Chapter 10

MIXED-MODE SORBENTS IN SOLID-PHASE EXTRACTION

N. Fontanals, F. Borrull, R.M. Marcé*

Departament de Química Analítica i Química Orgànica

Universitat Rovira i Virgili

Campus Sescelades

Marcel·lí Domingo, s/n

43007 Tarragona

Spain

*corresponding author:

Phone: (+ 34) 977 55 81 70

Fax: (+34) 977 55 84 46

E-mail: rosamaria.marce@urv.cat

ABSTRACT

In this chapter, a description is given of mixed-mode polymeric sorbents which enhance extraction selectivity and capacity in a single material. Different aspects of these materials are covered, including their synthesis, morphological and chemical properties, as well as their application in solid-phase extraction (SPE).

The SPE protocol for each type of mixed-mode sorbent (strong/weak and cation/anion-exchange materials) is discussed, since the protocol is crucial to the success of SPE for this kind of materials. Applications of the types of sorbent in different types of matrices are also discussed.

Abbreviations: Solid-phase extraction (SPE); strong-cation exchange (SCX); strong-anion exchange (SAX); weak-cation exchange (WCX); weak-anion exchange (WAX); ion-exchange capacity (IEC); high pressure liquid chromatography (HPLC); ultra high pressure liquid chromatography (UHPLC); hidrophylic interaction liquid chromatography (HILIC); gas chromatography (GC); capillary electrophoresis (CE); mass spectrometry (MS); tandem mass spectrometry (MS/MS); triple quadrupole (QqQ); ultraviolet (UV); diode array detector (DAD); fluorescence (FL);

10.1 Introduction

Over recent decades, solid-phase extraction (SPE) has emerged as the most commonly used and successful sample extraction technique, since it can enrich different type of analytes and extract them from their liquid matrices efficiently. One of the main advantages of SPE is its versatility, mainly due to the availability of different materials that cover the interactions with various analytes. Thus, one of the main aims of SPE research focuses on the development of novel materials that improve the properties of the already existing materials and, therefore, the results of SPE (Ramos 2012).

Firstly, chromatographic materials were adapted and used as SPE sorbents. Originally, the first SPE materials were silica-based and modified with groups such as C_{18}, C_8, phenyl, CH, CN and NH_2. However, silica-based materials present several disadvantages, such as instability at extreme pHs, low recovery in the extraction of polar analytes and the presence of some residual silanol groups. Carbon-based sorbents were another type of materials used, including graphitized carbon blacks (GCBs) and porous graphitic carbon (PGCs). However, the disadvantage of these sorbents is that they involve some difficulty when eluting certain compounds, and some of them even remain irreversibly adsorbed. Polymer-based sorbents were the latest development in materials. However, the conventional hydrophobic macroporous polystyrene-divinylbenzene (PS-DVB) still displays poor capacity and selectivity, as it has a specific surface area up to 800 m^2/g and interacts with the analytes through the hydrophobic interaction that occurs (Van der Waals force and π-π interactions of the aromatic rings that make up the sorbent structure).

To improve capacity, hypercrosslinked sorbents, with higher specific surface areas (up to 2,000 m^2/g) provide enhanced interaction with the analytes and, therefore, higher retention (Tsyurupa and Davankov 2002). The hydrophobic structure of the original porous polymers has also been improved by generating both hydrophilic macroporous and hypercrosslinked sorbents, which can also display hydrophilic interactions (hydrogen bonding and dipole-dipole interactions). The hydrophilicity of the sorbents can be introduced either by a hydrophilic precursor monomer or by chemically modifying the PS-DVB polymer skeleton (Fontanals et al. 2007).

With respect to selectivity, some tailor-made sorbents have been designed to interact selectively with the target compound(s) but remove all other analytes, including interferences. The first sorbents to be considered exclusively selective were immunosorbents (ISs), which have an immobilized antibody, which presents specific and selective interactions for the target compound (antigen). However, ISs have several drawbacks, such as being time-consuming to prepare, irreproducibility between batches, instability and limited use in aqueous media. To overcome these drawbacks, the 1990s saw the emergence of molecularly imprinted materials (MIPs), which are synthetic polymers that have specific cavities designed for a template molecule. During SPE, and because of their molecular recognition retention, MIPs are selective with respect to the target analyte or structurally-related compounds (Martín-Esteban 2013).

In recent years, research into SPE sorbents has focused on improving capacity and selectivity within a single material, leading to the emergence of what are known as mixed-mode polymeric sorbents. These sorbents combine a polymeric skeleton with ionic groups, with two types of interactions available: reverse-phase (RP) -from the skeleton- and ionic-exchange -from the ionic groups-. Mixed-mode sorbents are classified depending on whether the ionic group attached to the resin is cationic or anionic and, at the same time, whether it is strong or weak. The most common of these are sulphonic and carboxylic acid for strong and weak-cation exchange sorbents, respectively, and amines, quaternary for strong, and tertiary and secondary for weak anion-exchange sorbents. The benefit of mixed-mode sorbents is that the ion-exchange interaction between the sorbent and the analytes and/or interferences is turned on and off by the careful selection of washing and elution solvent pH, resulting in the selective protonation or deprotonation of the analytes or interferences, and even the sorbent (in the case of weak ion exchange sorbents). Thus, the interferences and analytes can be eluted separately during the washing and elution steps, respectively, thanks to the careful selection of pH and the solvent in each SPE step (Fontanals et al. 2010a).

At present, mixed-mode sorbents are one of the main focuses of research for manufacturers and companies. One of the reasons for this is, generally, the need for cleaner extracts from SPE and, in particular, preventing ion suppression/enhancement when these extracts are injected into liquid chromatography-mass spectrometry (LC-MS) systems. Therefore, despite being relatively new, they have been applied in various fields to extract different types of analytes in a selective manner from the matrix interference usually present in complex samples, such as those of biological, foodstuff and environmental origin (Fontanals et al. 2010a).

In view of the wide acceptance of these mixed-mode materials in SPE, in the present chapter, all of the aspects related with them are covered. The chapter is divided into three main sections that include: a description of the different approaches for their preparation, a discussion of the interactions displayed and the SPE protocol responsible for these interactions, and a selection of applications in different field analyses.

10.2 Fundamentals

As stated above, depending on the ionic group that functionalizes the mixed-mode polymeric sorbent, they are classified and intended for selectively retaining certain types of compounds. So, on one side, strong cation-exchange (SCX) sorbents have a polymeric skeleton modified with a strong anion-exchange (SAX) group, with the sulphonic group being most commonly used. The anionic group promotes cation-exchange interactions with the cationic compounds in the sample, while, most of the analytes (whatever their ionic state) and the interferences in the sample can also establish RP interactions with the skeleton. These features make them suitable for the selective extraction of (weak) basic compounds from complex matrices. In contrast, strong anion-exchange (SAX) sorbents have a polymeric skeleton modified with a strong cation-exchange (SCX) group, such as quaternary amine. The cationic moiety promotes anion-exchange interactions with the anionic compounds in the sample, while, in a similar way to the SCX sorbents, most of the analytes (whatever their ionic state) and the interferences in the sample can also establish RP interactions with the skeleton. These features of SAX sorbents make them suitable for the selective extraction of (weak) acidic compounds from complex matrices.

With respect to weak ionic exchange sorbents, the difference in comparison to their strong counterparts is that, in weak ion-exchange technology, the weak ionic group in the sorbent may be charged or not depending on the pH whereas, in strong ion-exchange, the ionic moiety always remains charged. Specifically, weak cation-exchange (WCX) sorbents are modified with weak anion-exchange (WAX) groups, with carboxylic acid being one of the most common. These groups promote cation-exchange interactions with the anionic compounds in the sample, and, like other mixed-mode sorbents, the rest of compounds can establish RP interactions. WCX sorbents are designed to extract (strong) basic compounds selectively from complex samples. Weak anion-exchange (WAX) sorbents are modified with weak cation-exchange (WCX) groups, such as tertiary or secondary amines. These protonated amines promote anion-exchange interactions with the anionic compounds in the samples, while the rest of the compounds can also be interacted through RP interactions. This type of mixed-mode sorbents are designed to extract (strong) acidic compounds selectively from complex samples.

10.3 Preparation of the Mixed-Mode Sorbents

This section, which is divided into commercially available and in-house sorbents, describes the preparation of the different mixed-mode polymeric sorbents or the moiety that modifies them. It also includes, when available, information regarding the morphology of the different mixed-mode sorbents as well as their ion-exchange capacity. This information is really useful for predicting the retention mechanism involved with each type of sorbent.

10.3.1 Commercial Sorbents

To the best of our knowledge, all of the commercially available mixed-mode sorbents are based on a previously designed and commercialized polymeric sorbent that displays hydrophobic and/or hydrophilic interactions with the skeleton itself and then further modified with ion exchange groups. Table 1 summarizes the commercially available mixed-mode ion exchange sorbents together with their main features.

Table 1:

One such sorbent is Oasis HLB (Waters Corporation, Milford, MA, USA), which is based on polyvinylpyrrolidone-divinylbenzene (PVP-DVB) and has a specific surface area of 800 m^2/g. It has been further modified with each of the four different types of ions in order to be converted into each type of mixed-mode sorbent: SCX, SAX, WCX and WAX, the resulting versions being known as Oasis MCX, Oasis MAX, Oasis WCX and Oasis WAX, respectively. In particular, Oasis MCX is modified with sulphonic groups (pK_a <1) after reacting the precursor resin (Oasis HLB) with concentrated sulphonic acid (Brousmiche et al. 2008). Figure 1 outlines the synthetic routes to obtaining all of the Oasis-based mixed-mode sorbents. Oasis MAX is modified with a dimethylbutylamine (DMBA) moiety, which is the amine that worked best in comparison to the rest of the amines tested: triethylamine, diethylethylamine, dimethylmethylamine and DMBA. Oasis MAX is obtained from an intermediate chlorinated resin from the precursor (Oasis HLB). In addition, the reaction conditions have been also optimized, and research revealed that the amination reaction was complete after two hours at reflux (93ºC), since no significant increase in the ion exchange capacity (IEC) took place beyond this point (Brousmiche et al. 2008). In a similar way, Oasis WAX is obtained by further modifying the chlorinated Oasis HLB skeleton with the piperazine group, which display a higher IEC (0.86 meq/g) compared to the other amines tested: dibutylamine (0.35 meq/g), diisopropylamine (0.15 meq/g) and morpholine (0.59 meq/g). In all cases, however, the reaction time rose to 18 h compared to the 2 h reaction time for Oasis MAX. Oasis WCX is also obtained from the intermediate chlorinated resin, which is further oxidized into carboxylic groups in presence of hydrogen peroxide and various catalysts (Brousmiche et al. 2008).

Figure 1:

It should be mentioned that the preparation procedure of the Oasis-based mixed-mode sorbents is reported in a published study (Brousmiche et al. 2008), and this is why it is possible to describe the preparation procedure in detail. This is not the case for the rest of the mixed-mode sorbents detailed in Table 1, for which, in most cases, the maximum information available is the moiety that modifies the resin. However, not even this information is available in other cases. Nevertheless, from the properties and the interactions displayed, significant differences in their preparation are not expected in comparison to those already reported for Oasis-based mixed-mode sorbents.

10.3.2 In-House Sorbents

Our research group was the first to pioneer the preparation of in-house mixed-mode polymeric sorbents. Resins were synthesized for each type of mixed-mode sorbent (i.e. SCX, SAX, WCX and WAX). Table 2 lists all of the in-house mixed-mode sorbents described in this section. The unique feature compared to commercially available sorbents comes from the polymeric skeleton. While commercially available sorbents are prepared from macroporous structures, our in-house mixed-mode sorbents are prepared from hypercrosslinked structures, which enhance RP interactions through their greater specific surface area (1,000 – 1,500 m^2/g). Specifically, the hypercrosslinked resins are prepared from vinylbenzylchloride (VBC)-DVB precursors obtained by precipitation polymerization (PP), which produces low micron size particles that are more suitable for SPE. The particles obtained, named as PP, were further hypercrosslinked (HXLPP) by means of the Friedel-Crafts reaction, where the chlorine moiety in the VBC monomer acts as an internal electrophile. Figure 2 shows the different approaches adopted to obtain the various in-house mixed-mode sorbents.

<u>Table 2:</u>

In particular, for the synthesis of SCX, the HXLPP resins were post-functionalized with either acetyl sulphate or lauroyl sulphate, with the latter proving to be the most effective reagent for the sulphonation. After optimizing different variables affecting the synthesis, three HXLPP-SCX resins were prepared with different contents in terms of the percentage of the sulphate reagent (i.e. 15%, 20% and 50%). Finally, the HXLPP-SCX sorbent modified with 50% sulphate reagent displayed the highest IEC (2.5 meq/g) and the largest specific surface area (1370 m^2/g) which might be indicative of sulphone bridge formation (Cormack et al. 2012).

<u>Figure 2:</u>

The procedures to obtain HXLPP-SAX (modified with 5% and 10% dimethylbutylamine – DMBA) resins were slightly different. In this case, the DMBA in the appropriate ratio was firstly reacted with the VBC-DVB precursor (named PP), that had been already obtained via the usual PP procedure, to obtain the PP-SAX particles. The quaternized precursors (PP-SAX) were then hypercrosslinked following the usual procedure and HXLPP-SAX resins were obtained. The authors discovered that amination was less efficient after hypercrosslinking due to the bulky nature of the tertiary amine (DMBA) restricting the amine's access to the free chloromethyl groups: therefore, amination was conducted before hypercrosslinking (Bratkowska et al., 2012b). Figure 2A outlines the synthetic route for obtaining the HXLPP-SAX sorbents as an example of the pre-hypercrosslinked chemical modification.

In contrast, post-hypercrosslinked chemical modification was used in order to prepare HXLPP-WAX (modified with piperazine, HXLPP-WAX-piperazine, and ethylenediamine, HXLPP-WAX-EDA) (Figure 2B). In these cases, the amine moieties were introduced by modification through the remaining chlorine groups already present in the HXLPP resin. Specifically, firstly HXLPP resin was swollen in dried toluene and then a 5 molar excess of the secondary amine (either EDA or piperazine) relative to chlorine was reacted for 18 h at 85°C (Fontanals et al. 2008).

Alternatively, in the synthesis of the HXLPP-WCX sorbent, the carboxyl moiety was introduced in the precursor monomer (methacrylic acid - MAA) used in the hypercrosslinking process. In other words, the terpolymer MAA-VBC-DVB in a 10/50/40, w/w% ratio was the precursor polymer in this hypercrosslinking reaction (Bratkowska et al. 2010). In this way, the carboxylic moieties were already present from the beginning of the reaction, and, as their incorporation into the HXLPP-WCX was satisfactory (equivalent to 0.72 meq/g as IEC), the authors did not evaluate the possibility of introducing the carboxylic group in a post-hypercrosslinked modification. Moreover, it should be mentioned that the IEC obtained for the HXLPP-WCX (0.72 meq/g) was similar to that reported for the commercially available sorbents, such as Oasis WCX (0.75 meq/g) and Strata-X-CW (0.74 meq/g). However, the specific surface area was larger (1125 m^2/g for HXLPP-WCX compared to ~800 m^2/g).

In an initial study, N-vinylimidazole-DVB, which was designed as hydrophilic sorbent, prepared by conventional suspension polymerization, was subsequently classified as a SAX sorbent because the imidazole group that it contains may or may not be protonated depending on the pH, and thus may interact ionically with the analytes (Fontanals et al., 2006). The main difference with NVIm-DVB as the SAX sorbent compared to the others is that the positive charge in the imidazole group of NVIm-DVB is delocalized across the imidazole ring whereas, with the other SAX sorbents, the quaternary ammonium group bonds to the polymer network through an aliphatic carbon and, as such, cannot be delocalized because the charge centre is not in conjugation with the polymer.

Another group of materials that have been also classified as SAX mixed-mode polymeric sorbents are supported ionic liquid phases (SILPs), as their nature is mostly based on imidazolium-based functional groups immobilized onto silica or polymer supports, giving rise to SAX interactions though the nitrogen moiety cation. To date, the SILPs have been prepared onto a polymeric support that is based on VBC-DVB combining N-methylimidazole cations with trifluoroacetate (Fontanals et al. 2009), tetrafluoroborate and trifluoromethanesulphonate (Bratkowska et al., 2012a), and N-butylimidazole with chloride (Zhu 2011). Figure 2C shows the synthetic route for obtaining one of these SILPs modified with tetrafluoroborate. One feature of these SILPs is that they are prepared from a polymer support that contains 2% of crosslinker (DVB), which has a very low specific surface area. Therefore, their main retention mechanisms are through the cationic groups attached to this polymer. It should be noted that, to date, there are no commercially available SILPS. In addition, there are more SILPs that were prepared and evaluated under SAX interaction mechanisms (Kirchner 2009, Vidal et al. 2012). However, they are silica-based and are not therefore considered in this chapter, which is more dedicated to mixed-mode polymeric sorbent.

Along similar lines, other research groups have developed mixed-mode materials, but without a polymeric skeleton. An interesting example of this is the hybrid organic-inorganic silica monolith initially modified with mercapto moieties, which after oxidation were converted into sulphonic acid groups that provide SAX interactions in the monolith (Zheng et al. 2009). Multi-walled carbon nanotubes have been non-covalently functionalized with poly(diallyldimethylammonium chloride) to create SAX sorbents (Kanaujia et al. 2011). The mixed-mode properties of the admicelles (based on sodium dodecyl sulphate – tetrabutylammonium) have also been reported as an alternative material for retaining ionic compounds (Luque and Rubio 2012).

10.4 Solid-Phase Extraction Conditions

As mentioned above, one of the critical aspects of mixed-mode sorbent technology is the selection of a suitable SPE protocol which, as is well-known, includes 4 steps, namely (1) conditioning, (2) sample loading, (3) washing and (4) elution. In this section, the SPE protocols recommended for each type of mixed-mode sorbent are described. It should be mentioned that the volumes in each step are not indicated, as they depend on different factors, such as the amount of sorbent, the type and complexity of sample, type of application, and the strength or weakness of the interaction, among others. Schematically, Figure 3 also summarizes all four protocols and the general properties of the analytes most likely to ionically interact with each type of sorbent. It should remembered that the conditioning step is the same in all four protocols and consists of organic solvent followed by aqueous solution, under conditions very similar to those applied for the sample.

Figure 3:

It should be also pointed out that, in some studies, the mixed-mode polymeric sorbents are evaluated or applied using an SPE protocol that differs from the recommended protocol, insofar as the pH and solvent are not optimized in each SPE step. This might lead to drawing erroneous conclusions from the performance of mixed-mode polymeric sorbents. This issue will be addressed after presenting each of the recommended protocols.

10.4.1 SCX Sorbents

When working with SCX sorbents, the aim of the various SPE steps is to switch the chargeability of the analytes (or interferences) because of the SAX properties ($pK_a<1$) of the modifying group in the sorbent, which remains deprotonated under all of the conditions of the SPE steps. In this respect, weak basic compounds are the most suitable analytes for extraction with SCX sorbents (as they are cations that can switch chargeability).

After conditioning, the sample is adjusted to a low pH (usually about pH 3) in order to protonate the analytes and establish ionic interactions with the SAX moieties in the sorbent, and then loaded. The washing step can then be split into two parts. Firstly, the acidic aqueous solution is compromised with the aim of removing the water-soluble compounds poorly retained by RP interactions as well as further activating the ionic interactions between the analytes and the sorbent. This step is not included in all protocols. The second part of the washing always forms part of the protocol and involves adding pure organic solvent (usually pure MeOH or ACN) that disrupts the RP interactions between the neutral and acidic compounds or interferences in the sample and they elute. Finally, the basic analytes elute with basic solution (generally from 2% to

10% NH_4OH) in organic solvent (MeOH or ACN). The basic solution ensures the protonation of the analytes (becoming neutral) and the elution strength of the solvent elutes the analytes, while the sorbent remains deprotonated.

Most of the examples in the literature use these guidelines as the optimal SPE protocol that ensures the efficient use of the sorbent. However, there are some studies that vary slightly. For instance, in the extraction of illicit drugs from wastewaters using Oasis MCX in all instances, some authors (Bijlsma et al. 2009, Pedrouzo et al. 2011) performed the washing step only with a basic aqueous solution, while the recommended solution should be acidic. In any case, this basic solution might further deprotonate the acidic analytes and release them from the cartridge, if these analytes are water-soluble. Other studies (González-Mariño et al. 2011, Sousa et al. 2011), however, performed the washing step with the solutions recommended in the protocol (i.e. acidic aqueous solution followed by pure MeOH), and the only observation is that the acidic illicit drugs (e.g. THC and its metabolite THC-COOH) eluted with the pure MeOH (González-Mariño et al. 2011, Sousa et al. 2011). In any case, under similar detection instrumentation (i.e. ESI-QqQ-MS/MS), when the methanol washing step was included, the matrix effect is lower and, consequently, the levels of detection achieved are lower (Pedrouzo et al. 2011, Sousa et al. 2011).

Certain other studies performed a detailed optimization of the parameters affecting SPE. For instance, for the extraction of a group of benzodiazepines from blood using Oasis MCX, the authors tested different organic solvents (MeOH, ethanol, isopropanolol, isobutanol, ACN and ethyl acetate) combined with an initial aqueous HCl to wash out the interferences. The authors found that the mixture based on 40% 0.15 M HCl aqueous and 60% isopropanol yielded significantly cleaner extracts (Karlonas et al. 2013).

10.4.2 SAX Sorbents

In a similar way to that described above, the aim of the different SPE steps in the SAX sorbents is to switch the chargeability of the analytes (or interferences) because the SCX properties ($pK_a > 18$) of the quaternary amine moiety in the sorbent remains protonated under all of the conditions in the SPE steps. In view of this, weak acidic compounds are suitable candidates for extraction by SAX sorbents (as they are anions that can switch chargeability).

To enhance the potential of the SAX sorbents, the sample is loaded at pH 7 to ensure that the acidic analytes become deprotonated and can establish ionic interactions with the quaternary amines in the sorbent. The subsequent washing step optionally includes basic aqueous solution for rinsing the water-soluble compounds and further activating the ionic interactions. The second part of the washing step is based on pure organic solvent (MeOH or ACN), which washes the basic and neutral analytes or interferences that only interact with the sorbent by RP interactions. The elution step is performed with acidic solution (2-10% HCOOH) in MeOH or ACN. This acidic solution ensures that the acids are protonated (become neutral) and the ionic interactions with the sorbent (that remains charged) are disrupted, while the elution strength of the organic solvent elutes these acidic analytes.

There are some examples, however, that modified the recommended protocol. Some of them refer to loading the sample in organic solvent that has been basified. This practice is quite usual when the sample has already been treated in another extraction step, such as the protein precipitation with ACN (Xia et al. 2009) or with MeOH (Landberg et al. 2009). The analytes now dissolved in the organic solvent can be loaded directly into the SAX cartridge, thereby avoiding the time-consuming step of evaporating and re-dissolving the sample in an aqueous-based solvent.

In another example, a group of benzotriazoles in aqueous matrices were extracted using Oasis MAX and eluting the analytes merely with MeOH. These basic analytes were not able to interact ionically with Oasis MAX sorbent. However, the authors found that MAX cartridges provided cleaner extracts with lower complexity compared to Oasis HLB, which might be attributed to the proper interaction of the acidic interferences with the amine moieties of the sorbent (Emotte et al. 2012). The authors, however, did not test any cationic-exchange sorbent, which might produce cleaner extracts and selective extraction of the target compounds.

It should be pointed out that when SILPs were tested as SAX sorbents, the volume and percentage of the acidic additive was larger, which might be necessary to disrupt stronger ionic interactions between the analytes and the sorbent (Bratkowska et al. 2012a). For instance, for the elution of a group of acidic pharmaceuticals from SILPs (200 mg packed in a cartridge), it was necessary to use 15 mL of 10% HCOOH in MeOH, whereas the most common elution conditions involve 5 mL of 5% HCOOH in MeOH (Bratkowska et al. 2012a). Meanwhile, it should be also noted that SILPs also tolerate washing with larger volumes of MeOH (e.g. 20 mL (Fontanals et al., 2009) and 10 mL (Bratkowska et al. 2012a;) without losses, while the usual washing volume is not higher than 5 mL in other SAX sorbents.

10.4.3 WCX Sorbents

In weak ion exchange technology, the aim of the different SPE steps is to switch the chargeability of the analytes or that of the sorbents, since the ionic group that modifies the sorbent has more tunable pK_a, which enables more successful changes depending on the pH in the different SPE steps. Specifically, in WCX, the carboxylic acid (which is the most usual anionic group for modifying the resins) can be protonated (neutral) or deprotonated (anionic) depending on the pH. Under these circumstances, the most suitable compounds to be extracted with WCX sorbents are (strong) basic compounds which might be in their cationic form, and therefore able to establish cationic interactions.

Following the protocol's recommendations, the sample is loaded under neutral pH conditions so that the carboxylic groups in the sorbent are deprotonated and the basic compounds protonated, as they are able to interact ionically. Apart from the ionic interactions, all of the compounds can be bonded to the sorbent through RP interactions. The subsequent washing step optionally begins with aqueous ammonium solution in order to further enhance the ionic interactions. However, at the same time, all of the water-soluble compounds that are weakly bonded are released. In addition, the compulsory second part of the washing step involves pure organic solvent that elutes (or washes out) all of the non-protonated (neutral and anionic acidic) analytes merely bonded through RP interactions in the sorbent. Finally, in the elution step, an acidic (2-10% HCOOH) solution in organic solvent ensures the protonation of the carboxylic groups in the sorbent and, thus, the ionic interactions with the basic (protonated) analytes are disrupted. The organic solvent elutes these basic analytes from the sorbent.

Some studies further optimize the SPE protocol. For instance, Bratkowska (Bratkowska et al. 2010) evaluated the performance of the extraction of an in-house HXLPPWCX sorbent for a group of basic pharmaceuticals. One part of the study focused on investigating the elution efficiency of different acidic solvent compositions that included: 5 mL aliquots of 2% HCOOH in MeOH, 2% trifluoroacetic (TFA) in MeOH and 2% TFA in MeOH/ACN (1/4). Eventually, 2% TFA in MeOH was selected for use in the elution step as it delivered the best results (higher recoveries than with 2% HCOOH in MeOH) and did not cause any significant disturbance in the LC separation of the analytes. TFA acted as the acidic additive in a solution composed of

ACN/H_2O (80/20, v/v) which was also used as an elution solvent to elute a group of peptides from Oasis WCX (Shi et al. 2009).

Some other studies evaluated WCX sorbents without using the recommended protocol (Allanson et al. 2007, Batt et al. 2008, Tylová et al. 2011, Weigel et al. 2004). For instance, in the determination of a group of pharmaceutical compounds from environmental waters, Oasis WCX, Oasis HLB and Oasis MCX were evaluated and compared using the recommended SPE protocol for SCX sorbents in all of the sorbents, which led to confusing results. Finally, Oasis MCX was the sorbent of choice, probably because it was the only option that was evaluated under its optimal conditions (Batt et al. 2008).

10.4.4 WAX Sorbents

Similarly to the WCX sorbents, the aim of WAX technology is to switch either the chargeability of the weak cation moieties (i.e. secondary or tertiary amines) in the sorbent, or the chargeability of the analytes/or interferences during the SPE steps. Indeed, the suitable candidates for WAX sorbents are (strong) acid compounds, which might be in its anionic form all or most of the time, and therefore able to establish anionic interactions.

The recommended protocol started with loading of the sample into the cartridge at neutral pH, with the aim of deprotonating (anionic) the acidic compounds, while the amines in the sorbent remain protonated (cationic) and are therefore able to establish ionic interactions. Similarly to the other mixed-mode sorbents, the rest of analytes and interferences can also display RP interactions with the skeleton of the sorbent. As in the other mixed-mode protocols, the washing step can be divided into two parts. Firstly, an aqueous acidic solution (normally using HCOOH as the acidic additive) is responsible for the solubilization, and so washing out, of water-soluble compounds, as well as for reinforcing the ionic interaction points. The second part of the washing step consists of pure methanol solvent (most commonly) which disrupts the RP interactions between the sorbent and analytes/interferences (neutrals and cationic basics) and washes them from the cartridge. Finally, the elution step is performed with a basic solution (2-10% NH_4OH) in organic solvent. The basicity neutralizes the sorbent and disrupts the ionic interaction, to elute the acidic analytes thanks to the elution strength of the organic solvent.

In the following examples, the WAX protocol used was the same as the one recommended. However, the authors take advantage of the dual properties of the sorbent to elute two groups of analytes separately and selectively, one group in the washing step and the other in the elution step. It should be noted that this strategy is also feasible with the other mixed-mode sorbents. In this line, a group of pharmaceuticals that includes basic and acidic compounds were loaded together in the in-house HXLPPWAX sorbents. Subsequently, 4 mL of MeOH eluted the basic pharmaceuticals, as well as washing out all the interferences. Finally, 2 mL of 2% NH_4OH in MeOH/ACN (1/4, v/v) eluted the acidic pharmaceuticals of the group (Fontanals et al. 2008). A similar strategy was used for the selective elution of the glucuronide and sulphate steroid conjugates. The glucuronide form was neutralized and eluted with 4mL of 10% HCOOH in $MeOH/H_2O$ (95/5, v/v) (which also served as the washing solution), whereas the sulphate species remained charged and retained in the cartridge. Then, 4 mL of 5% NH_4OH $MeOH/H_2O$ (90/10, v/v) was passed through, which neutralized the secondary amine in the cartridge and activated the elution of sulphate conjugates as the interactions between the polymer and the sulphate moieties were disrupted (Strahm et al. 2008a).

10.5 Application of the Mixed-Mode Sorbents

10.5.1 SCX Sorbents

Mixed-mode SCX polymeric sorbents are the type of mixed-mode sorbent that have been applied most often. One reason might be due to the fact that they are more widely available in different commercial forms or because their features make them more suitable for selective application to extract a group of compounds from their complex matrices. Table 3 shows selected examples of different applications of SCX sorbents to extract different types of compounds from samples with different complexities and the techniques used to determine the compounds. It should be noted that this table (as well as other tables of applications for each type of mixed-mode in this chapter) aims to illustrate the disparity of applications, rather than duplicates of the same application.

Table 3:

In most cases, the SCX sorbents are used strictly in off-line mode in SPE cartridges, as detailed in Table 3. In other cases, on-line SPE-LC (Li et al. 2011, Zhou et al. 2009) and 96-well plate (Cunliffe et al. 2009, Fountain et al. 2009, Harris et al. 2004, Xu et al. 2005) approaches have been also been successful in their applications. In particular, in the on-line SPE-LC set-up using Oasis MCX for the determination of a group of steroidal alkaloids, the challenge was to find a suitable solution that acts at the same time as acting as an eluting solution for the analytes ionically trapped on the SCX sorbent and also a mobile phase to separate them in the LC. The different solutions tested included 0.1% NH_4OH aqueous solution or 0.05% diethylamine aqueous solution. The latter gave the best results and peak performance and was selected for the study (Zhou et al. 2009).

Other examples included, for instance, Bond Elut Plexa PCX being used in a flow-injection column followed by flame atomic absorption spectrometry to preconcentrate and determine trace amounts of metals from urine (Anthemidis et al. 2012). Another example is the hyphenated technique LC-SPE-NMR for analysing alkaloids of complex mixtures, in which the role of the SPE with SCX sorbent was to retain these basic alkaloids selectively, while excluding the remaining interferences from the LC (Johansen et al. 2012).

Other studies focused on the comparison between different SCX sorbents (Klinke and Linnet 2007, López et al. 2011, Msagati and Nindi 2006). In particular, five different sorbents that include three different mixed-mode SCX polymeric sorbents (Strata-X-C, Oasis MCX and Bond Elut Plexa PCX) along with Bond Elute Certify (SCX silica-based sorbent) and Lichrolut EN (hypercrosslinked polymeric sorbent) were compared for the extraction of piperazine derivatives in wine. Initial experiments pointed to all of the mixed-mode SCX polymeric sorbents tested as the best, as the results did not show significant differences between them. However, when the volume of wine was increased, only Bond Elut Plexa PCX was able to be loaded with volumes up to 200 mL of wine, while the breakthrough volumes of Oasis MCX and Strata-X-C were lower than 40 mL and 120 mL, respectively (López et al. 2011).

When the duality of the mixed-mode polymeric sorbent is not sufficient to extract all of the target analytes selectively and quantitatively, the tandem approach can be adopted. A number of studies have tested how the performance of the whole SPE method improves by connecting one mixed-mode SCX sorbent in series with a C18 sorbent (Ivanov Dobrev and Kaminek 2002, Park et al. 2007, Tauxe-Wuersch et al. 2006) or with a polymeric sorbent (Díaz-Cruz et al. 2008, Gros et al. 2009, Izumi et al. 2009, Roberts and Bersuder 2006). In these studies, the main aim of the tandem approach is both to clean up the matrix of interferences (usually achieved by the mixed-mode sorbent) and to recover the target analytes. For example, Izumi et al. (Izumi et al. 2009) compared Oasis HLB with the tandem Oasis HLB and MCX for the extraction of hormones from plants. The purification procedure was more efficient when using the tandem approach and it was selected for further analysis. It should be mentioned, however, that some studies rejected the tandem option, since the SPE protocol resulted more complex and the results were not significantly better compared to those with a single cartridge (Díaz-Cruz et al. 2008, Gros et al. 2009, Roberts and Bersuder 2006).

10.5.2 SAX Sorbents

Mixed-mode SAX sorbents, and in particular Oasis MAX due to its long use in the field, have also been extensively applied for the extraction of different types of acidic analytes in different fields. Table 4 summarizes the details for the application of SAX sorbents which, as mentioned above, most of them corresponds to the use of Oasis MAX that has been used in a variety of applications.

Table 4:

For instance, closantel, an antiparasitic drug administrated to animals, was analysed in animal tissue, using Oasis MAX for the SPE in all cases (Lai et al. 2011, Sun et al. 2007, Yeung et al. 2010). Initially, it was adopted the off-line SPE mode (Sun et al. 2007, Yeung et al. 2010), but later, authors easily adapted the SPE protocol to the on-line mode (Lai et al. 2011), as the acidic organic mobile phase used in LC also worked as an elution solvent in the SAX protocol. Comparing both SPE modes, the authors found that the on-line mode provides higher sensitivity, and a less painstaking and time-consuming procedure. Interestingly, Hewitt et al. (Hewitt et al. 2011) used a 20 x 2.1 mm precolumn packed with Oasis MAX as the stationary phase in LC to separate a group of non-ionic surfactants commonly used in the formulation of protein pharmaceuticals, monitoring its hydrolysis in different formulations from different vendors.

In other studies, its performance has been compared to other sorbents. For instance, Koijima (Kojima et al. 2004) compared Oasis MAX with the silica-based SAX sorbent (Bond Elut SAX) for the extraction of alkylphenols from river water samples. The authors ruled out Bond Elut SAX because it provided lower recoveries, which were attributed to the decomposition of the sorbent due to the strong alkaline solutions (1 M NaOH in aqueous sample). The Cela research group compared the performance of Oasis MAX and Oasis HLB in two separated studies (Carpinteiro et al. 2010, Montes et al. 2010) to extract neutral compounds (trans-resveratrol -a phytochemical present in vegetables (Carpinteiro et al. 2010, Montes et al. 2010), and a group of fungicides (Carpinteiro et al. 2010, Montes et al. 2010)) in wine samples. Despite extracting neutral compounds, in both studies, Oasis MAX was the sorbent of choice, as it provided cleaner extracts. The authors justified these results because, after loading the sample, the basic washing step enabled acids and phenols (present in wine and acting as interferences) to become ionized and retained through ionic interaction with the amine moieties in the MAX cartridges. Subsequently, in the elution step with 1 mL of MeOH, just neutral and weak bases (as the target analytes) were recovered, free from acidic and phenolic interferences.

Other interesting studies compare different classes of mixed-mode sorbents, particularly those that compared SCX sorbents to SAX ones (Ge et al. 2006, Josefsson and Sabanovic 2006, Lara et al. 2006, Lavén et al. 2009, Siwek et al. 2008). For example, Oasis HLB, Oasis MCX and Oasis MAX were compared for the extraction of a group of quinolones from raw milk. The authors found that Oasis MAX provided better recoveries (%R ~ 90%) compared to Oasis MCX (0%) or Oasis HLB (%R ~ 50%). However, the elution conditions in Oasis MAX were not suitable for subsequent electrophoretic analysis. For this reason, a two-step procedure was adopted. This involved a first step with Oasis MAX to eliminate the proteins and remaining fat through the washing step, and a second step in which the cleaner extract was loaded into Oasis HLB, which offered an elution solution that was more suitable for capillary electrophoresis (CE) (Lara et al. 2006). Another study compared Oasis MAX and Oasis MCX for the extraction of a group of beta-agonists and beta-antagonists from blood. With Oasis MCX, most of the analytes eluted in the elution step after an effective washing step with methanol solution. In contrast, with Oasis MAX, the target analytes were fractionated and some eluted in the washing step. Therefore, Oasis MCX was selected for this study (Josefsson and Sabanovic 2006).

In multiresidue analysis, a development of a generic analytical method remains difficult when a high number of compounds have to be considered simultaneously. As a result, testing different sorbents with different properties is a common practice in multiresidue analysis, in which the main difficulty is selecting the best SPE sorbent and conditions to give acceptable recoveries for all compounds with different physicochemical properties (Batt et al. 2008, Culleré et al. 2010, Fauvelle et al. 2012, Kasprzyk-Hordern et al. 2007, Marchi et al. 2009, Musenga and Cowan 2013, Tylová et al. 2011, Weigel et al. 2004). For instance, Culleré et al. (Culleré et al. 2010) tested the retention ability of 13 different sorbents (7 RP sorbents, 4 mixed-mode SCX and 3 mixed-mode SAX) and 18 different aroma compounds (covering neutral compounds, organic acids and organic bases) at different pHs. Results showed that two of the RP polymeric sorbents presented the highest retention for most of the compounds. These results partly surprised the authors, who expected that basic compounds at acid pHs and acid compounds at basic pHs would have been more efficiently extracted by mixed-mode SCX and SAX, respectively. In any case, this suggests that ion-exchange interactions for these volatile compounds might be weak, and the RP interactions may be the most important mechanisms in this extraction. It should be noted that Tables 3 - 6 (which show the applications for the different mixed-mode sorbents) only provided those applications where the mixed-mode sorbents were selected after a comparative evaluation with other sorbents.

10.5.3 WCX Sorbents

In a similar way, WCX sorbents have been also applied for the extraction of basic compounds, all of which summarized in Table 5.

Table 5:

As can be seen in this table, they mainly focus on the determination of therapeutic and illicit drugs in environmental samples and fluids. Just two of these studies focus on the determination of biological compounds, using the 96-well plate mode. In the rest of studies, the WCX sorbent is used in the off-line SPE approach, with the exception of five studies that applied the on-line SPE mode. The on-line SPE with WCX sorbent is perfectly feasible since the acidic elution solvent (described in the WCX protocol) is matched with a typical LC mobile phase. These circumstances have been exploited by different authors that applied the on-line-SPE (WCX)-LC approaches (Chiuminatto et al. 2010, de Jong et al. 2007, Fontanals et al. 2013). In addition, in the determination of a group of therapeutic and illicit drugs from human urine using Strata-X-CW in on-line SPE mode, the authors claimed that changing of the pH value from 7 (SPE loading) to 2.5 (chromatographic mobile phase) enhances the separation in terms of both resolution and analysis time since it increases the elution of two of the drugs from the SPE column and decreases their retention time, as well as avoiding the peak tailing (Chiuminatto et al. 2010). Another study (Fontanals et al. 2013) that also determined a group of illicit drugs from complex wastewater samples using Oasis WCX in on-line SPE-LC-MS reported the reduction of the matrix effect when a mixed-mode sorbent involved an effective washing step based on methanol. In this way, signal suppression and ion enhancement up to 95% and 256% were reported, respectively, when the washing step was not included. Meanwhile, the matrix effect was considered negligible (10-20% at highest) when the washing step was included. Indeed, the washing step permitted the proper quantification of the target analytes without the use of expensive deuterated internal standards.

With respect to the sorbent comparison, in one study, Oasis WCX was the sorbent of choice from among different types of silica and polymer-based sorbents (no specific details were given), for the quantitative determination of six potential breast cancer biomarker peptides in human serum. The negatively charged WCX sorbent appeared to be only sorbent able to retain one of the peptides (bradykinin), which exhibited a high pK_a value. Therefore, it displayed good recoveries for all of the peptides studied (Shi et al. 2009). Huq (Huq et al. 2006) also compared how Strata-X, Strata-X-C and Strata-X-CW performed in the extraction of a group of tetracyclines from honey samples. In this comparison, they demonstrated that the neutral sorbent, Strata-X, was not efficient at extracting compounds like tetracyclines and also that the elution conditions used to extract tetracyclines from a SCX sorbent such as Strata X-C were not suitable for the MS detector. Therefore, the WCX sorbent, Strata X-CW, was a suitable alternative for efficiently extracting tetracyclines under appropriate conditions, as well as for obtaining cleaner extracts. A similar conclusion was drawn in other studies in which a group of fluoroquinolones were extracted from wastewaters using Oasis WCX as sorbent in both cases, which was selected after comparing it with Oasis HLB and Oasis MCX in terms of recoveries and cleanness of the extract (Lee et al. 2007b, Zorita et al. 2008). The above results might indicate that both WCX sorbents have similar features. In the results from the comparison, one should bear in mind that a balanced option should be adopted. For instance, when Oasis HLB, Oasis MCX and Oasis WCX were compared for the extraction of tetracyclines in wastewaters, on one hand, the neutral sorbent provided good recoveries for all the compounds, but the extracts contained a lot of interferences, which affect the ion-suppression in the tandem MS detection. On the other hand, Oasis MCX strongly retained the target analytes, and it was not possible to quantify them properly. Therefore, the alternative was Oasis WCX, which provided better recoveries and cleaner extracts than its neutral analogue (Oasis HLB) (Lee et al. 2007b, Zorita et al. 2008).

10.5.4 WAX Sorbents

WAX sorbents have been used to some extent for the extraction of compounds with weak acidic or strong acidic; the latter, in principle, are more suitable for extraction with this type of sorbent. Table 6 presents all of the detailed information which, in principle, covers all the examples in the literature that uses WAX sorbents.

Table 6:

For the extraction of domoic acid (a neurotoxin that causes amnesic shellfish poisoning) that carboxylic acid groups have (weak anion moieties), Regueiro et al. (Regueiro et al. 2011) selected WAX sorbent instead of the SAX sorbent previously reported in literature for the extraction of domoic acid from shellfish. The authors justified their selection because SAX sorbents are always charged at any pH, which leads to irreversible retention of strong acids present in the sample matrix and so its extraction efficiency is reduced after every extraction. In contrast, WAX sorbents can be easily reactivated by neutralizing their charge with a basic solution, which results in much longer lifespan.

The performance of Oasis HLB, Oasis MAX and Oasis WAX were compared for the determination of estrogens and their conjugates in river sediments. The results obtained showed that, when the targets were extracted from ultrapure matrices (e.g. Milli-Q water), both Oasis HLB and Oasis WAX displayed a similar performance. However, when dealing with the sediments, better results were achieved with Oasis WAX, which allowed a washing step to clean up the matrix (Matejicek et al. 2007). Similar arguments (cleaner extracts obtained in WAX sorbent) were also used for the selection of Oasis WAX sorbent instead of Oasis HLB, for the extraction of fluorescent whitening agents from environmental waters (Chen et al. 2006) or perfluorinated acids in water and biota (Taniyasu et al. 2005).

Other studies, however, compared the performance of the four types of mixed-mode sorbents. For instance, eight different sorbents: Oasis HLB, MAX, MCX, WAX, WCX, Chromabond C_{18}, Isolute ENV+ and Isolute HCX (a silica-based strong cation-exchange sorbent) were compared for the determination of 28 therapeutic and illicit drugs from surface waters. It was found that Oasis MCX provided the best extraction since the extracts were cleaner (suitable for injection into the UPLC-MS/MS) and higher recoveries were achieved for all the compounds studied (Kasprzyk-Hordern et al. 2007). The Oasis MCX option was also considered among the other mixed-mode Oasis-based sorbents (i.e. MAX, WCX and WAX) for the extraction of a group of

34 pharmaceuticals from urine. In this case, MAX and WAX sorbents were ruled out because they were unable to retain basic analytes, whereas WCX was unable to retain acidic compounds during the loading (Marchi et al. 2009).

As well as their comparison, to further exploit the complementary features of the different mixed-mode sorbents, they have been combined in a highly feasible tandem approach that consists of the combination of two (Lavén et al. 2009) or three (Yang et al. 2011) mixed-mode polymeric sorbents in series. For instance, Yang et al. (Yang et al. 2011) developed an SPE procedure that consisted of three mixed-mode polymeric sorbents: (1) Oasis MCX, (2) Oasis MAX, (3) Oasis WAX to recover 51 out of 56 metabolites involved in biological pathways (including carboxylic acids, sugar phosphates, amino acids, acyl-derivatives and nucleotides) because, individually, Oasis MCX was unable to retain carboxylic acids, which were suitably retained in Oasis MAX, and Oasis WAX was the only option that enabled the retention of phosphate sugars. Therefore, the tandem approach might be suitable when analytes with disparate physicochemical properties need to be extracted simultaneously.

On other occasions, the mixed-mode sorbents do not provide the best performance (Culleré et al. 2010, Emotte et al. 2012, Fauvelle et al. 2012, Gilart et al. 2012, Gros et al. 2006, Kusch et al. 2006, Seitz et al. 2006, Van De Steene et al. 2006, Weigel et al. 2004). For example, a group of artificial sweeteners in environmental water were determined by SPE/LC-MS/MS. At first, seven different sorbents (Oasis HLB, Isolute ENV+, Bond Elut Plexa, Strata-X as hydrophilic polymeric sorbents; Oasis WAX, Oasis MAX and Bond Elut Plexa PAX as mixed-mode sorbents) were compared. After preliminary experiments, the authors discarded these tested mixed-mode sorbents due to either excessive or poor retention of the analytes. Moreover, the recovery results obtained with the RP sorbents were unaffected by the matrix effect when dealing with more complex samples (Seitz et al. 2006). In another study (Gilart et al. 2012), Oasis HLB, Oasis MAX, Oasis WAX and Affinilute MIP – NSAIDs (a commercially available MIP selective for non-steroidal anti-inflammatory drugs) were compared for the determination of a group of acidic pharmaceuticals in terms of selectivity and capacity. In preliminary results involving SPE/LC-UV, Oasis MAX and Oasis WAX were discarded because, in both cases, a broad band appeared at the beginning of the chromatogram. This was explained due to the elution steps in basic and acidic media, which promoted the removal of acidic interferences from the matrix. Finally, the MIP was selected since it provided a very effective reduction of matrix interferences and selective extraction of target analytes. In any case, it should be highlighted that MIPs are not always available or designed for broader classes of compounds. In such cases, mixed-mode sorbents can play an important role since they have a certain degree of selectivity and greater capacity than MIPs.

In general, the selection of one type of sorbent depends on parameters such as the type of analyte and sample, the extraction conditions, and the compatibility between solutions. Moreover, there are other variables, such as the degree of selectivity or sensitivity achieved in each particular study. In view of this diversity of factors, selecting a suitable sorbent is not a simple or trivial matter, and some experimental tests are recommended to be performed before selecting one type of mixed-mode polymeric sorbent.

10.6 Conclusions

The development of mixed-mode polymeric sorbents that combine selectivity and capacity is a growing topic of research in SPE. This is widely demonstrated by the range of commercially available materials as well as numerous applications in the field. The applications of mixed-mode polymeric sorbents mainly focus on enhancing sensitivity while reducing the matrix effect usually encountered in complex samples analysed in MS/MS detectors. The optimization of the parameters involved in the extraction should be carefully performed and should take into account the type of analytes and matrix, the type of mixed-mode sorbent, and the SPE protocol.

Although the application in mixed-mode polymeric sorbents and its suitable protocols are starting to become well-established, further applications should be expected involving a broader range of extraction techniques (e.g. SPME, SBSE) or by preparing other materials with more sophisticated features.

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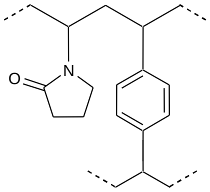
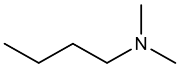
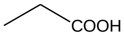
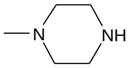
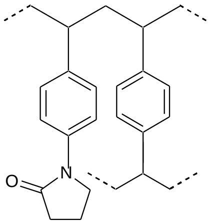
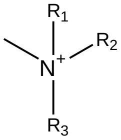
Figures legend

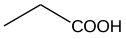
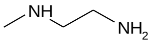
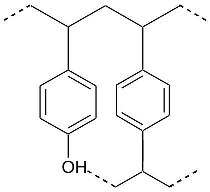
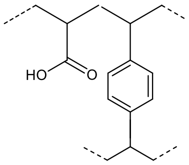
Figure 1. Examples of the synthetic route for a commercial available mixed-mode polymeric sorbent, (i) tertiary amine, Δ , (ii) H2SO4, r.t./Δ, (iii) secondary amine, Δ , (iv) 30% H2O2, Δ. Reprinted from [6] with permission of Elsevier.

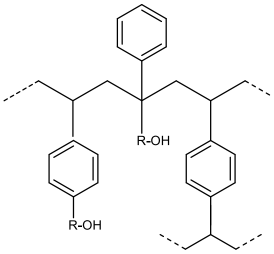
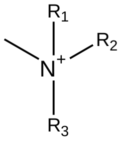
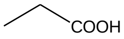
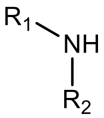
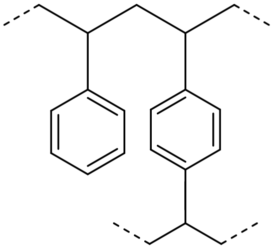
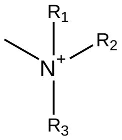
Figure 2. Synthetic approaches to prepare different “in-house” mixed-mode polymeric sorbents: A) HXLPP-SAX; B) HXLPP-WAX-EDA and HXLPP-WAX-piperazine; and C) SILPs. See the text for the synthetic conditions and sorbent properties.

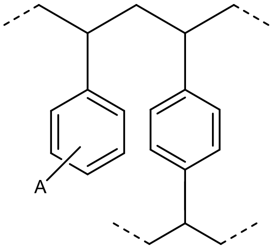
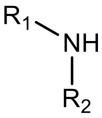
Figure 3. SPE protocols and type of analytes recommended for each type of mixed-mode ion-exchange polymeric sorbent. In brackets an example of most usual conditions.

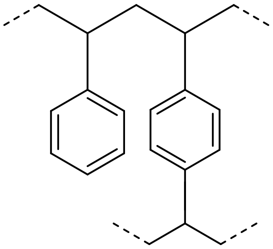
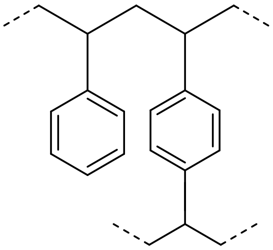
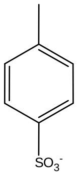
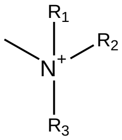
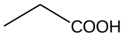
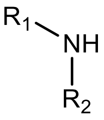
Table 1. Structure and properties of mixed-mode polymeric sorbents commercially available.

Supplier	Sorbent	Type of mixed-mode	Sorbent structure	
			Polymer based	Ionic group
Waters	Oasis MCX	SCX	Oasis HLB PVP-DVB (~800 m^2/g) 	—SO$_3$H
	Oasis MAX	SAX		
	Oasis WCX	WCX		
	Oasis WAX	WAX		
Phenomenex	Strata-X-C	SCX	Strata-X PS-DVB chemically modif. pyrrolidone (~ 800 m^2 g^{-1}) 	—SO$_3$H
	Strata-X-A	SAX		

	Strata-X-WC	WCX		
	Strata-X-AW	WAX		
Agilent Technol.	Bond Elut Plexa PCX	SCX	Bond Elut Plexa PS-DVB modif. hydroxyl groups (~550 m^2/g) 	n.d.
	Bond Elut Plexa PAX	SAX		n.d.
	Abselut NEXUS WCX	WCX	Abselut NEXUS (MAA-DVB) (~575 m^2/g) 	MAA
	Not available	WAX		

Biotage	Evolute CX	SCX	Evolute ABN PS-DVB hydroxylated 	$-SO_3H$
	Evolute AX	SAX		
	Evolute WCX	WCX		
	Evolute WAX	WAX		
J.T.Baker	Speed H_2O-Phobic SC-DVB	SCX	Speed H_2O-Phobic DVB PS-DVB 	$-SO_3H$
	Speed H_2O-Phobic SC-DVB	SAX	Speed H_2O-Philic DVB Hydrophilic PS-DVB	

	Not available	WCX	 (A = polar group)	
	Speed H_2O-Phobic WA-DVB	WAX		
Bonna- Agela Technol.	Cleanert PCX	SCX	No information	n.d.
	Cleanert PAX	SAX		n.d.
	Cleanert PWCX	WCX		n.d.
	Cleanert PWAX	WAX		n.d.
Polyintell	AttractSPE SCX	SCX	No information (~600 m^2/g, 1 meq/g)	n.d.
	AttractSPE SSX	SAX	No information (~600 m^2/g, 0.3 meq/g)	n.d.

	AttractSPE WCX	WCX	No information (~850 m^2/g, 0.77 meq/g)	n.d.
	AttractSPE WAX	WAX	No information (~650 m^2/g, 0.5 meq/g)	n.d.
Sharlau	Extrabond ECX	SCX	Extrabond PS-DVB (~ 700 m2/g) 	n.d.
	Extrabond EAX	SAX		n.d.
Macherel-Nagel	CHROMABOND HR-XC	SCX	CHROMABOND HR-X PS-DVB 	
	CHROMABOND HR-XA	SAX		
	CHROMABOND HR-XCW	WCX		
	CHROMABOND HR-XAW	WAX		

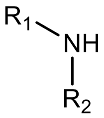
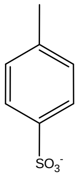
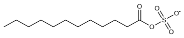
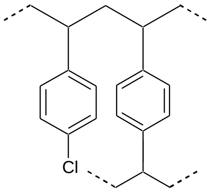
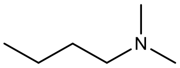
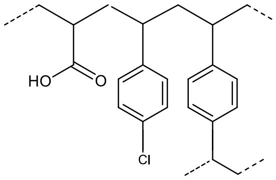
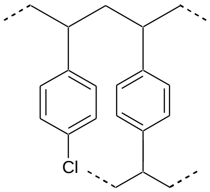
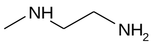
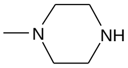
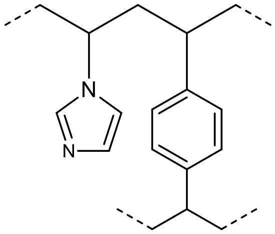
UTC	Styrene Screen BXC	SCX	STYRENE SCREEN PS-DVB	$-Si-(CH_2)_2-C_6H_6-SO_3H$
	Styrene Screen QAX	SAX		$-Si-(CH_2)_3N\ (CH_3)_3$
	Styrene Screen CCX	WCX		$Si-CH_2COOH$
	Clean Screen THC	WAX	Clean Screen Hybrid (polymer + silica)	
	Clean Screen DAU	SCX		

Table 2. Structure and properties of "in-house" mixed-mode polymeric sorbents.

Sorbent	Type of mixed-mode	Sorbent structure				Ref.
		Polymer based	Ionic group	IEC (meq/g)	Specific surface area (m^2/g)	
HXLPP-SCX	SCX	HXLPP VBC-DVB		2.5	~ 1370	(Cormack et al., 2012)

HXLPP-SAX	SAX			0.2	~ 1470	(Bratkowska et al., 2012b)
HXLPP-WCX	WCX	HXLPP MAA-VBC-DVB 	MAA	0.72	~ 1125	(Bratkowska et al., 2010)
HXLPP-WAX-EDA	WAX	HXLPP VBC-DVB 		0.75	~ 1000	(Fontanals et al., 2008)
HXLPP-WAX-piperazine	WAX			0.90	~ 1000	(Fontanals et al., 2008)
NVIm-DVB	SAX	NVIm-DVB 	NVIm	n.d.	~ 625	(Fontanals et al., 2006)

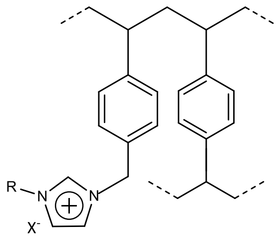
SILPs	SAX		Imidazolium	2.7	< 5	(Bratkowska et al., 2012a; (Fontanals et al., 2009)

Table 3. Applications of the mixed-mode SCX polymeric sorbents.

Compounds	**Application**	**Sorbent**	**SPE mode**	**Analysis**	**Ref.**
Therapeutic drugs	Environment	Strata-X-C	Off-line	LC-MS/MS(QqQ)	(Van De Steene et al., 2006)
		Strata-X-C	LLE + off-line	GC-MS	(Tauxe-Wuersch et al., 2006)
	Biological fluids	Oasis MCX	Off-line	CE-UV	(Lehtonen et al., 2004)
		Oasis MCX	PPT + off-line	LC-MS/MS(QqQ)	(Xue et al., 2006)
		Oasis MCX	On-line	LC-MS/MS(QqQ)	(Li et al., 2011)
		Oasis MCX	96-well-plate	LC-MS/MS(QqQ)	(Cunliffe et al., 2009)
		Bond Elute Plexa PCX	96-well-plate	LC-Orbitrap	(Musenga and Cowan, 2013)

		Cleanert PCX	Off-line	GC-MS	(Wu et al., 2009)
	Food	Oasis MCX	Off-line	LC-UV	(Nochetto et al., 2009)
		Bond Elute Plexa PCX	Off-line	HILIC-MS/MS(QqQ)	(Wu et al., 2012)
		Strata-X-C	Off-line	LC-MS/MS	(Huq et al., 2006)
Illicit drugs	Environment	Oasis MCX	Off-line	UHPLC-MS/MS(QqQ)	(Bijlsma et al., 2009; Gheorghe et al., 2008; González-Mariño et al., 2011; Pedrouzo et al., 2011)
	Biological fluids	Oasis MCX	Off-line	UHPLC-MS/MS(QqQ)	(Berg et al., 2009; Fountain et al., 2009)
		Cleanert PCX	Off-line	LC-QTRAP	(Dowling and Regan, 2011)
Biological comp.	Biological fluids	Oasis MCX	Off-line	CE-MS	(Sentellas et al., 2004)
		Strata-X-C	Off-line	LC-MS/MS(QqQ)	(Coles and Kharasch,

					2007; Kollroser and Schober, 2002; Lin et al., 2005; Wang et al., 2007; Zhao et al., 2004)
		Oasis MCX	96-well-plate	HILIC-MS/MS(QqQ)	(Heinig and Wirz, 2009)
	Food	Oasis MCX	96-well-plate	HILIC-MS/MS(QqQ)	(Xu et al., 2005)
		Oasis MCX	Off-line	LC-FL-MS	(Malakova et al., 2007)
		Bond Elute Plexa PCX	Off-line	LC-MS/MS(QqQ)	(Zheng et al., 2010)
Herbicides	Environment	Oasis MCX	Off-line	LC-MS/MS(QqQ)	(Nanita et al., 2008)
	Food	Oasis MCX	PLE + off-line	CE-DAD or LC-DAD	(Carabias-Martínez et al., 2007; Rodríguez-Gonzalo et al., 2009)
Additives	Environment	Strata-X-C	Off-line	LC-MS/MS(QqQ)	(Bermudo et al., 2006)
	Biological	Evolute CX	Off-line	LC-	(Williams and

	fluids			MS/MS(QqQ)	Caulfield, 2009)

Table 4. Applications of the mixed-mode SAX polymeric sorbents.

Compounds	**Application**	**Sorbent**	**SPE mode**	**Analysis**	**Ref.**
Therapeutic drugs	Environment	Oasis MAX	Off-line	LC-DAD	(Benito-Peña et al., 2006; Gil-Garcia et al., 2008; Tansupo et al., 2010)
		HXLPPSAX	Off-line	LC-UV	(Bratkowska et al., 2012b)
	Biological fluids	Oasis MAX	Off-line	LC-MS/MS(QqQ)	(Abdel-Hamid et al., 2006)
Biological comp.	Biological fluids	Oasis MAX	96-well-plate	LC-MS/MS(QqQ)	(Li et al., 2006)
	Tissue	Oasis MAX	Off-line	LC-MS/MS(QqQ)	(Kakimoto et al., 2008)
	Hair	Oasis MAX	Off-line	GC-MS	(Kharbouche et al., 2009)
	Food	Oasis MAX	Off-line	CE-MS	(Ge et al.,

					2007)
Fungicides	Food	Oasis MAX	Off-line	GC-MS	(Montes et al., 2010)
Additives	Environment	Oasis MAX	Off-line	LC-MS/MS(QqQ)	(García-López et al., 2010)
	Biological fluids	Oasis MAX	Off-line	GC-MS	(Landberg et al., 2009)
Organic acids	Environment	Chromabond HR-AX	Passive sampler	LC-MS	(Fauvelle et al., 2012)

Table 5. Applications of the mixed-mode WCX polymeric sorbents.

Compounds	**Application**	**Sorbent**	**SPE mode**	**Analysis**	**Ref.**
Therapeutic drugs	Environment	Oasis WCX	Off-line	LC-FL	(Lee et al., 2007b)
		HXLPPWCX	Off-line	LC-UV	(Bratkowska et al., 2010)
		Oasis WCX	Off-line	LC-MS/MS(QqQ)	(Lee et al., 2007b; Zorita et al., 2008)
		Oasis WCX	Off-line	UHPLC-MS/MS(QqQ)	(Batt et al., 2008)

		Oasis WCX	Off-line	HILIC-MS/MS(QqQ)	(Peru et al., 2006)
	Biological fluids	Strata-X-WC	Off-line	LC-MS	(Allanson et al., 2007)
	Food	Strata-X-WC	Off-line	LC-MS/MS(QqQ)	(Huq et al., 2006)
Illicit drugs	Environment	Oasis WCX	On-line	LC-MS	(Fontanals et al., 2013)
	Biological fluids	Strata-X-WC	On-line	LC-DAD	(Schonberg et al., 2006)
		Strata-X-WC	On-line	LC-MS/MS(QqQ)	(Chiuminatto et al., 2010)
Biological comp.	Biological fluids	Oasis WCX	96-well-plate	LC-MS/MS(QqQ)	(Shi et al., 2009)
		Strata-X-WC	96-well-plate	LC-MS/MS(QqQ)	(Clark and Frank, 2011)

Table 6. Applications of the mixed-mode WAX polymeric sorbents.

Compounds	**Application**	**Sorbent**	**SPE mode**	**Analysis**	**Ref.**
Therapeutic drugs	Environment	Oasis WAX	Off-line	LC-MS/MS(QqQ)	(Matejicek et al., 2007)
		HXLPPWAX		LC-UV	(Fontanals et al., 2008)
		HXLPPWAX	On-line		(Fontanals et al.,

					2010b)
	Biological fluids	Oasis WAX	Off-line	GC-MS	(Strahm et al., 2008a)
		Oasis WAX	Off-line	LC-MS/MS(QqQ)	(Carli et al., 2009; Strahm et al., 2008a; Strahm et al., 2008b)
Biological comp.	Food	Biobasic AX	On-line	LC-MS/MS(QqQ)	(Regueiro et al., 2011)
Additives	Environment	Oasis WAX	Off-line	LC-MS/MS(QqQ)	(Chen et al., 2006; Taniyasu et al., 2005)
		Strata-X-AW	Passive sampler	LC-MS	(Kaserzon et al., 2012)
	Paper & clothes	Oasis WAX	Off-line	LC-MS/MS(QqQ)	(Chen and Ding, 2006)
Phenolic comp.	Environment	Oasis WAX	96-well-plate	LC-MS/MS(QqQ)	(Zedda et al., 2010)
Natural organic matter		Strata-X-AW	Off-line	^{13}C-NMR	(Ratpukdi et al., 2009)