three in-house coatings contained a low content of crosslinker (20-50%), obtaining rigid but not fragile monolithic coatings. In the literature, the most common values of crosslinker content are between 70% and 90% when DVB is used [1,3,4]. In contrast, when *N,N'*-methylenebisacrylamide (MBAA) was used as crosslinker, a level of just 35% was required [5,6]. Therefore, this parameter is important to evaluate in order to obtain a compromise between rigidity and polarity. Although the synthesised monolithic coatings were physically stable, the stirring rates were kept at 500 rpm (lower than those applied using commercial stir bars) in order to keep the integrity of the coating. Moreover, the main disadvantage of a low content of crosslinker in these in-house monolithic coatings was the decrease in the specific surface area of the polymeric material. The higher specific surface area was attributed to the poly(HEMA-*co*-DVB) monolith (160 m^2 g^{-1}) due to the use of DVB as a crosslinker, which, thanks to its

structure, enabled the polymer network to be crosslinked, creating small pores that increase the specific surface area. In contrast, the other two in-house monoliths, in which the crosslinker was PETRA, presented really low specific surface areas, below 5 m^2 g^{-1}. This fact was attributed to the type of crosslinker used, since PETRA is more flexible than DVB and so the formation of pores is less usual. However, the type of porogen used was also a factor. The porogen also plays an important role because it must dissolve the monomers and participate in the pore formation. In poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA), methanol and 1-dodecanol were used to ensure complete dissolution of the monomers, even though they would provide large pores in the monolithic structure. Although they did not display good specific surface area in the dry state, these in-house monolithic coatings were characterised by their high swelling capacity, allowing the entrance of the analytes into the monolithic structure and so increasing the number of interactions sites. The poly(PEGMA-*co*-PETRA) monolith presented the highest swelling capacity.

More recently, a study reported by Ochiai et al. [7] has demonstrated the wear of the coating of the new commercial stir bar EG Silicone Twister under stirring conditions. In this study, a novel analytical methodology was proposed, in which the sample preparation involved a multi-SBSE, including the simultaneous use of EG Silicone and PDMS Twisters for extracting the analytes of interest from water samples. In this study, the PDMS-coated stir was used to stir the sample and extracting phase, while two PEG-coated stir bars and another PDMS-coated stir bar were fixed to the glass wall with a magnetic clip. With this SBSE set-up, several compounds with a wide range of polarities (log $K_{O/W}$ values ranging from 0.56 to 4.21) could be extracted in the same extraction process. The authors selected the PDMS-coated stir bar for stirring the sample because they observed that the PEG-coated stir bar suffered damage to the coating due to the friction with the sample vessel. Fortunately, in our study presented in this section in which EG Silicone was used, no physical damage of the coating was observed, with the whole analytical method being performed without any problems. The multi-SBSE procedure improved the individual performance of each stir bar greatly, increasing the enrichment factor between 2 and 7 for the most polar compounds. In this study, it is also noteworthy that those the individual performance of the EG Silicone Twister is not suitable at all for extracting polar compounds, showing recovery values below 28% for compounds with log $K_{O/W}$ values below 2.

The dimensions of the stir bars and the amount of extracting phase are completely different with respect to commercial and in-house stir bars. It is a well-known fact that the higher the amount of extracting phase, the higher the capacity and extraction efficiencies obtained. Table 1 shows the dimensions of the commercial and in-house

coatings used for SBSE. For instance, all three commercial stir bars presented low volumes of extracting phases, ranging from 24 µL to 32 µL, and their dimensions were 10 mm in length and a coating thickness of 0.5 mm. In contrast, the design of the in-house stir bars was slightly different with higher dimensions: 12 mm in length and polymer thickness of 1 mm, resulting in approximately 225 µL of polymer volume. It is important to note that the thickness of the coating was obtained thanks to a glass tube with a higher diameter, inside which the glass bar with the spring was placed. Therefore, the difference in the amount of extracting phase was huge and it resulted in higher extraction efficiencies for the analytes of interest, even with a shorter extraction time (1 h), than those obtained using the commercial stir bars.

Table 1. Dimensions of all commercial and in-house coatings for SBSE

Stir bar	**Twister (PDMS)**	**EG Silicone Twister (PEG)**	**Acrylate Twister (PA)**	**In-house coating (poly(PEGMA-*co*-PETRA))**
Dimensions				
Length (mm)	10	10	10	12
Thickness (mm)	0.5	0.5	0.5	1
Polymer volume (µL)	24	32	25	225

Once all three in-house coatings were synthesised, they were tested as coatings for the SBSE of a group of pharmaceuticals and personal care products (PPCPs) from aqueous samples. In preliminary tests, the results demonstrated the different sorption behaviours of these monolithic materials. Among them, the poly(HEMA-*co*-PETRA) coating provided the worst SBSE performance, even for the most apolar compounds. In contrast, poly(HEMA-*co*-DVB) and poly(PEGMA-*co*-PETRA) provided similar results, due to the presence of π-π interactions and the high number of polar functionalities, respectively. Although both monolithic coatings were unable to extract the most polar compounds, they could recover compounds with moderate to low polarity. In the end, poly(PEGMA-*co*-PETRA) monolithic material was the coating of choice for subsequent SBSE application, and is reported in the last study included in this section.

All three commercial stir bars together with the in-house monolithic coating, poly(PEGMA-*co*-PETRA), were evaluated for SBSE and tested for extracting PPCPs from wastewater samples and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The selected compounds presented different polarities, with log $K_{O/W}$ values between -0.6 and 6.1, and different polar functional groups. In Table 2,

Table 2. Optimised SBSE conditions for all commercial and in-house coatings.

Stir bar	Twister (PDMS)	EG Silicone Twister (PEG)	Acrylate Twister (PA)	In-house coating (poly(PEGMA-*co*-PETRA))
Extraction				
Sample volume (mL)	50	50	50	50
Sample pH	5	5	5	7
Salt addition (%)	-	-	-	15
Stirring rate (rpm)	1000	1000	1000	500
Extraction time (h)	4	4	4	1
Liquid desorption				
Desorption solvent	MeOH	MeOH	MeOH	MeOH/ACN (1/1)
Solvent volume (mL)	1	1	1	5
Desorption mode	Sonication	Sonication	Sonication	Stirring (500 rpm)
Desorption time (min)	15	15	60	10

the optimised SBSE parameters using the different commercial and in-house coatings are shown. For instance, the sample pH is an important parameter if the compounds and/or the coating structures contain acidic or basic functionalities. In particular, the analytes of interest presented several acidic and basic functional groups. Therefore, it was necessary that these compounds were in their neutral form to interact with both commercial and in-house coatings. Since the majority of the analytes had pK_a values between 7 and 9, most of the compounds showed their maximum signal response at pH 5 and 7. For commercial stir bars, no significant differences were observed between 5 and 7 while, for the poly(PEGMA-*co*-PETRA) coating, pH 7 seemed to be the most suitable for the studied compounds, although their signal responses did not vary as much in comparison to pH 5. The stirring rate is a parameter that was expected to be different between the commercial and in-house coatings for their different mechanical stabilities. While the commercial stir bars could support a stirring rate of 1000 rpm, the in-house stir bar lost part of its coating at stirring rates higher than 500 rpm. Moreover, since the extraction time was only 1 h, the stirring rate was high enough to bring about equilibrium. The most relevant parameter in SBSE is the extraction time, which is the time that the analytes need to reach equilibrium between the extracting phase and the liquid sample. The results derived from studying this parameter demonstrated that this parameter is more significant for all those compounds with higher affinity towards the extracting phase. For instance, for the PDMS-coated stir bar (Twister), while the most polar compounds did not show a significant improvement in their signal responses with longer extraction times, the most apolar compounds, such as triclosan, presented an increasing tendency at longer extraction times. In contrast, using the PEG-coated stir bar

(EG Silicone Twister), the compounds with moderate polarity demonstrated better affinity for this extracting phase with an increase in the analyte responses at longer times, in comparison to the PDMS- and PA-coated stir bars, thanks to the hydroxyl groups that enabled hydrogen bondings. In the end, the analytes reached equilibrium using all three stir bars at 4 h. Although the poly(PEGMA-*co*-PETRA) coating is thicker than the others, the time necessary to reach equilibrium for most of the analytes was approximately 1 h. For some compounds, an increase was observed in the analyte response at higher extraction times. However, this increase was not significant and 1 h was selected as the optimal extraction time, resulting in a much more rapid SBSE method. It is important to note that 30 min should have been tested to check if the recovery values were similar than those with 1 h or an important decrease in analyte response was observed.

With respect to the LD conditions, the main difference between the commercial and in-house stir bars was the desorption mode. While the commercial stir bars can undergo sonication without any damage to the coating, when the in-house stir bar based on poly(PEGMA-*co*-PETRA) was desorbed using an ultrasonic bath, part of the coating was damaged and lost. The use of sonication for the LD also helped to reduce the solvent volume needed. For example, 1 mL of methanol (MeOH) was necessary to immerse the commercial stir bars whereas, for the desorption of the in-house coating using stirring, 5 mL of a mixture of MeOH and acetonitrile (ACN) was the minimum volume required to immerse it, due to its higher dimensions. A relevant feature in LD conditions is the long desorption time that the PA-coated stir bar (Acrylate Twister) required (60 min) in comparison to the other stir bars (10-15 min). A possible explanation could be the stronger interactions that the carboxyl group of the PA-coated stir bar was able to establish with the basic functionalities of some compounds.

With respect to the recovery values, under SBSE optimised conditions, the PDMS-coated stir bar was only able to extract the most apolar compounds, while the best SBSE performance was obtained using the PEG-coated stir bar, with recovery values between 10% and 80% for compounds with log $K_{O/W}$ values over 3. However, it is worth mentioning that the PEG-coated stir bar did not allow the extraction of the most polar compounds, those with a log $K_{O/W}$ below 3, with it becoming a suitable extracting phase for the SBSE of compounds with moderate to low polarity. In contrast, the poly(PEGMA-*co*-PETRA) coating was able to improve the sorption of the studied analytes in comparison to the new commercial stir bars. This novel in-house coating allowed a better extraction of both apolar and moderately polar compounds with recovery values between 19% and 64% in ultrapure water. The most polar compounds (paracetamol, caffeine, benzotriazole and antipyrine) with log $K_{O/W}$ values below 1.5 were hardly

recovered. The results obtained from this new in-house coating were not as successful as those obtained from two previous in-house coatings synthesised by our research group and based on poly(VPD-*co*-DVB) [1] and poly(MAA-*co*-DVB) [2], which enabled recovery values between 45% and 110% for most of the compounds. The main reason could be attributed to the presence of DVB as a crosslinker, which promoted the sorption of those analytes with aromatic rings in their structure through π-π interactions. However, the long chains of PEGMA molecules with hydroxyl and ether functional groups, as well as the polar moieties of PETRA, facilitated and improved the sorption of moderate and low polarity compounds in comparison to the classical PDMS-coated stir bar or the new PEG-coated stir bar. The improvements in the sorption of polar compounds on SBSE coatings are evident in the studies presented, but there is an extensive field of research to continue working on novel polar polymeric materials for SBSE.

Apart from the recovery values studied, the matrix effect was also studied for all four stir bars in influent and effluent wastewater samples. The results showed low ion suppression/enhancement (between -22% and 27%) for most of the compounds in the matrices studied. The fact that no relevant ion suppression/enhancement was observed may be attributed not only to the lower sample volume used (50 mL) but also to the use of SBSE as the extraction technique, which, in other studies, has been considered to provide less matrix effect than other extraction techniques, such as SPE [8-10]. However, using selective sorbents for SPE, such as the in-house SCX monolithic sorbent included in Section 3.1.3, with similar volumes of wastewater samples (25-50 mL), the ion suppression/enhancement values ranged from -12% to 21% for several basic pharmaceuticals and illicit drugs. Therefore, it can be concluded that the use of selective materials, low sample volumes and the type of extraction technique can help to overcome this undesirable effect. For example, using the poly(PEGMA-*co*-PETRA) coating, the compounds that underwent stronger ion suppression were the most polar ones, such as paracetamol (31-33%) and caffeine (43-48%). This fact could be attributed to the co-elution of these analytes with many other polar interferences. The rest of the compounds presented acceptable values of ion suppression/enhancement, which were compensated using a matrix-matched calibration curve in 50 mL of effluent wastewater samples. Of the commercial stir bars, the PEG-coated stir bar was the best SBSE coating and it was selected for the method validation, and quantification of those compounds could be partially recovered. Both analytical methods reported similar sensitivity with method detection limits (MDLs) ranging from 15 to 50 ngL^{-1} for the in-house stir bar and between 5 and 10 ngL^{-1} for the PEG-coated stir bar. Subsequently, the quantification of several influent and effluent wastewater samples was performed using the poly(PEGMA-*co*-PETRA) and PEG-coated stir bars. Both methods report the presence of

the majority of the compounds in environmental waters and both particularly in agreement with respect to the high levels of concentrations of propylparaben, DHB and benzophenone-3 in influent wastewaters. A few studies have applied SBSE using the PDMS-coated stir bar followed by LC-MS/MS for extracting pharmaceuticals and PCPs from environmental waters, observing similar levels of sensitivity at low ng L^{-1} as those reported in our study [8,11,12]. Therefore, the new coating for SBSE followed by LC-MS/MS offered a suitable analytical methodology, which can be further exploited for other types of polar compounds in complex matrices.

References

[1] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, F. Borrull, N. Fontanals, Anal. Chim. Acta 706 (2011) 135.
[2] D. Bratkowska, N. Fontanals, P.A.G. Cormack, F. Borrull, R.M. Marcé, J. Chromatogr. A 1225 (2012) 1.
[3] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1217 (2010) 2667.
[4] X.J. Huang, N.N. Qiu, D.X. Yuan, Q.M. Lin, J. Chromatogr. A 1216 (2009) 4354.
[5] X.J. Huang, L.L. Chen, D.X. Yuan, X.B. Luo, J. Sep. Sci. 34 (2011) 3418.
[6] X. Huang, L. Chen, D. Yuan, Anal. Bioanal. Chem. 405 (2013) 6885.
[7] N. Ochiai, K. Sasamoto, T. Ieda, F. David, P. Sandra, J. Chromatogr. A 1315 (2013) 70.
[8] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chim. 397 (2010) 2833.
[9] C. Margoum, C. Guillemain, X. Yang, M. Coquery, Talanta 116 (2013) 1.
[10] A. Juan-García, J. Mañes, G. Font, Y. Picó, J. Chromatogr. A 1050 (2004) 119.
[11] D.R. Klein, D.F. Flannelly, M.M. Schultz, J. Chromatogr. A 1217 (2010) 1742.
[12] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1174 (2007) 27.

CHAPTER 4

CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

The main conclusions that can be drawn from the studies included in the present Doctoral Thesis can be summarised as follows:

1. Both selectivity and sensitivity are required in the development of analytical methods for determining organic contaminants in environmental samples and they can be achieved through both selective sample preparation techniques and sensitive detection techniques.

2. Selectivity in solid-phase extraction (SPE) has been achieved with the application of a commercial molecularly imprinted polymer (MIP) specific for non-steroidal and anti-inflammatory drugs (NSAIDs) and an in-house strong cation-exchange (SCX) sorbent for extracting pharmaceuticals and illicit drugs from wastewater samples.

3. The proposed molecularly imprinted solid-phase extraction (MISPE) performance is able to provide a selective and effective clean-up of the sample thanks to the imprinting cavities, which keep compounds retained that belong to the NSAID family (salicylic acid, naproxen, diclofenac, fenoprofen and ibuprofen) and other acidic pharmaceuticals and metabolites that are structurally related (gemfibrozil and clofibric acid), while at the same time eluting many interferences.

4. MISPE provides much cleaner extract and chromatograms than the other SPE sorbents, reducing but not eliminating the matrix effect in liquid chromatography-tandem mass spectrometry (LC-MS/MS). This undesirable effect was further compensated by developing a matrix-matched calibration curve in wastewater samples.

5. The effectiveness of MISPE in eliminating interferences has enabled the development of a rapid and simple method, consisting of the direct coupling between the commercial MISPE sorbent for NSAIDs and MS/MS and having been successfully applied for determining acidic pharmaceuticals in environmental waters.

6. To promote selectivity in SPE, two in-house strong cation-exchange (SCX) polymeric materials have successfully been synthesised via traditional polymerisation to be applied as sorbents for SPE, based on AMPSA/HEMA/PETRA and post-sulphonated HEMA/DVB.

7. The presence of sulphonic moieties in SCX sorbents was confirmed by the excellent SPE performance which selectively extracted a group of basic pharmaceuticals and illicit drugs from wastewater samples and removed matrix interferences.

8. The sulphonated HEMA/DVB polymeric sorbent is able to establish ion-exchange interactions as well as π-π interactions with the aromatic rings present in the analytes' structures, providing better SPE performance than the AMPSA/HEMA/PETRA sorbent in terms of recovery values and matrix effect, and being the sorbent of choice for determining the studied analytes in environmental waters.

9. Two new polar commercial stir bars, based on poly(ethylene) glycol (PEG) modified silicone (EG Silicone Twister) and polyacrylate (PA) with a proportion of PEG (Acrylate Twister), have been evaluated for the stir bar sorptive extraction (SBSE) of a group of pharmaceutical and personal care products (PPCPs) with different polarities.

10. Both EG Silicone and Acrylate Twisters offer a better SBSE performance than the classic polydimethylsiloxane (PDMS)-coated stir bar (Twister) for the extraction of low and moderate polar compounds. In particular, EG Silicone Twister was the stir bar of choice for the method validation, since it provides the best recovery values for the studied compounds.

11. Three in-house monolithic coatings for SBSE, based on poly(HEMA-*co*-DVB), poly(HEMA-*co*-PETRA) and poly(PEGMA-*co*-PETRA), have been synthesised with polar functional groups in their structures. The poly(HEMA-*co*-DVB) and poly(PEGMA-*co*-PETRA) coatings presented the best SBSE performance, as well as physical stability, during a series of qualitative experiments.

12. The in-house poly(PEGMA-*co*-PETRA) monolithic material was the SBSE coating of choice for extracting a group of PPCPs from environmental waters, obtaining better extraction capacities for moderate and low polarity compounds and low values of ion suppression/enhancement in comparison to the commercial stir bars.

13. The studies presented in this Doctoral Thesis have provided great improvements in the synthesis and application of selective materials for SPE, as well as polar coatings for SBSE. The results derived from these studies encourage us to continue synthesising new materials for sorptive extraction techniques to deal with the matrix effect and the extraction of more polar contaminants.

APPENDIX

UNIVERSITAT ROVIRA I VIRGILI
PREPARATION AND APPLICATION OF NOVEL SELECTIVE AND POLAR MATERIALS FOR SORPTIVE EXTRACTION OF EMERGING CONTAMINANTS FROM ENVIRONMENTAL WATERS
Núria Gilart Alzuria
DL: T 1098-2014

Appendix I. Abbreviations used in this Doctoral Thesis.

4-CP	4-chlorophenol
4-NP	4-nitrophenol
4-VIm	4-vinylimidazole
4-VP	4-vinylpyridine
ACN	Acetonitrile
Al_2O_3	Alumina
AMPSA	2-acrylamido-2-methylpropane sulphonic acid
AN	Acrylonitrile
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photoionisation
APTES	3-aminopropyltriethoxysilane
BM	4,4'-bis(maleimido)diphenylmethane
CAR	Carboxen
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CH_3COOH	Acetic acid
CMSt	Cyanomethylstyrene
CNTs	Carbon nanotubes
CW	Carbowax
DCM	Dichloromethane
DI	Direct immersion
DLLME	Dispersive liquid-liquid microextraction
DMN	Di(methacryloyloxymethyl)naphtalene
DPX	Disposable pipette extraction
DSDME	Directly suspended drop microextraction
DVB	Divinylbenzene
DVPh	Divinylbiphenyl
ED	Electrochemical detection
EDCs	Endocrine disrupting compounds
EGDMA	Ethylene glycol dimethacrylate
EOCs	Emerging organic contaminants
ESI	Electrospray ionisation
FD	Fluorescence detection
GC	Gas chromatography
GCBs	Graphitised carbon blacks
H_2SO_4	Sulphuric acid
HCOOH	Formic acid

HEMA	2-hydroxyethyl methacrylate
HF-LPME	Hollow-fibre liquid-phase microextraction
HILIC	Hydrophilic interaction liquid chromatography
HRMS	High resolution mass spectrometry
HS	Headspace
ICPMS	Inductively coupled plasma mass spectrometry
IDLs	Instrumental detection limits
ILISs	Isotopically labelled internal standards
ILs	Ionic liquids
IQLs	Instrumental quantification limits
IT	Ion trap
$K_{O/W}$	Octanol-water partition coefficient
LC	Liquid chromatography
LD	Liquid desorption
LLE	Liquid-liquid extraction
LPME	Liquid-phase microextraction
LTQ-FT-Orbitrap	Linear ion trap-Fourier transform-Orbitrap
LVI	Large-volume injection
MAE	Microwave-assisted extraction
MAN	Methacrylonitrile
MASPE	Methacrylic acid-3-sulfopropyl ester potassium salt
MDLs	Method detection limits
ME	Matrix effect
MEDDE	*p,p'*-dihydroxydiphenylpropane diglycidyl methacrylic ester
MEMDE	*p,p'*-dihydroxydiphenylmethane diglycidyl methacrylic ester
MeOH	Methanol
MEPS	Microextraction by packed sorbents
$MgSiO_3$	Magnesium silicate or Florisil
Mini-SAE	Miniaturised syringe assisted extraction
MIPs	Molecularly imprinted polymers
MISPE	Molecularly imprinted solid-phase extraction
MQLs	Method quantification limits
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MTBSTFA	N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide
NaCl	Sodium Chloride
NSAIDs	Non-steroidal anti-inflammatory drugs
NVIm	N-vinylimidazole

PA	Polyacrylate
PAHs	Polycyclic aromatic hydrocarbons
PANI	Polyaniline
PBBs	Polybrominated biphenyls
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCPs	Personal care products
PDMS	Polydimethylsiloxane
PDPA	Polydiphenylaniline
PEG	Poly(ethyleneglycol)
PEGMA	Poly(poly(ethylene glycol) methacrylate
PETRA	Pentaerythritol triacrylate
PGCs	Porous graphitic carbons
PLE	Pressurised liquid extraction
PNMA	Poly-N-methylaniline
POPs	Persistent organic pollutants
PPCPs	Pharmaceuticals and personal care products
PPy	Polypyrrole
PS-DVB	Poly(styrene-divinylbenzene)
PTVs	Programmable temperature vaporizers
PVP-DVB	Poly(N-vinylpyrrolidone-divinylbenzene)
QqLIT	Quadrupole linear ion trap
QqQ	Triple quadrupole
QqTOF	Quadrupole time-of-flight
SAX	Strong anion-exchange
SBME	Solvent bar microextraction
SBSE	Stir bar sorptive extraction
SCX	Strong cation-exchange
SDME	Single drop microextraction
SILPs	Supported ionic liquid phases
SiO_2	Silica
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM	Selected reaction monitoring
TD	Thermal desorption
TDU	Thermal desorption unit
TPs	Transformation products
USE	Ultrasonic extraction
UV	Ultraviolet-visible detection

VBC	Vinylbenzyl chloride
VN	Vinylnaphtalene
VOCs	Volatile organic compounds
VPh	Vinylbiphenyl
VSCs	Volatile sulphur compounds
WAX	Weak anion-exchange
WCX	Weak cation-exchange
WWTPs	Wastewater treatment plants

Appendix II. List of publications

N. Gilart, F. Borrull, N. Fontanals, R.M. Marcé, *"Selective materials for solid-phase extraction in environmental analysis"*, Trends Environ. Anal. Chem. 1 (2014) 8-18 (Section 1.1.2.1.)

N. Gilart, R.M. Marcé, F. Borrull, N. Fontanals, *"New coatings for stir bar sorptive extraction of polar emerging organic contaminants"*, Trends Anal. Chem. 54 (2014) 11-23 (Section 1.2.2.1.)

N. Gilart, R.M. Marcé, F. Borrull, N. Fontanals, *"Determination of pharmaceuticals in wastewaters using solid-phase extraction-liquid chromatography-tandem mass spectrometry"*, J. Sep. Sci. 35 (2012) 875-882 (Section 3.1.1.)

N. Gilart, R.M. Marcé, N. Fontanals, F. Borrull, *"A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography"*, Talanta 110 (2013) 196-201 (Section 3.1.2.)

N. Gilart, P.A.G. Cormack, R.M. Marcé, N. Fontanals, F. Borrull, "Selective determination of pharmaceuticals and illicit drugs in wastewaters using a novel strong cation-exchange solid-phase extraction combined with liquid chromatography-tandem mass spectrometry", J. Chromatogr. A 1325 (2014) 137-146 (Section 3.1.3.)

N. Gilart, N. Miralles, R.M. Marcé, F. Borrull, N. Fontanals, *"Novel coatings for stir bar sorptive extraction to determine pharmaceuticals and personal care products in environmental waters by liquid chromatography and tandem mass spectrometry"*, Anal. Chim. Acta 774 (2013) 51-60 (Section 3.2.1.)

N. Gilart, R.M. Marcé, P.A.G. Cormack, N. Fontanals, F. Borrull, *"Synthesis and characterisation of new polar monolithic coatings for stir bar sorptive extraction"*, Talanta (s*ubmitted*) (2014) (Section 3.2.2.)

N. Gilart, P.A.G. Cormack, R.M. Marcé, F. Borrull, N. Fontanals, *"Preparation of a polar monolithic coating for stir bar sorptive extraction of emerging contaminants from wastewaters"*, J. Chromatogr. A 1295 (2013) 42-47 (Section 3.2.3.)