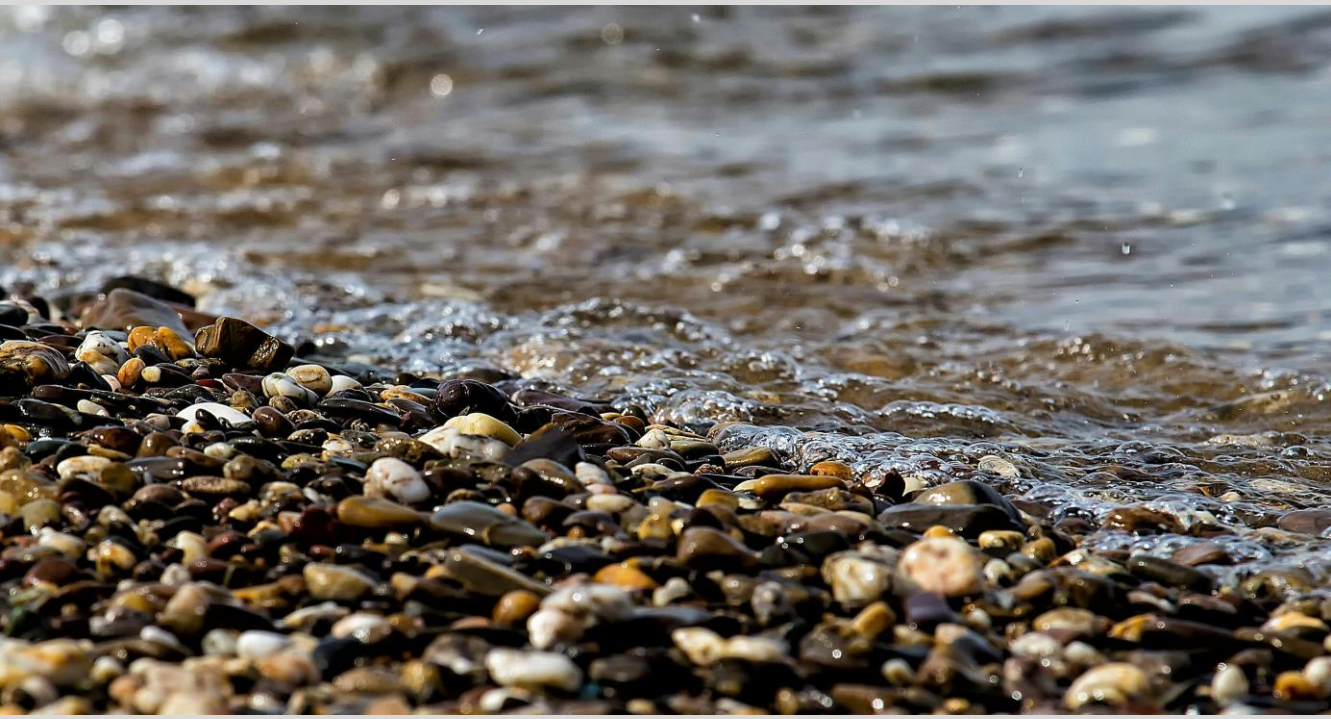
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES

Alberto Moral Ruiz

UNIVERSITAT ROVIRA i VIRGILI

In-house mixed-mode ion-exchange materials for the extraction of contaminants from environmental samples

ALBERTO MORAL RUIZ



DOCTORAL THESIS
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In-house mixed-mode ion-exchange materials for the extraction of contaminants from environmental samples

Alberto Moral Ruiz

Doctoral Thesis

Supervised by

Prof. Rosa Maria Marcé and Prof. Nuria Fontanals

Departament de Química Analítica i Química Orgànica

UNIVERSITAT ROVIRA i VIRGILI

Tarragona

2024

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Prof. NÚRIA FONTANALS, Professor at the Department of Analytical Chemistry and Organic Chemistry of the Faculty of Chemistry at Universitat Rovira i Virgili,

WE STATE:

That the present study, entitled “IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES”, presented by ALBERTO MORAL RUIZ for the award of degree of Doctor, has been carried out under our supervision at the Department of Analytical Chemistry and Organic Chemistry of this university.

Tarragona, June 27th 2024

Doctoral Thesis Supervisors

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te a Tropela. Laura “la mala”, te lo he dicho siempre, eres mi favorita, por eso a veces soy un poco duro contigo, pero te tengo un especial cariño. Por eso de vez en cuando, te pegaré cuatro bocinazos para que no bajes la guardia porque sé que vales mucho pero también te gusta relajarte. Marina, me fascinaba estar contigo en el laboratorio de preparativa, porque la conversación podía ir por cualquier sitio no esperado, y espero que siempre te brille el cutis. Agradecer también a todas las personas con las que he compartido el laboratorio: Joan Carles, Miriam, Nicole, Itsasne, Arnau, Lucía, Alba, Jun, Nuria, Juli…Queridos compañeros de CROMA, no sé qué nos deparará la vida, ni si nos veremos mucho en el futuro, pero la verdad es que estos tres años han sido la hostia, muchas gracias.

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ABSTRACT

Water contamination is one of the issues that must be addressed if human and environmental health is to be ensured. The impact of industrial activity, wastewater run-off and agriculture needs to be assessed so that the levels of pollution can be estimated and remediation measures taken when needed. In this regard, it is now a priority to determine the contaminants in environmental water samples. The chromatographic separation techniques (gas and liquid chromatography) combined with mass spectrometry quantification are powerful techniques for the quantification of the contaminants. However, since environmental water samples are complex matrices due to the presence of compounds with different properties and at low concentrations (ng/L), sample preparation techniques are required to increase the selectivity and sensitivity of the analysis. Therefore, the development of new sorptive materials to improve sample preparation procedures is one important line in analytical chemistry.

This Doctoral Thesis focuses on developing mixed-mode ion-exchange sorbents and applying them to the solid phase-extraction (SPE) of organic contaminants from environmental water samples. Mixed-mode ion-exchange sorbents can perform reversed-phase and ion-exchange interactions, so that they can retain a wide range of analytes. They can also selectively extract compounds retained through ion-exchange interactions if a washing step based on an organic solvent is included and rinses the compounds retained through reversed-phase interactions. The sorbents evaluated are divided into two types of materials: silica-based sorbents and polymer-based sorbents.

Regarding the evaluation of silica-based sorbents, they were mixed-mode zwitterionic ion-exchange sorbents. Several sorbents were functionalized with sulfonic acid groups and quaternary amines, so that they could perform strong cation- and anion-exchange interactions (SCX and SAX). These groups were introduced as two ionic functional groups or a single additive that combined them both. Some of these sorbents were also modified with activated carbon and graphene microparticles. In the case of the polymer-based sorbents, two types of mixed-mode ion-exchange sorbents were developed. The first type was based on hypercrosslinked sulfonated core shells, so that it could perform SCX interactions by exploiting a novel format of polymer core shells. On the other hand, the second material was based on amphoteric microparticles functionalized with primary amines and carboxylic acid groups using a novel approach based on the ring opening of maleic anhydride with ethylenediamine. Therefore, the material was able to perform weak anion- and cation-exchange interactions (WAX and WCX).

All the sorbents developed were successfully applied to the SPE of pharmaceuticals, high production volume chemicals and illicit drugs, mainly in environmental water samples. In one study, one of these sorbents was also applied to clean up of organic extracts from fish and dust samples. In all the studies, the samples were analyzed with either liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS).

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1. INTRODUCTION

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Environmental water pollution is one of the main problems that have to face humanity in the future years. Its relevance is so high that the 6th of the 17 Sustainable Development Goals from United Nations is related to “Clean Water and Sanitation”, being the 6.3 target: “By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally” [1]. In this sense, evaluating the levels of pollution is fundamental to ensure the safety of aquatic systems and human health. The balance of aquatic ecosystems can be disrupted, harming the species present, moreover, the contaminants can bioaccumulate in the water bodies, contaminating food chains and suppose a human threat in the case of consumption of the contaminated aquatic organisms. The organic contaminants present in environmental water do not have acute effects, the toxicity is due to the long exposure to them, therefore, knowing the concentration of contaminants help to assess the risks and evaluate the remediation operations needed. The contaminants can reach the environment through different ways, as can be observed in Figure 1.

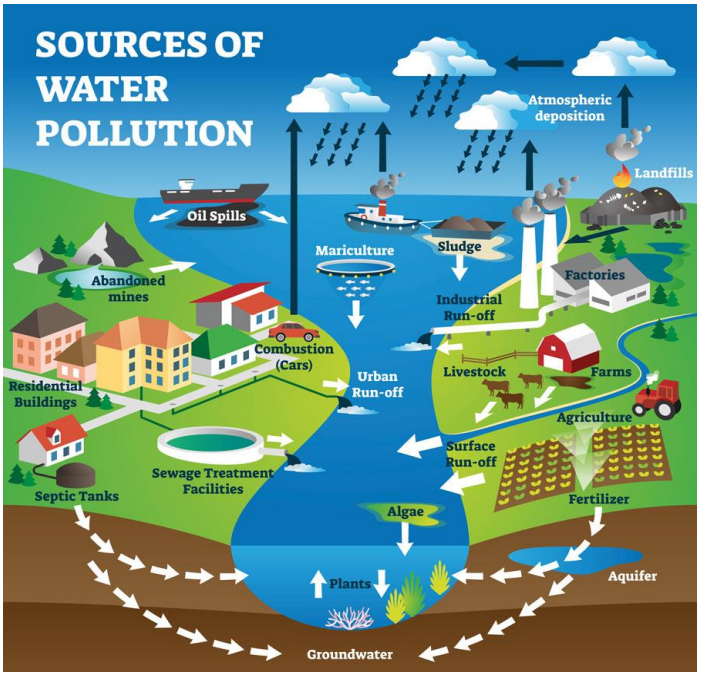

Figure 1- Sources of water pollution (stock image).

The most important ways are the industrial discharges (factories, mining, oil spills), the wastewater disposal, the agriculture run-off and the urban run-off. There are many different groups of organic contaminants, and in Figure 2, some relevant groups of organic contaminants are presented. Industrial waste products arrive to the environment after industrial discharges, polycyclic aromatic hydrocarbons (PAHs), perfluorinated alkyl

substances (PFASs) and high-volume production chemicals (HVPCs) can be highlighted among others. The pharmaceuticals and personal care products (PPCPs) arrive to the environment through wastewater disposal and urban run-off. Pharmaceuticals are a very diverse group with many families: antidepressants, antibiotics, β-blockers… Apart from pharmaceuticals, hormones and personal care products present for hygiene and grooming purposes like parabens used as preservatives can be highlighted. Another remarkable group of pollutants are endocrine disruptor compounds (EDCs). These compounds have an increasing concern during the recent years, since they can mimic, block or alter the behaviour of hormones, leading to health effects on human and environmental health. Among them, bisphenols, phthalates and polychlorinated biphenyls (PCBs) can be highlighted. On another note, pesticides are compounds that arrive to the environment after agricultural activities. The field of pesticides is in continuous change, since environmental and health studies generate regulatory changes. For instance, organochlorine pesticides (OCPs) were substituted by organophosphorus ones (OPPs), being also replaced by other families such as neonicotinoids or pyrethroids [2]. Another relevant group of pollutants are de disinfection byproducts (DBPs), which are generated when disinfectants like chlorine or ozone react with the compounds present in water during the production of drinking water. The DBPs can be genotoxic and carcinogenic, and for this reason, some of them are regulated such as some trihalomethanes, haloacetic acids or haloamides [3,4].

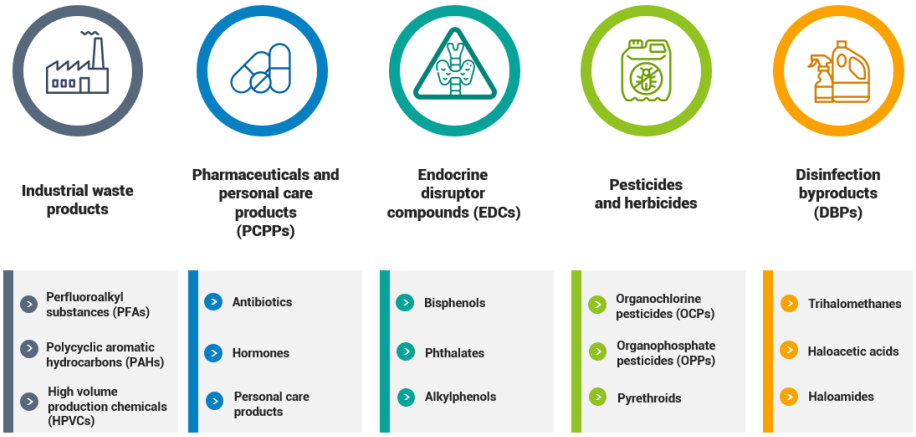

Figure 2. Some groups of relevant contaminants in environmental water samples and examples of families.

In this Doctoral Thesis, the compounds determined belong to three families: pharmaceuticals, HPVCs and illicit drugs. Focusing on pharmaceuticals, their production and consumption have significantly increased with the development of medicine and, therefore, the amount of these compounds discharged into wastewater post consumption and from production industries have also increased. The most detected pharmaceuticals in the environment are anti-bacterial, non-steroidal anti-inflammatory drugs (NSAIDs),

antiretroviral, steroid hormones and antidepressants. It should be noted that the early life stages of various aquatic organisms are particularly susceptible to the adverse reproductive effects of pharmaceutical exposure. This calls for immediate action and increased focus on mitigating this growing threat. Moreover, chemical stability and low concentrations make that its removal in wastewater treatment plants difficult, therefore they are released to the environment [5,6]. Another group of compounds determined are the HPVCs, they are defined by the Environmental Protection Agency (EPA) as chemicals manufactured or imported into the USA in amounts greater than 1 million pounds per year. These chemicals are characterized by high industrial applicability and versatility. Moreover, since their industrial applications are significantly high, its determination and toxicity assessment must be carried out in order to evaluate their environmental impact. Since this group of compounds is based on industrial use rather than chemical properties present diverse families of compounds such as benzothiazoles, phthalate esters, organophosphate esters, triazines, benzotriazoles or benzenesulfonamides are examples of families of compounds present in the “United States High Production Volume Challenge Program List” from EPA [7]. On another note, illicit drugs are psychoactive substances that stimulate or inhibit the central nervous system. The consumption of these compounds has escalated in the recent years and once taken, they are excreted as unaltered forms or metabolites. Moreover, they can produce serious impact since they arrive to the environment through wastewater disposal due to the inefficient removal of them in wastewater treatment plants [8,9]. The presence of illicit drugs must be tracked in order to evaluate the risks they can suppose to environmental species and human health.

As it has been pointed out, there are several types of pollutants and regarding their potential harmful effects, their levels of concentration should be tracked in order to evaluate the possible toxicity and carry out the pertinent remediation measures. Some organizations, such as Environmental Protection Agency (EPA) in United States or Water Framework Directive (WFD) in the European Union, that regularly publish lists of compounds that should be monitored; in the case of EPA, this list is named Contaminant Candidate List, which identifies chemical and microbials contaminants that are not subjected to national primary drinking water regulations but may suppose health risks. Focusing on the European equivalent, the WFD publishes the Priority Substances Watch List each two years [10–12], as can be observed in Table 2. The substances included in the Watch Lists are considered contaminants of emerging concern (CECs) and their determination is particularly relevant for the potential impact on aquatic ecosystems and human health. The member states are required to monitor the presence of these substances and report the concentrations found. To date, four different Watch Lists have been published since 2016, and it is expected that during 2024 the 5th one will be published. Attending to Table 2, it can be observed that the compounds present in the lists are diverse. In all four lists, pesticides, hormones and PPCPs are present. For instance, focusing on the pesticides, in the first list, methiocarb, acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, oxadiazon and triallate are present. These pesticides belong to different groups: carbamates (triallate and methiocarb), neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam) and oxadiazoles (oxadiazon). All of them were maintained for the second list except for oxadiazon and triallate; however, all of them were removed in the third list,

triazole pesticides (imizalil, ipconazole, metconazole, penconazole, prochloraz, tetraconazole, tebuconazole), dimoxystrobin (strobilurin family) and famoxadone (oxazolidinedione family) were included instead. In the last list published, all the pesticides from third list were maintained and three more were added: azoxystrobin (also from strobilurin family) and diflufenican and fipronil (from phenilpyrazol family). Another group of contaminants that have been always present in these Watch List are the PCPPs. For instance, it can be highlighted the case of 2-ethylhexyl-4-methoxycinnamate, which was present in first and third Watch Lists, a compound which is used as ultraviolet B filter in sunscreens. Focusing on pharmaceuticals, antibiotics have always been present in the lists (erythromycin, clarythromicin, azithromycin, amoxiclin, ciprofloxacin, trimethoprim, sulfamethoxazole, clindamycin and ofloxacin) since the widespread use of antibiotics can led to the emergence of antibiotic-resistant bacteria. Apart from antibiotics there have been present compounds from other families such as diclofenac (non-steroidal anti-inflammatory drugs, NSAIDs), venlafaxine (antidepressant), metformin (antidiabetic) or clotimazole, fluconazole and miconazole (antifungals) are also included. Moreover, hormones are also part from PCPPs were already present in the first three Watch Lists (17-α-ethinylestradiol, 17-β-estradiol, estrone and norethisterone). Hormones are also considered EDCs because they can interfere with the hormonal balance leading to serious health problems such as reproductive or developmental problems.

Table 2.- Compounds present in the four Priority Substances Watch List published by WFD.

Watch List	Compounds
1st Watch List (2016)	17-α-ethinylestradiol, 17-β-estradiol, estrone, diclofenac, 2,6-di-*tert*-butyl-4-methylphenol, 2-ethylhexyl-4-methoxycinnamate, erythromycin, clarythromicin, azythromycin, methiocarb, acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, oxadiazon, triallate
2nd Watch List (2018)	17-α-ethinylestradiol, 17-β-estradiol, estrone, erythromycin, clarythromicin, azythromycin, methiocarb, acetamiprid, clothianidin, imidacloprid, thiamethoxam, thiacloprid, metaflumizone, amoxicilin, ciprofloxacin
3rd Watch List (2020)	2-ethylhexyl-4-methoxycinnamate, bifenthrin, deltamethrin, esfenvalerate, permethrin, metaflumizone, amoxicilin, ciprofloxacin, sulfamethoxazole, trimethoprim, clotimazole, fluconazole, miconazole, venlafaxine, norethisterone, imizalil, ipconazole, metconazole, penconazole, prochloraz, tetraconazole, tebuconazole, dimoxystrobin, famoxadone, chromium (III and VI), cyanide
4th Watch List (2022)	Sulfamethoxazole, trimethoprim, venlafaxine, clotrimazole, fluconazole, imizalil, ipconazole, metconazole, miconazole, penconazole, prochloraz, tebuconazole, tetraconazole, dimoxystrobin, azoxystrobin, famoxadone, diflufenican, fipronil, clindmycin, oflxacin, metformin, avobenzone, oxybenzone, octocrylene

The Watch Lists also publish the predicted no-effect concentration (PNEC) of the compounds present in the lists, which use to be low; for instance, for clindamycin and ofloxacin, the concentrations are 0.044 and 0.026 µg/L respectively. For this reason, the use of chromatographic instruments combined with mass spectrometry or tandem mass spectrometry detection is recommended in the mentioned lists. Focusing on liquid chromatography (LC), the most basic detectors are ultraviolet detector (UV), diode array detector (DAD) or fluorescence detector (FLD), which are robust, simple and cheap. However, instrumental limits are in the range of µg/L and the selectivity is low. For this reason, when lower limits of detection are needed (in the level of ng/L) or the complexity of the sample is high, mass spectrometry detectors are used. LC combined with tandem mass spectrometry (LC-MS/MS) or with high resolution mass spectrometry (LC-HRMS) are the most commonly used techniques for the analysis of environmental complex samples [13,14]. LC-MS/MS thanks to the multiple reaction monitoring (MRM) mode have an improved sensitivity. Meanwhile with LC-HRMS, thanks to the mass accuracy measurements, it is possible to have high confirmation power in the quantification, and Q-HRMS, which improves the sensitivity compared with traditional HRMS, being a quantification technique with enhanced selectivity and sensitivity [15]. Another remarkable technique is ion mobility spectrometry (IMS), enhancing the separation of complex samples. IMS adds the fourth dimension for separation (time, chromatography, ion mobility and mass spectrometry), based on the mobility of the ions through a gas phase under an electric field [16]. The chromatographic separation combined with Q-HRMS or IMS-MS emerge as powerful tools which allow the analysis of samples with high. Another interesting application is the untargeted or suspected screening analysis, semi-quantitative types of analysis aimed to have a wide information of the compounds that can be present in complex samples [17,18].

However, despite LC-MS is a powerful tool, complex matrices or sensitivity requirements need for sample preparation. Sample preparation is one of the most relevant steps in the analytical procedure, because samples can be transformed into a format more suitable for the analysis, cleaned-up to remove interfering matrix species or preconcentrated to increase the sensitivity of the quantification. For these reasons, sample preparation has become a major part of the analysis time (taking even 80% of the total time of the analysis). One of the most important operations of the sample preparation is the sample extraction, aimed to isolate the analytes from the matrix to facilitate their quantification. Examples of extraction techniques are liquid-liquid extraction (LLE) or solid-phase extraction (SPE) [19]. Since it is a very important part of the analytical procedure, one of the most interesting research lines in analytical chemistry is the improvement of this step with the development of new solvents and materials, the creation of innovative techniques and the automatization or miniaturization of this step.

It should be highlighted that in the recent years, in the framework of Green Chemistry, the trends is towards greener sample preparation methods. The concern about reducing the environmental impact of chemical activities is gradually gaining concern at analytical chemistry. Two lists with recommendations have been published, one is “The 12 Principles of Green Analytical Chemistry (GAC)”, referring to the analytical procedures in general, giving guidelines to improve the greenness of sampling, methods, instruments, reagents

and waste management [20]. In addition, GAC presented some recommendations as “The ten principles of Green Sample Preparation” which are depicted in Figure 3 [21]. Some examples of what they claim are look for safer solvents and reagents, minimization of waste, chemical compounds and energy consumption, use of sustainable and reusable materials and promote automatization of the processes [21].

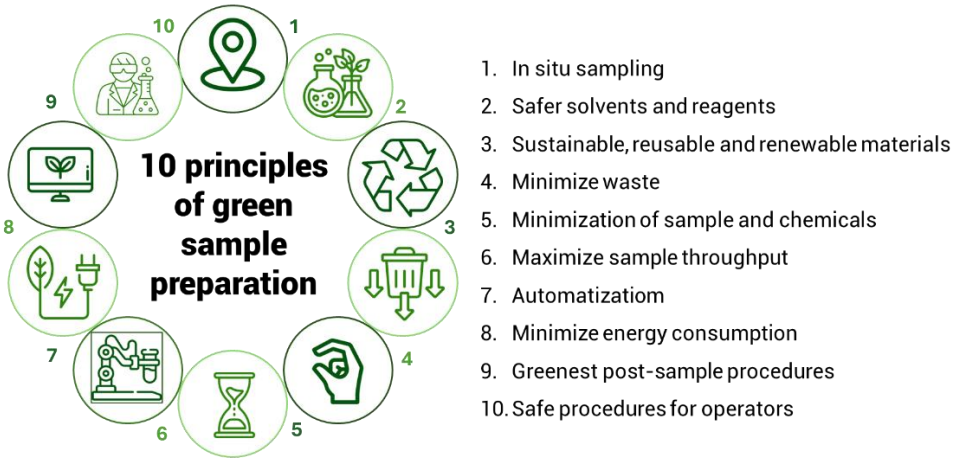

Figure 3.- The ten principles of green sample preparation.

During the following sections of the introduction, the most interesting extraction techniques will be described, either liquid- or sorptive-based, showing some examples of its applications. Moreover, the novel liquid phases applied will be mentioned and briefly described. Regarding the novel sportive materials, a review study discussing the application of novel materials applied in sorptive techniques for the analysis of environmental water samples was included and another section, describing sorptive materials that were not included in the review.

1.1. Liquid-based extraction

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Liquid-based extraction techniques

Liquid-liquid extraction (LLE) was first applied in the petroleum industry in the early 1900s by Lazar Edeleanu. This technique is based on the transference of compounds between two liquid phases, being one of them an aqueous solution and the other one, an organic solvent. Thanks to stirring or shaking, one liquid is dispersed into the other occurring the matter exchange, and then the phases are separated [22]. Its simplicity and wide applicability made LLE one of the most relevant extraction techniques in the past and it is still applied like in Aguilar-Méndez *et al.* [23] or Amiridou *et al.* [24] studies. who used dichloromethane to extract volatile compounds from tequila and endocrine disruptors from water samples, respectively.

LLE have many drawbacks like the limitations to automatize or the consumption of long times, but the most significant inconvenient is the use of large volumes of toxic organic solvents. For instance, in the mentioned study from Amiridou *et al.* [24] used 150 mL of dichloromethane (3 x 50 mL) for each sample which is a high volume of a highly toxic solvent. With the purpose to develop fast, efficient, and more environmentally friendly liquid-based extraction method, liquid-phase microextraction (LPME) was introduced in 1990s. It was similar to LLE but using lower volumes of sample and organic solvents and improving the extraction efficiency and the enrichment [25]. The most relevant LPME techniques are: dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME) and hollow fiber liquid-phase microextraction (HF-LPME).

In 2006, Rezae *et al.* [26] added a dispersant into the mixture, developing the DLLME. 8 µL of tetrachloroethylene as extraction solvent and 1 mL of acetone were added to 5 mL of water sample to extract PAHs. This approach increased the enrichment and allowed to decrease the extractant volume since the dispersant increased the mass exchange. The dispersant facilitates the formation of fine droplets in the aqueous phase, enhancing the mass exchange. To separate the extraction solvent from the aqueous phase, centrifugation is used, since most of the solvents applied have more density than water and sediment in the bottom of the vial [27]. An scheme of DLLME is shown in Figure 4,.where it can be observed how the dispersed small droplets are sedimented in the bottom of the vial, being easily removed with a syringe. For these reasons, this approach has been widely applied in the recent years [28]. An example of an application of DLLME is the study performed by Khodadoust *et al* [29] when determined carbamate pesticides in water samples using chloroform as extractant and acetonitrile as dispersing solvent. The volume of chloroform used was 126 µL, being significantly lower than when LLE is applied.

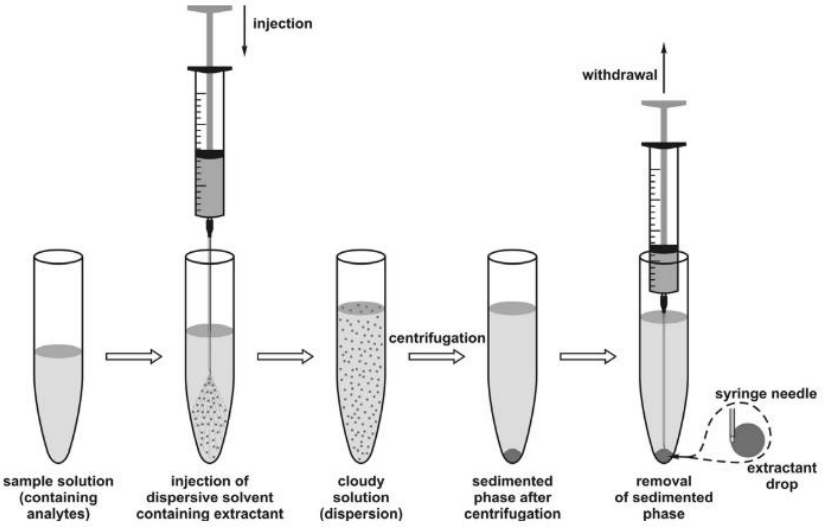

Figure 4. Scheme showing the different steps of DLLME [27].

Another relevant LPME technique is SDME, which is based on a drop suspended on a fiber and then it can be immersed in a liquid solution being a two-phase SDME (direct immersion SDME) or held on the headspace being a three-phase SDME (headspace SDME) like is shown in Figure 5. It is characterized for its simplicity, since the only needed setup is a common micro-syringe, making it possible to easily automatize. Moreover, since the volume of the droplets ranges from 0.2 to 5 µL, high enrichment factors can be obtained. However, it should be highlighted that the instability of the drops is the most relevant disadvantage, which can lead to low reproducibility [30]. An example of an application of direct immersion SDME is the study performed by Dos Anjos *et al.* [31] where pesticides from different families (carbamates, OPPs, OCPs, pyrethroids and strobilurin) were determined in coconut water. Three different extraction solvents were tested: toluene, cyclohexane and isooctane. It was observed that the three solvents showed a similar performance, and the authors selected toluene due to their lower toxicity, although it is also very toxic. Only 1 µL of toluene was needed to perform the extraction and the drop was directly injected into the GC-MS instrument. Regarding headspace SDME, it can be highlighted the study performed by Qin *et al.* [32] where the performance of head-space solid-phase microextraction (HS-SPME) and HS-SDME were compared for the extraction of methanol from wine samples, being both methods optimized. The solvents selected for HS-SDME were ethylene glycol, dimethyl sulfoxide and dimethylformamide, being the last the best performing one. It was observed that the detection limit with HS-SDME was significantly lower than the one obtained with HS-SPME, being 1 µg/L and 500 µg/L, respectively. Moreover, the method based on HS-SDME showed better precision than the one based on HS-SPME. Another advantage from HS-SDME over HS-SPME is the lack of carryover problem.

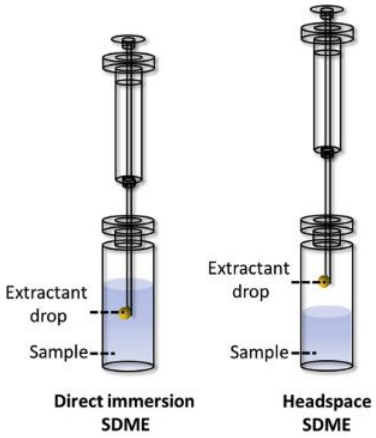

Figure 5. Scheme of direct immersion and HS-SDME procedures [30].

Talking about hollow fiber liquid phase microextraction (HF-LPME), it was introduced by Pedersen-Bjergard in 1999 [33]. It is based on a porous hydrophobic hollow fiber phase filled with the acceptor phase and with an organic solvent immobilized in the pores of the fiber through capillary forces. The analytes diffuse through the walls of the hollow fiber thanks to the concentration gradient, being recovered in the acceptor phase inside the fiber. This technique has been widely applied thanks to its simplicity, low volume consumption, cheapness and efficiency [33–36] There are two types of HF-LPME: two-phase HF-LPME [35] and three-phase HF-LPME [34]. As can be observed in Figure 6a, in the two-phases HF-LPME, the acceptor phase is the same solvent that is adsorbed in the fiber. This type is more focused on analytes with organic-solvent affinity. The mentioned first application of HF-LPME [33] is an example of three-phase type, where methamphetamine was extracted from urine and plasma with a fiber where 1-octanol was immobilized and with 25 µL a 0.1 M solution of HCl as acceptor phase. Apart from the preconcentration, the clean-up of the sample was also achieved since large molecules, acidic compounds and neutral components were not extracted into the acidic acceptor phase, fact that was especially interesting when a complex sample like plasma was analyzed. Another application of two-phase HF-LPME is the study performed by Wang *et al.* [37] where organophosphate esters were determined in river samples. Three different solvents were evaluated: toluene, cyclohexane and 1-octanol, being tolune the best performing one. Moreover, it can be noted that the extraction method was automatized. In the case of three-phase HF-LPME, as can be observed in Figure 6b, the acceptor phase is an aqueous solution, being an aqueous-organic-aqueous system. This type of HF-LPME is focused on compounds soluble in water. An example of an application of two-phase HF-LPME is the study performed by González-Sálamo *et al.* [36] where phthalic acid esters were determined in water samples. 40 µL of 1-octanol were used for the extraction of the analyte since 1-octanol is a suitable solvent to extract this type of analytes thanks to its low polarity compared to water.

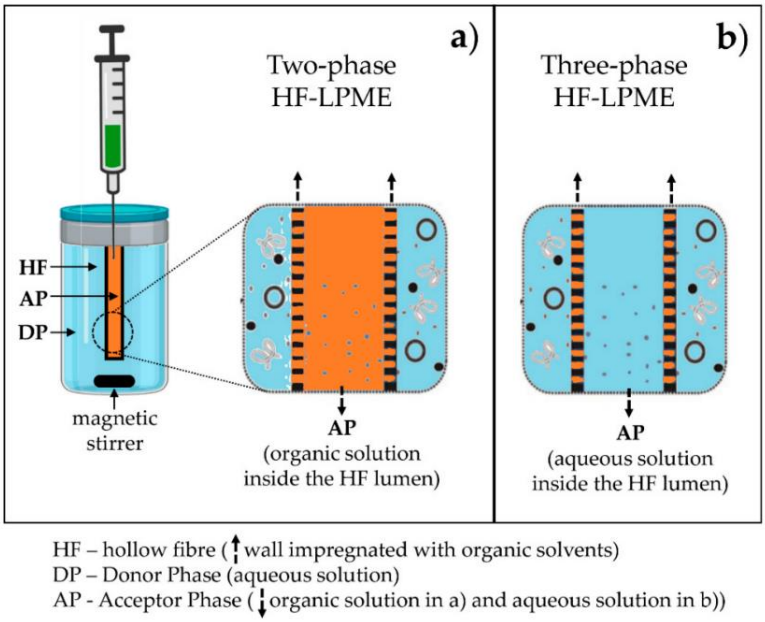

Figure 6. Schemes of tow phase (a) and three-phase (b) HF-LPME [38].

The last liquid-based technique to be highlighted in this section is cloud point extraction (CPE), developed by Watanabe and Tanaka in 1978 [39]. The extraction technique is based on the property of micellar solutions to become cloudy and form a new phase at a critical temperature, known as cloud point temperature. In the study performed by Watanabe and Tanaka the surfactant used was polyoxyethylene nonyl phenyl ether (PONPE) and it was used to extract zinc from water sample. The use of non-ionic surfactants, such as polyethyleneglycol (PEG) is more common than the cationic or anionic ones, such as cetyltrimethylammonium bromide (CTAB) or sodium dodecyl sulphate (SDS). The technique is characterized by high enrichment factors, good selectivity and simplicity, being the biggest drawback the need to heat during relatively long times [40]. The search of methods to obtain the CPE at room temperature is interesting, for instance, Luo *et al.* [41] combined PEG 6000 with acetonitrile and Na_2SO_4 to generate the CPE at room temperature for the extraction of alkylphenols in water samples. As can be observed in Figure 7, the addition of acetonitrile and Na_2SO_4 makes it possible to generate the CPE, and then, the centrifugation facilitates the separation of micelles and the easy removal with a syringe. It can be highlighted that relatively low detection limits were obtained (0.17 – 0.39 µg/L) when the quantification was done with LC-FLD.

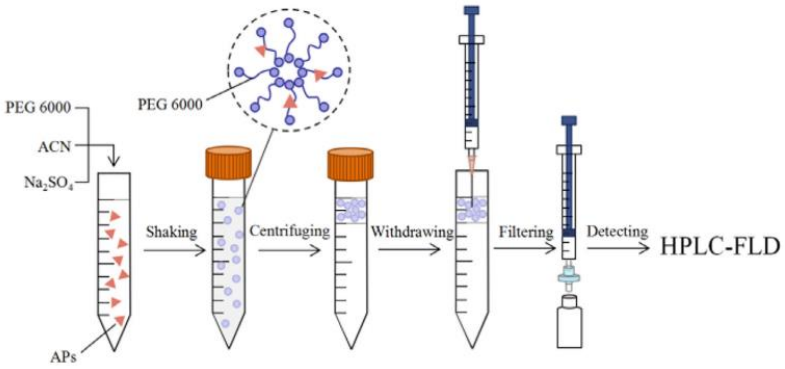

Figure 7. Scheme of an example of CPE protocol [41].

Alternative solvents

After introducing briefly the liquid-based extraction techniques, it can be interesting to glance over some new liquids that have started to replace the "classic solvents" like dichloromethane, chloroform or toluene, which are characterized for its harmful environmental impact [42,43]. The solvents that will be described will be long-chained alcohols, switchable solvents, supramolecular liquids, ionic liquids and deep eutectic solvents.

Starting with long-chained alcohols, these solvents are usually applied through solidification of floating organic droplet combined with dispersive liquid-liquid microextraction (SFOD-DLLME). Their density (lower than water) makes it difficult to recover the extractant phase with DLLME. However, as can be observed in Figure 8, since their fusion point is easy to achieve with an ice bath, the droplet is solidified, and the removal is easy with a spatula. Despite adding more steps to the extraction procedure (freezing and melting the organic droplet) and needing an ice bath to solidify the solvent, the method continues being simple and easy to carry out in any laboratory [44]. One example of an application is the study performed by Wang *et al.* [45] who used 1-dodecanol over 1-decanol and 1-undecanol for the extraction of strobilurin fungicides from water samples. 1-dodecanol showed higher extraction yields. Moreover, since the freezing points of these compounds increase when they have more carbon atoms (1-decanol: 7 ºC, 1-undecanol: 16 ºC, 1-dodecanol: 23 ºC), the formation of the solid droplet and its melting for chromatographic analysis is faster in comparison with the other solvents. Another examples is the study performed by Hoisang *et al.* [46] who applied 1-undecanol for the determination of insecticides in fruit juice, vegetables and agricultural runoff. Apart from 1-undecanol, 1-dodecanol, cyclohexane (freezing point: 6.5 ºC) and cyclohexanol (freezing point: 26 ºC) were tested, but the two latter were discarded because of their low surface tension, which led to the formation of small droplets instead of a single drpo. It can be highlighted that only 20 µL of 1-undecanol were used, achieving enrichment factors ranging from 355 to 509.

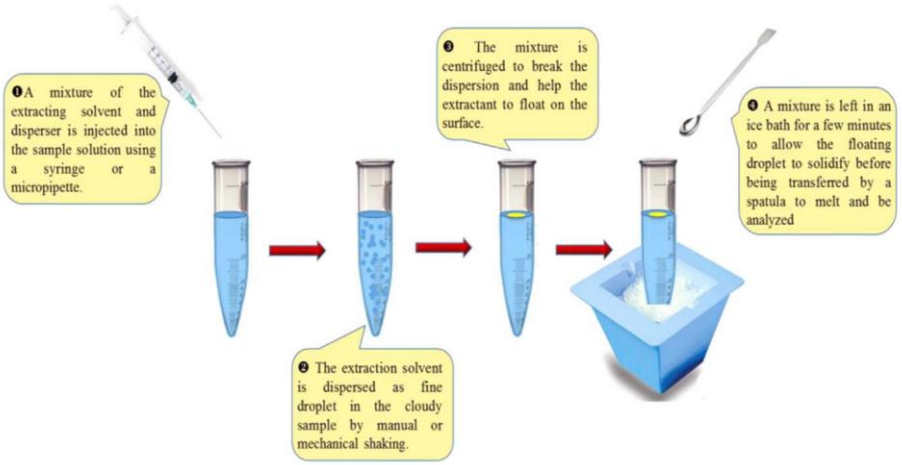

Figure 8. Scheme showing the steps of SFOD-DLLME [44].

Switchable solvents are liquids that have physicochemical properties that can be sharply changed reversibly. The property that is modified is the polarity of the liquid, turning hydrophilic solvents into hydrophobic forms or vice versa. Since the change is reversible, the solvents can be recovered and reused, making them interesting in the context of GAC. The more common solvents are amines (secondary and tertiary) and fatty acids. The switch used with the amines is the addition of CO_2 through bubbling or adding dry ice, and the amines react with the carbonic acid that is created when CO_2 is bubbled in water. The result of the reaction is an ammonium carbonate salt, which is hydrophilic. To recover the hydrophobic amines, it is only needed to remove the CO_2. [47–50]. For instance, Chen *et al.* [48] evaluated five different tertiary amines for the extraction of *Polygonatum sibiricum* polysaccharides. It was observed that *N,N*-dimethylbutylamine provided the best results over triethylamine, *N,N*-ethyldiisopropylamine, *N,N*-dimethylbenzylamine and *N,N*-dimethylcyclohexylamine. As it has been mentioned, CO_2 was used to trigger the change in the polarity, being possible to reuse the solvent up to 60 times. Turning to fatty acids, the switch is triggered changing the pH by adding sodium hydroxide, that through an acid-base reaction generates the sodium salt, which is hydrophilic. The reaction can be reverted adding an acid (e.g. HCl), recovering the hydrophobic specie [49]. An example of the application of fatty acids is the study performed by Hu *et al.* [50] where hexanoic acid was used to extract pyrethroid insecticides from water samples. As it has been pointed out, the trigger to obtain the hydrophilic form was the addition of NaOH meanwhile sulfuric acid was used to recover the hydrophobic form.

Ionic liquids (IL) are another type of interesting solvents, since they are ionic species that are liquids at room temperature with low vapor pressure. The wide variety of anions and cations that can be chosen allows to tune the properties of the IL applied, being the most common cations imidazolium, pyridinium, pyrrolidinium, phosphonium, ammonium, or sulfonium; in the case of anions, the most common ones are hexafluorophosphate,

tetrafluoroborate, halides, alkylsulfates, tosylate, methanesulfonate, or bis(trifluoromethylsulfonyl) imidazolium [42,43,51]. Despite being a type of liquid known since 1914, when Walden synthesized ethylammonium nitrate [52], they were not applied in extraction techniques until 2009, when Liu *et al.* [53] developed a method for the extraction of insecticides using an IL for the first time. The IL applied was 1-hexyl-3-methylimidazolium hexafluorophosphate and the extraction technique used was DLLME using MeOH as dispersant. Only 18 µL of the IL were applied, that were dissolved in 50 µL of MeOH, obtaining a good enrichment factor. Another example of an application of ILs is the study from Pestana *et al.* [54], who used 1-octyl-3-methylimidazolium hexafluorophosphate to extract UV filters from water samples through DLLME. ILs are considered green solvents by some authors, however, it should be noted that components of the synthesis or the final IL can be toxic, so despite being greener than conventional solvents, there are greener solvents than ILs [42,43]. One way to overcome the toxicity of ILs is to use natural precursors such as amino acids to create the anions or alicyclic cations like choline [55]. In this sense, Feng *et al.* [56] applied an IL based on the combination of choline with several amino acids (glutamic acid, alanine, proline, histidine, tyrosine, phenylalanine, glycine and tryptophan) for the extraction of scutellarin, and rosmarinic acid from *Perilla frutescens* leaves. It was observed that the best performing IL was the combination of choline with glutamic acid.

Supramolecular solvents (SUPRAs) are 3D nano-structured liquids non-soluble in water that are based on amphiphilic molecules that are aggregated through non-covalent interactions. To form the SUPRAs, amphiphiles are added to a solution until reach a certain concentration known as critical micellar concentration (CMC). When this concentration is achieved, the micelles are formed. These isotropic mixtures suffer a process called “coacervation” that generates a new phase which is non-soluble in water, which is the SUPRAs as can be seen in the second draw of Figure 9. The coacervation can be induced through five different methods, which lead to the five different types of SUPRAs: water-induced SUPRAs, temperature-induced SUPRAs, pH-induced SUPRAs, counterion-induced SUPRAs and salt-induced SUPRAs. Depending on the nature of the amphiphiles that form the SUPRAs, they can interact differently with the analytes (ion-exchange, hydrogen bonding, π-cation or hydrophobic interactions) [42,55,57]. The first application of SUPRAs to extract analytes was reported in 2009 by Ballesteros-Gómez *et al.* [58], where decanoic acid was used to form the micelles in tetrahydrofuran and the addition of water induced the formation of the SUPRAs. This system was evaluated for the extraction of three different contaminants: bisphenol A, ochratoxin A and benzo[*a*]pyrene from several beverages (wine, vinegar, tea, soft drinks,…). These SUPRAs allowed they direct injection of crude extracts since major matrix components are not dissolved in the extractant phase. Since 2009 there have been numerous applications of SUPRAs in extraction techniques as reported in a recent review [57]. For instance, Yu *et al.* [59] developed an interesting SUPRAs using citronellol as amphiphile and heptafluorobutanol as coacervation agent for the extraction of sulfonamides in sediment samples. This study was aligned with the recommendations of GAC, since citronellol is an environmentally friendly molecule with low toxicity. Moreover, it was evaluated the change of heptafluorobutanol over hexafluoroisopropanol, which is

significantly less toxic. However, it should be noted that heptafluorobutanol is still a fluorinated compound, so its toxicity is considerably high.

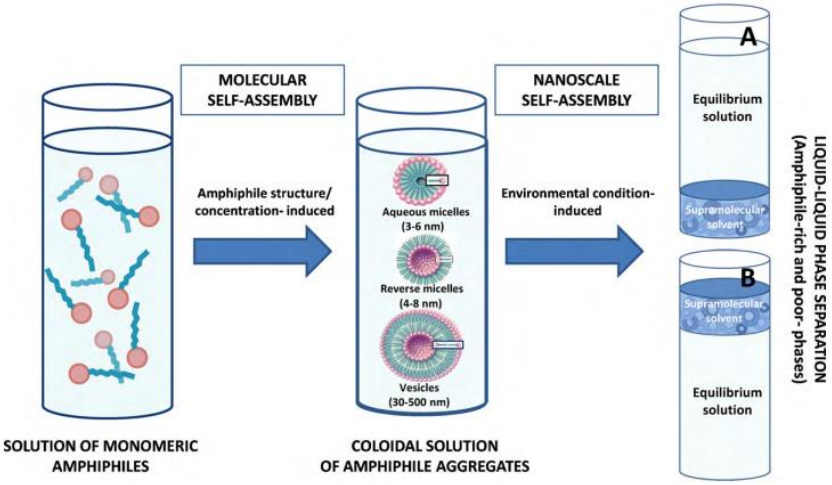

Figure 9. Scheme of the formation of a SUPRAs [60].

Deep eutectic solvents (DES) are the current trend in terms of new solvents to be applied in analytical chemistry. They are mixtures of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) discovered by Abbot *et al.* [61] when they mixed urea as HBD and choline chloride as HBA. The mixture of HBD and HBA creates a system that melts and freezes at temperature lower than the individual components due to hydrogen bonding and van der Waals interactions [43,62]. They are characterized by low cost, easy synthesis, low vapour pressure and low toxicity. As HBD, urea, ethylene glycol or glycerol are commonly applied, meanwhile, as HBA, the most common compounds are quaternary amines, being choline chloride the most used one [63]. For instance, Moggadam *et al.* [64] combined choline chloride with three different HBDs (glycerol, ethylene glycol and phenol) to extract pesticides from fruit juices. One of the most interesting points of DES is the possibility to use natural derivatives, named natural deep eutectic solvents (NADES), following the recommendations of GAC. Figure 10 shows several examples of natural derivatives used as HBDs such as alcohols (glycerol or ethyleneglycol), sugars (fructose or glucose) or amines (urea or dimethylurea); as HBAs, quaternary amines such as betaine or choline derivatives are applied. There are compounds such as terpenes (menthol or thymol), amino acids or organic acids (lactic or citric acid) that can be used either as HBDs or HBAs [62]. For instance, Pochialov *et al.* [65] applied a NADES to determine fluoroquinolone antibiotics in water samples, in their study, thymol was used as HBA and three fatty acids (hexanoic, heptanoic, octanoic and nonanoic acids) were tested as HBDs. It was observed that the best performing NADES was the one with nonanoic acid, due to its least solubility in water. On another note, Seetha *et al.* [66] applied a NADES based on thymol as HBA and camphor as HBD to extract bisphenols in food samples through DLLME.

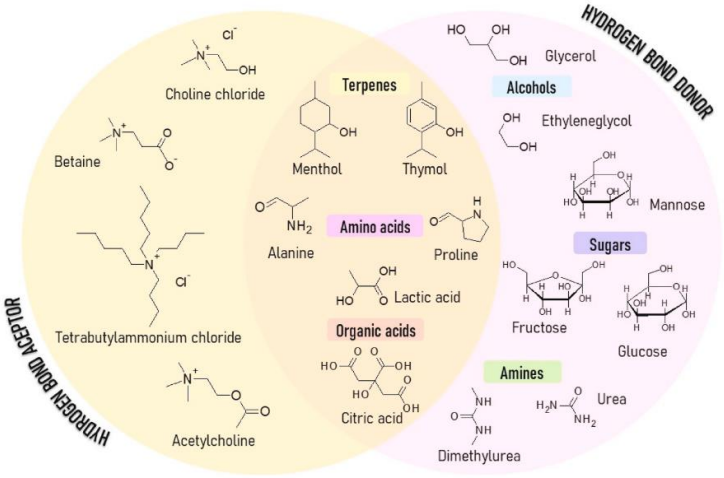

Figure 10. Examples of hydrogen bond acceptors and hydrogen bond donors commonly used for NADES synthesis [62].

To sum up, there is a wide variety of liquid-based extraction techniques that combined with the new solvents that have been developed in the recent years, provide a broad array of tools to be applied. Moreover, it is a field that experiences a lot of research activity meaning that new advancements can be unlocked in the coming years.

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

1.2. Sorptive extraction techniques

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Alberto Moral Ruiz

Nowadays, solid-phase extraction (SPE) is probably the most widely used extraction technique, and its use is so established that it is applied in reference methods such as ISO 21675:2019 for the determination of PFAs (perfluoroalkyl substances) [1] or ISO 20179:2005 for the determination of microcystins [2]. This extraction technique is based on the retention of compounds in a solid that is packed in a cartridge. Figure 11 shows a scheme of a SPE procedure. The cartridge is conditioned, then, the sample is loaded, and the compounds are retained in the sorbent. Then, a washing step can be included to remove the interferences and lastly, the analytes are eluted with a solvent able to disrupt the interactions that retained the analytes. Its robustness, simplicity, versatility, and ease to automatize made it a valuable tool.

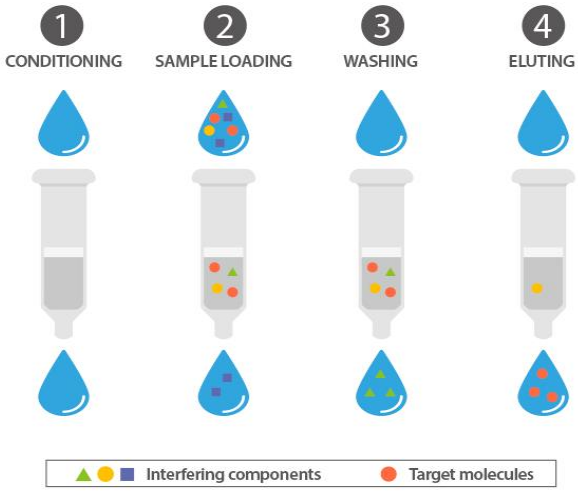

Figure 11. Scheme of a SPE procedure (stock image).

SPE was developed in the 1950s, and the first reported application was the packing of activated carbon to extract organic compounds from water samples [3]. During the initial years of SPE, activated carbon was used but due to some problems like heterogeneity, irreversible adsorption or modification of the analytes, more appropriate sorbents were sought. The first alternative material were the polymers, polystyrene, polystyrene-co-divinylbenzene and polyethylene-co-methacrylate resins (Amberlite XAD) were started to apply in the 1960s. Moreover, in the 1970s, it was found out that the silica-based used as stationary phases for liquid chromatography were suitable for SPE. This event promoted the development of the first commercial silica-based SPE cartridges, being the most applied the one with octadecyl-bonded silica. In the 1980s, the improvements in preparation technology, allowed the reappearance of carbonaceous materials since it was possible to obtain more homogeneous structures and higher more reproducible properties. Therefore, the classic materials that have been applied in SPE are polymer-based, silica-based and carbon-based [4,5].

One important research line in the field of SPE have been the evolution of the previously mentioned sorbents. In the field of polymeric materials [6,7], the initial advances were in the way of developing hydrophilic materials, for example, functionalizing the polymer with acetyl- or hydroxyl groups [8]. Parallelly, ion-exchange groups such as sulfonic acid groups [9] or amines [10] were also included. The development of crosslinked and hypercrosslinked polymers made it possible to obtain materials with high surface area. This increase in the surface area allows to improves the capacity extraction since the higher availability of sites increases the efficiency and the kinetics of the extraction [11]. The development of hypercrosslinked polymer materials has been evaluated in our research group, for instance, hydrophilic sorbents using as monomers divinylbenzene (DVB), vinylbenzene chloride (VBC) and 2-hydroxyethyl methacrylate (HEMA) [12]. Thanks to the chlorine groups from VBC, it was possible to obtain a hypercrosslinked material, obtaining materials with a specific surface area of 670, 850 and 925 m^2/g, dependeding on the % of VBC (i.e. 25%, 40 and 50%, respectively).

The next evolution of polymer-based sorbents was the development of molecular imprinted polymers (MIPs) [13]. MIPs are a group of materials that has attracted a special interest since its appearance [14]. They are materials based on the specific recognition of molecule, then, these materials have high selectivity. To achieve this selectivity, the polymerization of the monomers is carried out in the presence of a template molecule. After the polymerization, the template is removed from the polymer, leaving cavities complementary in terms of size, shape and chemical functionalities, then, the developed polymer will be able to selectively retain the template or related compounds [15,16]. An example of a study were a MIP is applied can be the one performed by Díaz-Álvarez *et al.* [17] where methacrylic acid and 2-hydroxyethyl methacrylate were used as comonomers and propazine was used as template for the extraction of triazine herbicides (propazine, desethylatrazine, desisopropylatrazine, simazine, cyanazine and atrazine). The synthesized material showed excellent results for the extraction of the selected triazines in comparison with the same material prepared without including the template, presenting recoveries larger than 40%. Moreover, the triazine herbicides were extracted from surface water samples with the MIP and a commercial C_{18} sorbent. When comparing the chromatograms, the one corresponding to the C_{18} material showed considerable noise hindering the identification of the peaks, meanwhile with the MIP, the peaks were narrow and easy to identify, demonstrating the excellent selectivity provided by the MIP.

Regarding materials with enhanced selectivity, the use of antibodies as functional groups should be highlighted. Antibodies are applied to immunosorbents; they are based in the molecular recognition thanks to the antigen-antibody interaction. This technique is manly focused on the extraction of large molecules (proteins, hormones, enzymes…) since the antigen-antibody interaction is based on steric interactions, for this reason, the selectivity could not be achieved for small molecules [18–20]. An example of an application of immunosorbents is the study performed by Vállez-Gomis *et al.* [21] where two different immunosorbents were developed to selectively extract thyroxine from serum samples through SPE. The antibodies were grafted on “(CNBr)-activated-Sepharose” which is a commercial agarose polysaccharide. To evaluate the selectivity, the chromatograms of a sample with or without applying SPE were compared, the chromatogram after SPE

presented lower signal-to-noise ratios and lower baseline. In addition, significantly higher matrix effects were observed without applying SPE, proving the enhanced selectivity when the immunosorbent was applied.

Another type of sorbents that has been widely evaluated in our research group are the mixed-mode sorbents [22–24]. They are based on the combination ion-exchange and reversed-phase interactions thanks to the dual functionalization with ion-exchange groups and alkyl chains. They are a versatile option, since they can extract diverse compounds exploiting both type of interactions. Moreover, a washing step based on an organic solvent can rinse the compounds retained through reversed-phase interactions, extracting selectively the analytes retained through ion-exchange interactions [22–25]. The mixed-mode sorbents will be applied in the present Doctoral Thesis and will be deeply discussed in following sections. In addition, as can be observed in Table 2, there have been developed several commercial polymeric sorbents with mixed-mode ion-exchange moieties such as Oasis MCX and MAX or Strata X-AW and Strata X-CW.

Nowadays, one of the most relevant research lines in polymeric sorbents is focused on the development and application of greener materials, examples of biopolymers such as cellulose or chitosan will be discussed section 1.3.2.

Regarding silica materials, the initial silica sorbents were functionalized with C_{18} chains, in the following years more functional groups were included such as C_2, C_4 , C_8, cyclohexyl, diol, cyanopropyl, phenyl, diphenyl or phenethyl [4]. Similarly to polymer-based materials, the development of ion-exchange and, subsequently, mixed-mode sorbents was carried out. As can be observed in Table 2, there have been also developed commercial silica-based sorbents functionalized with the moieties mentioned such as Strata C_{18} (reversed phase), Strata SCX and SAX (ion-exchange) or Isolute HAX and HCX (mixed-mode ion-exchange). In the recent years, the development of mesoporous silica has attracted attention. Mesoporous silica has an ordered porous structure where the pores have size uniformity [26]. The higher surface area and the tunable pore size (2 – 50 nm) made mesoporous silica an interesting material to be applied, and some applications of this material will be presented in section 1.3.1. Following the principles of GAC, greener sources of silica are starting to be evaluated [27] being the most relevant rice husk, an example of an application is the study performed by Zadeh *et al.* [28] for the extraction of herbicides from water samples.

In the case of carbonaceous materials, the activated carbon was substituted by graphitized carbon blacks (GCBs) and porous graphitic carbons (PGCs). GCBs are obtained by heating carbon blacks at 2700 – 3000 ºC, being an interesting material due to their high surface area and the ability to extract polar compounds due to the oxygen chemisorption during the heating, enabling an anion-exchange mechanism. On the other hand, GCBs are composed by a highly homogeneous crystalline structure made of large graphitic sheets. The graphitic sheets are characterized by high π delocalized density, being able to retain compounds through π-π interactions [4,29]. In the recent years, the trends in carbonaceous materials have been focused in the use of carbon nanotubes (CNTs), multi-walled carbon nanotubes (MWCNTs), graphene and graphene oxide. The properties of these materials and several applications of them will be discussed in section1.3.1.

As has been pointed out, several commercial polymer-based and silica-based sorbents have been developed. Table 2 presents examples of some of the most applied sorbents classified by brands, presenting the material and their interactions.

Table 2.- Selection of some commercially available sorbents.

Commercial brand	Name of the material	Characteristics
Waters	Oasis HLB	Polymeric
	Oasis HLB Prime	Reversed phase
	Oasis MAX	
	Oasis MCX	Polymeric
	Oasis Prime MAX	Mixed-mode strong ion-exchange
	Oasis Prime MCX	
	Oasis WCX	Polymeric
	Oasis WAX	Mixed-mode weak ion-exchange
	Sep-Pak Plus	Silica
	Sep-Pak Classic	Reversed phase
Phenomenex	Strata C_{18}	Silica
	Strata C_8	Reversed phase
	Strata PH	
	Strata SCX	Silica
	Strata SAX	Strong ion-exchange
	Strata WAX	Silica
	Strata NH_2	Weak ion-exchange
	Strata NH_2/C_{18}-E	Silica Mixed-mode weak-anion exchange
	Strata X	Polymeric
	Strata XL	Reversed phase
	Strata X-A	Polymeric
	Strata X-C	Mixed-mode strong ion-exchange
	Strata X-AW	Polymeric
	Strata X-CW	Mixed-mode weak ion-exchange
Biotage	Evolute ABN	Polymeric Reversed phase
	Evolute AX	Polymeric
	Evolute CX	Mixed-mode strong ion-exchange
	Evolute WAX	Polymeric
	Evolute WCX	Mixed-mode weak ion-exchange
	Isolute ENV+	
	Isolute C_{18}	Polymeric
	Isolute C_8	Reversed phase
	Isollute C_4	
	Isolute HCX	Silica
	Isolute HAX	Mixed-mode strong ion-exchange
	Isolute SCX	Polymeric
	Isoluite SAX	Mixed-mode strong ion-exchange
	Isolute CBA	Polymeric
	Isolute NH_2	Mixed-mode weak ion-exchange

The most common format of SPE is the cartridge, however, apart from traditional off-line cartridges, new formats of SPE have appeared. An example is on-line SPE, this format couples the sample extraction technique with the analysis instrument (commonly LC), allowing to automate the extraction. The automatization of the process increases the time efficiency and the sample throughput. Moreover, the sample and solvent volumes needed are also reduced [30]. However, it should be pointed out that on-line SPE leads to lower enrichment factors due to the low loading volumes and that high back pressure can be generated in the system. There are several commercially available cartridges for on-line SPE, for example, the cartridge PLRPs (from Agilent) or Oasis HLB (from Waters). For instance, an on-line SPE cartridge packed with Oasis HLB was applied for the extraction of multiresidue pesticides in water samples [31]. In the mentioned study, the sample volume was 5 mL, leading to low matrix effects for most of the analytes despite using a sorbent with low specificity.

To address multiple extractions is parallel, an interesting commercially format of SPE is the multi-well set-up. It is based on 96 or 384 wells in a tray, containing from small amounts of sorbent and having low working volumes. The possibility of loading several samples in parallel increases significantly the sample throughput and automatize the extraction [32]. These trays are designed to extract low sample volumes with well having low amount of sorbent. For instance, the commercially available 96-well packed with Oasis HLB have from 2 to 60 mg, on the other hand, the cartridges contain from 10 mg to 6g of sorbent. This format is interesting to be applied with samples with low availability such as blood serum [33] or plasma [34].

The miniaturization of SPE led to the creation of techniques such as pipette-tip SPE (PT-SPE) or in-syringe SPE. The sorbent is packed inside a pipette-tip or a syringe. These formats allow the reduce of sorbent amount and loading volume, moreover, these designs are simple and user-friendly and compatible with common laboratory equipment such as pipettes or syringes. Another interesting point is that these formats are portable, thus, the in-situ sampling can be performed [35,36]. An example of an application of PT-SPE is the study performed by Wang *et al.* [37] where three biomarkers of lung cancer (4-methoxyphenylacetic acid, cortisone and cortisol) were extracted from urine samples. Only 1.5 mg of a commercial strong anion-exchange sorbent were packed in a 200 µL pipette tip, the loading volume was only 1 mL of urine and the elution was done with 0.4 mL of 2% acetic acid in methanol. It was possible to quantify the three biomarkers in all samples from patients with lung cancer, proving the suitability of the developed method. Previously in our research group, PT-SPE was applied for the determination of drugs of abuse in urine samples [38]. 10 mg of a commercial silica-based mixed-mode ion-exchange sorbent functionalized with strong cation-exchange moieties was packed in a pipette tip. It can be highlighted that a washing step based on methanol was included to increase the selectivity of the extraction. This washing step made it possible to obtain low matrix effects, being lower than ±30% for most of the analytes.

Dispersive solid phase extraction (dSPE) and magnetic solid phase extraction (MSPE) are two techniques based on the dispersion of a sorbent in a liquid. These two techniques use reduced amount of sorbent and volumes of solvents. Thanks to the dispersion of the sorbent

in the liquid, the mass transfer is fast and present some advantages over traditional SPE such as the removal of the conditioning step and avoiding problems such as back-pressure or clog of the cartridge. The dispersion of the sorbent can be assisted using vortex, ultrasonic or air to reduce the time needed to achieve the matter transfer. One of the main drawbacks of dSPE is that to separate the sorbent from the sample or from the eluate need filtering or centrifugation. However, as can be observed in Figure 12, this operation is significantly easier and simple in MSPE, since a magnet facilitates the separation between the sorbent and the liquids. To achieve this, the sorbents for MSPE are based on a magnetic material (commonly magnetite), which interacts with the magnet. For these reasons, dSPE and mainly MSPE are widely applied nowadays and the development of sorbents for these techniques is one of the most important research lines in the field of novel sorptive sorbents [39–41]. In the review present in section 1.3.1., several applications of these techniques will be discussed.

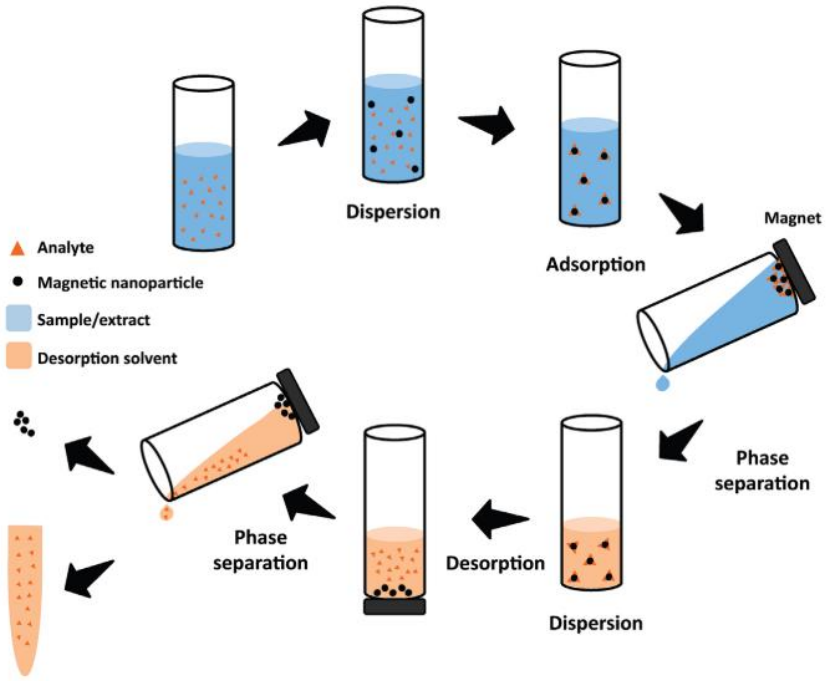

Figure 12. Scheme of the steps involved in MSPE [39].

Solid-phase microextraction (SPME) is a technique developed by Pawliszyn *et al.* [42] in the 1990s. The technique is based on a small amount of extracting phase placed on a solid support (commonly a fiber) which is exposed to the sample for the extraction of compounds. Unlike SPE which is an exhaustive technique, SPME is a non-exhaustive technique, based on the equilibrium between the sample and the extractant sorbent. The most important advantages from SPME are solvent-free techniques, easy-to-use, easy to integrate with

chromatographic instruments, automatable and portable. Regarding the integration with chromatographic instruments, it is especially interesting the combination with GC using thermal desorption, allowing to introduce directly to the gas chromatograph the analytes without needing an additional redissolving step. This combination allows to reach low limits of detection and quantification. In SPME there are two types of exposure: direct or headspace. Direct exposure consists of the immersion of the extraction phase on the sample occurring the transportation of analytes. In the case of headspace (HS), the extraction phase is inserted into the headspace over the sample, extracting only the volatile or semivolatile compounds from the sample. Figure 13 presents an example of a HS-SPME extraction combined with thermal desorption. The HS-SPME is interesting since there is no contact between the sample and the extraction phase, causing low impact of non-volatile interferences and less damaging of the fiber. In both cases, an equilibrium between the sample and the extraction phase must be reached, using stirring and in some cases heating to reduce the time to reach it [43–45]. The most significant drawbacks are that despite the fibers are reusable, they degrade and may have carryover problems when reused, the long times needed to reach the equilibrium and the low surface area that present the fibers.

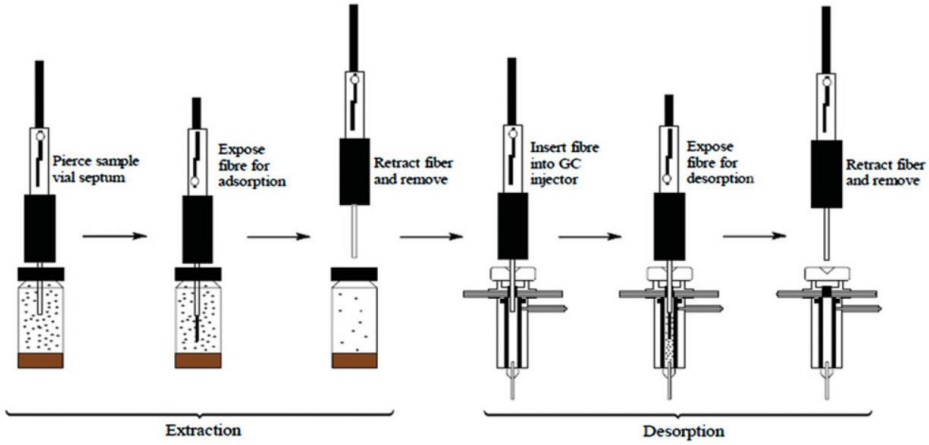

Figure 13. Scheme of SPME combined with thermal desorption [46].

The most common format of SPME are the fibers, like the example shown in Figure 13. Fibers have been widely applied since the beginning of this technique when Arthur and Pawliszyn created SPME [42]. A silica fiber was coated with a polyimide film to extract volatile chlorinated compounds from water samples. The importance and applicability of SPME made that different SPME fibers commercially available, such as polydimethylsiloxane (PDMS), divinylbenzene (DVB), polyacrylate (PA) or polyethylene glycol (PEG). There are also fibers that combine more than one material such as DVB/CAR/PDMS or PEG/PDMS. Moreover, the development of new in-house fibers is an ongoing area of research as will be pointed out in the review present in section 1.3.1. where several examples of novel fibers will be presented.

Apart from traditional fibers, there are other formats to exploit SPME such as SPME-arrow [47], in-tube (IT)-SPME [48] or thin film (TF)-SPME [49], which apply a capillary tube or a flat thin film, respectively, instead of a fiber. These approaches present higher surfaces area for the extraction, leading to higher capacity and higher enrichment factors, achieving lower limits. In our research group, a method based on SPME-arrow was developed for the extraction of synthetic musk fragrances in fish samples [47]. When the method was compared with conventional SPME, it was observed that the extraction efficiencies obtained with SPME arrow were up to ten times higher than when conventional SPME was applied thanks to the larger surface area.

Another example of a non-exhaustive extraction technique is stir bar sorptive extraction (SBSE), developed when Pat Sandra and their collaborators observed that during SPME extraction, the analytes were not only being retained in the SPME fiber, but also in the Teflon coated stir bar used to facilitate the mass transfer [50]. When the stir bars were introduced in the thermal desorption desorption chamber, it was observed that the concentration of the analytes in the stir bars was up to ten times higher than in the SPME fiber. Then, they coated a stir bar with PDMS and first applied SBSE for the extraction of PAHs from water samples in the late 1990s [51]. As have been mentioned previously, one of the main drawbacks of SPME is the low surface area, moreover, stir bars present higher surface area and can be an interesting alternative to conventional SPME. The technique is based on coating a magnetic stir bar with a sorptive phase and place it in the sample solution, where it is stirred to extract the analytes. Then, either thermal or liquid desorption can be applied. When thermal desorption is used, the method is a solvent-free technique and provides higher enrichment factors but it is only suitable for volatile or semivolatile analytes. In the case of liquid desorption, ultrasound are commonly applied to facilitate the mass transfer from the stir bar to the desorption solvent. Currently, there are two commercially available stir bars: PDMS and PDMS/PEG. During the recent years, new materials have been evaluated as coating for SBSE [52,53]. For instance, in our research group some polymer-based materials have been evaluated. The last of them consisted of a weak anion-exchange mixed-mode polymer using as monomer glycidyl methacrylate and ethylenediamine to functionalize the polymer with the ion-exchange groups [54]. This coating was evaluated for the extraction of acidic analytes from river samples obtaining acceptable extraction efficiencies (ranging from 20 to 47%) and low matrix effects (ranging from -15 to +17%).

Despite being a relatively novel technique, SBSE inspired the creation of other formats or techniques. For example, rotating disk sorptive extraction (RDSE) applies a disk with a diameter of 1.5 cm instead of a stir bar. As can be observed in Figure 14, there are two types of sportive phases: laminar and particulate. The laminar configuration, presented in Figure 14A, can be considered similar to a traditional SBSE. On the other hand, the particulate configuration, presented in Figure 14B, it is quite interesting because is based on packing a sorbent inside the disk. Any sorbent applied in SPE can be packed in the disk and during the extraction, the sample is recirculated through the sorbent, maximizing the contact between the sample and the sorbent [55]. Most of the applications of this set-up are based on packing the commercial sorbent Oasis HLB; for instance, when only 50 mg of the commercial sorbent were used to extract NSAIDs from 25 mL of wastewater samples, with 60 min of extraction time, it was possible to reach detection limits in the range of low ng/L [56].

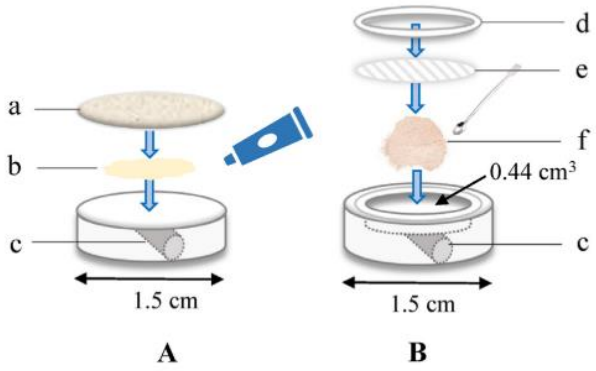

Figure 14. Schemes of laminar (A) and particulate (B) configurations of RSDE [55].
a) laminar sorptive phase, b) adherent, c) magnetic stirrer,
d) teflon ring, e) glass-fiber filter, f) particulate sorptive phase.

Another interesting extraction technique related to SBSE is stir bar sorptive dispersive microextraction. This technique, presented in 2014 by Benedé *et al.* [57], can be considered a mixture between MSPE and SBSE. As can be observed in Figure 15, a magnet is used to disperse and collect magnetic nanoparticles. This technique overcomes drawbacks from SBSE like long extraction time since it is an exhaustive technique, but without losing advantages of SBSE such as the possibility to combine it with thermal desorption or the ease to use it, becoming a versatile technique. It should be pointed out that the magnet should be specially strong (neodymium magnet) to generate a strong magnetic field [58].

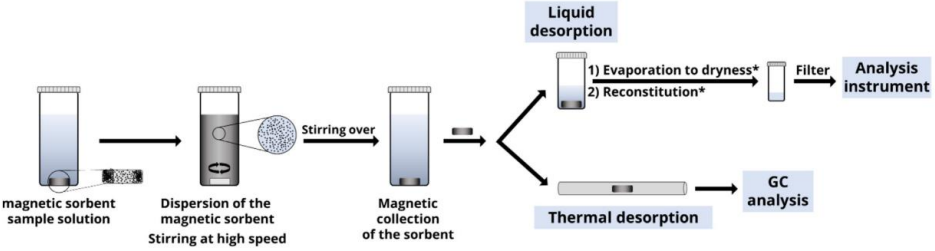

Figure 15. Scheme of different formats of SBSDME [58].

Fabric phase sorptive extraction (FPSE) is a technique developed by Kabir and Furton in 2014 [59]. The technique is based on exploiting the high surface area from fabric due to their porous and fibrous structure combined with the benefits of sol-gel coating technology. Since its development many different sol-gel coatings have been evaluated such as PDMS, polycapralactone, Carbowax 20M or combinations of more than one, creating multi-mode fabric phases, enabling the extraction of analytes with different properties [60]. For instance,

Celeiro *et al.* [61] evaluated different fabric coated with PDMS, PCAP-PDMS-CAP (poly(caprolactone-dimethylsiloxane-caprolactone)), Carbowax 20M and polytetrahydrofuran for the extraction of multiclass pesticides. The two first functionalities introduced non-polar groups, Carbowax 20M polar groups and polytetrahydrofuran medium polar groups. It was observed that since the Carbowax 20M was able to perform hydrogen bonding, the recoveries with this coating were significantly higher than with the other.

Capsule phase microextraction (CPME) was also developed by Kabir and Furton based on two permeable porous cylindrical membranes, one of which contains a sorbent meanwhile the second one contains a magnet. Therefore, one of the porous membranes contains the material that performs the extraction meanwhile the other one, facilitates the stir of the other membrane thanks to the magnet [62]. The advantage of this technique is that any type of sorbent can be packed inside the membrane, for example, in a study some sol-gel materials were packed: PDMS, octadecyltrimethoxysilane, PEG 300, polytetrahydrofuran and poly(dimethyldiphenylsiloxane) for the extraction of OCPs from environmental water samples [63]. It was observed that the PEG 300 material was the most suitable sorbent, obtaining recoveries ranging from 14 to 75%. CPME was also evaluated in our group in two studies [64,65], in the first of which, capsules packed with different polymeric materials were evaluated for the extraction of PPCPs from environmental waters. The best performing material was Carbowax 20M, which is a polymer highly functionalized with hydroxyl groups.

As highlighted in this section, SPE remains as the predominant technique in sorptive extraction. Additionally, dSPE, MSPE and SPME are also extensively used. Although innovation in this field has been traditionally limited, during the recent years novel techniques such as SBSDME, CPME or FPSE have emerged. In addition, in the following chapter, applications of novel materials with the techniques that have been presented in this chapter will be discussed.

1.3. Novel materials for sorptive extraction techniques

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

As it has been pointed out, the development of novel materials to be applied to sorptive extraction techniques is one relevant research line in the field of analytical chemistry. Therefore, this chapter is devoted to present novel materials applied to sorptive extraction techniques, section 1.3.1. discusses the several in-house materials applied for the extraction of pollutants in water samples from 2018. The materials are polymer-based, silica-based, carbon-based (graphene, CNTs and multiwalled carbon nanotubes (MWCNTs), metal organic frameworks (MOFs), covalent organic frameworks (COFs) and composites combining them are discussed. This information is presented in article format since it has been submitted for its publication in Trends in Analytical Chemistry.

However, some interesting materials were mentioned briefly in the review, such as layered double hydroxides (LDHs) or they presented few or none application to environmental water samples. In this sense, section 1.3.2. complements the information presented in section 1.3.1. with a brief description of aptamers, LDHs, polyoxometalates (POMs) and MXenes. Additionally, some sustainable materials that aligned with the principles of Green Analytical Chemistry are presented: biopolymers (cellulose, chitosan, agarose, alginate and starch), biochar, cork and wooden toothpicks.

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1.3.1. Novel materials for sorptive techniques for the analysis of environmental water samples

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Alberto Moral Ruiz

Novel materials for sorptive extraction techniques for the analysis of environmental water samples

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Abstract

Developing new materials for improving the sample preparation step in analytical procedures is one of the most important research lines in analytical chemistry in order to improve the sensitivity or selectivity in this step. The sorptive extraction techniques most commonly used are solid-phase extraction (SPE), magnetic solid-phase extraction (MSPE) and solid-phase microextraction (SPME). The most important materials can be grouped into polymer-based, silica-based, carbon-based, metal organic frameworks (MOFs) and covalent organic frameworks (COFs). These materials can also be combined.

In this review, we present an overview of the most relevant in-house materials used in sorptive extraction techniques that have been reported for extracting contaminants from water samples since 2018. The most important properties that characterize each type of material and how they are exploited to extract the analytes are also presented. In addition, we discuss several examples of applications of these materials and the extraction procedures and highlight the most relevant aspects.

Keywords: novel materials, sorptive extraction techniques, environmental water samples, sample extraction protocol

1. Introduction

The presence of contaminants in environmental water can affect public health and environmental integrity. There are many different groups of organic contaminants, such as pesticides [1], pharmaceuticals and personal care products (PCPPs) [2], endocrine disruptor compounds (EDCs) [3] and polycyclic aromatic hydrocarbons (PAHs) [4], among others. These contaminants reach environmental water through three main sources: urban wastewater disposal, industrial waste and agricultural runoff. Therefore, it is important to determine their levels to evaluate the possible effects on human health and the environment [5].

Although liquid chromatography (LC) and gas chromatography (GC) combined with mass spectrometry (MS) are powerful tools, in order to obtain reliable determinations, the sample preparation must be applied to improve the selectivity and sensitivity of the determination. Sample preparation is often one of the most time-consuming steps of the analytical procedure. Therefore, one of the most important research lines in the field of analytical chemistry is improving the current sample preparation procedures, focusing on improving the extraction methods.

The extraction techniques for liquid samples can be divided into liquid-liquid extraction techniques and solid-liquid or sorptive extraction techniques. In the field of liquid-liquid extraction techniques, examples of the most commonly used solvents are chloroform, dichloromethane, toluene and hexane. However, due to their toxicity and environmental impact, they have started to be replaced by less toxic solvents, such as long-chained alcohols like 1-decanol or 1-dodecanol. Moreover, new liquid systems have also been developed with solvents such as switchable solvents, supramolecular solvents, ionic liquids and deep eutectic solvents [6]. The improvements for sorptive extraction techniques have usually focused on silica-based [7], polymer-based [8] and carbon-based materials [9,10]. Moreover, in recent years, other supports have appeared, such as covalent organic frameworks (COFs) [11] or metal organic frameworks (MOFs) [12]. These solid materials are commonly applied in sorptive extraction techniques such as solid-phase extraction (SPE), magnetic solid-phase extraction (MSPE), dispersive solid-phase extraction (dSPE) and solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE).

In this review, we give an overview of the novel in-house materials that have been developed and applied since 2018 for determining contaminants in environmental waters. The materials are divided into polymer-based materials, silica-based materials, carbon-based materials, COFs and MOFs. The combination of two or more types has also been explored and there is also a miscellaneous section for materials not included in the the mentioned groups. In addition, magnetic nanoparticles (MNPs) functionalized with the mentioned materials are included in the sections referring to the materials, since the MNPs are used to magnetize the sorbent.

A table is included in each section with selected examples of each type of material and some information about the material applied in each study as well as the extraction technique and its

procedure, and the analytical technique used for the determination. In addition, each section includes the description of the most relevant properties of each material and some interesting examples in which we discuss how these properties are exploited in several applications.

2. Polymer-based sorbents

Polymers are a type of material that has been widely used in sorptive extraction techniques for many years [8]. Their applicability led to the development of several commercially available materials that are widely applied. Examples of materials applied in SPE are Oasis, Bond Elute and Strata families. The most remarkable one is Oasis HLB, which, as we will point out in this review, has been widely used as a comparison with in-house materials. In SPME there are a few commercially available fibers, such as polydimethylsiloxane (PDMS) fiber, polyacrylate (PA) fiber and polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber. In SBSE, we can highlight the PDMS stir bar, which is also widely applied. Molecular imprinted polymers (MIPs) are another group of polymers that are used in different extraction techniques due to their enhanced selectivity. However, since they have been discussed in depth in other review studies [13,14], they will not be included in the present study.

Polymers give a wide range of flexibility, there is a huge diversity of monomers that can be used as building blocks, from simple molecules such as pyrrole [15] to the use of β-cyclodextrin [16], as can be observed in Table 1. It is very common to use two different monomers in order to combine their properties, such as N-vinylpyrrolidone and divinylbenzene [17] or divinylbenzene and vinylbenzene chloride [18]. Divinylbenzene is generally used as crosslinker monomer to form the structure, while the other monomers (i.e. N-vinylpyrrolidone or vinylbenzene chloride) are introduced to add functional groups. The variability can increase even more with the possibility to functionalize the polymer after the synthesis, and it is possible to add carboxylic acid groups [19], amines [20], a Schiff base [21], perfluorooctanoic acid groups [22] or γ-cyclodextrin [23], among others. Using polymers from natural sources has also been explored in recent years, such as amylodextrin [24], chitosan [25] and luteolin [26], following the principles of Green Analytical Chemistry.

According to the conditions shown in Table 1, there are diverse applications of in-house polymeric materials in SPE, as well as different extraction conditions. For instance, the amount of sorbent ranged from low quantities in the range from 25 to 60 mg, to higher quantities in the range from 150 to 500 mg. The loading volume ranged from 8 to 250 mL; however, it is surprising that the lowest loading volume (8 mL) was used with the largest amount of sorbent (150 mg) [16], as it is usually the opposite tendency. It is also surprising that in the study the quantity of the sorbent is expressed in mL [27]. The reason is that the polymer was prepared directly in the cartridge through a thermal polymerization of 2-hydroxymethacrylate.

Different types of interactions can be exploited to extract the analytes, which is related to the extraction conditions shown in Table 1. For instance, Guo *et al.* [26] exploited the π-π stacking and the Lewis acid-base interactions between the

hydroxyl groups in the sorbent, which behaved as Lewis bases, and the NO_2 or CN groups in the neonicotinoid insecticides, which behaved as Lewis acids. Therefore, the pH selected was 6, ensuring that the analytes were in neutral form; and the compounds were eluted with acetonitrile (ACN), an adequate solvent to disrupt these interactions. Ning *et al.* [19] evaluated the exploit of ion-exchange interactions with a polystyrene-divinylbenzene (PS/DVB) polymer functionalized with mercaptosuccinic acid to perform weak cation-exchange interactions. Nadal *et al.* [18] also evaluated this with a hypercrosslinked polydivinylbenzene/vinylbenzene chloride (PDVB/VBC) polymer functionalized with amino acids to perform cation- and anion-exchange interactions simultaneously. In both cases, it was possible to include a washing step based on MeOH to remove the compounds retained through reversed-phase interactions, increasing the selectivity of the extraction. In the study performed by Wang *et al.* [28] with a triazine based polymer, the performance of the in-house material for extracting EDCs was compared with some commercial sorbents (Oasis HLB, MWCNTs, graphitized carbon and C_{18}). They obtained the highest recoveries with the in-house sorbent. This sorbent was also evaluated for extracting phenyl urea herbicides, chlorophenols and PAHs. Since the most important interactions were hydrogen bonding interactions, the sorbents showed good performances for all the compounds except the PAHs, which could not form this type of interaction.

Polymers have also been widely applied in dispersive techniques (MSPE and dSPE). As can be seen in Table 1, the loading volume ranged from 5 to 30 mL, according to the amount of sorbent, which ranged from 4 to 50 mg, except for the study performed by Liu *et al.* [17], which used 500 mL. Despite selecting a large extracting volume considering the amount of sorbent (50 mg) and the extraction time (15 min ultrasound assisted extraction), the recoveries are good. The performance of this sorbent (a copolymer of N-vinylpyrrolidone and DVB) was compared with a cartridge with 500 mg of Oasis HLB sorbent, presenting slightly higher recoveries, with ten times less sorbent. Another example is the study performed by Fan *et al.* [21], which presents an interesting functionalization of a polyethylene glycol sorbent that consisted of a Schiff base network (a system with high π density), which made it possible to extract benzoyl urea pesticides by exploiting π-π interactions and hydrogen bonding. In addition, Huang *et al.* [23] exploited hydrophobic interactions to extract microcystins with a sorbent functionalized with a γ-cyclodextrin. These interactions were also exploited in another study to extract polychlorinated biphenyls (PCBs) with a chitosan sorbent [25]. Other authors exploited ion-exchange interactions, such as Li *et al.* [15], who extracted lactams with a polypyrrole material, applying an acidic pH during the extraction step and a basic solution of MeOH to elute the analytes. In another study [29], hormones and insecticides were retained through electrostatic interactions, π–π stacking and hydrogen bonding with an o-hydroxyazobenzene polymer. However, in the elution step (1 mL MeOH), the π–π stacking and

hydrogen bonding seem to be more relevant than the electrostatic interactions for retaining the analytes.

Polymeric materials have been applied in other techniques, as seen in Table 1, such as headspace SPME (HS-SPME) [30], pipette tip SPE (PT-SPE) [24], stir bar sorptive extraction (SBSE) [31] and stir bar sorptive dispersive microextraction (SBSDME) [32]. In the HS-SPME study [30], three different polymeric ionic liquids were developed for extracting UV filters. The best performing sorbent was the one in which 1-vinylbenzyl-3-hexadecylimidazolium bis[(trifluoromethyl)-sulfonyl]imide was used as the monomer. Moreover, using thermal desorption in the GC-MS made it possible to reach low detection limits (low ng/L), which can be seen in Table 1. Thermal desorption was also used for determining nitro musks through SBSDME [32], and low detection limits were also obtained. In this study, the extraction was performed with polydopamine MNPs. In both cases, they exploited the π–π stacking and hydrogen bonding interactions. In the SBSE study [31], the sorbent was rich in phenolic groups, which allowed it to interact with the benzotriazoles through π–π stacking, hydrogen bonding and hydrophobic interactions. Therefore, recoveries obtained with the in-house stir bar were higher than the ones obtained with the commercial PDMS stir bar it was compared with, and which can only perform hydrophobic interactions.

As highlighted, polymer-based materials have been widely used with different applications and it is expected that in the future novel materials will appear, new monomers will be tested and novel functional groups will be evaluated.

3. Silica-based materials

Silica is an interesting material that has been applied in sorptive extraction for many years, it can be obtained at a low cost and it is easy to functionalize [7]. The silanol groups present on the surface of silica can be easily modified by inserting different functionalities, such as quaternary amine groups C_{18} chains [33], [34] or a Schiff Base [35]. As can be seen in Table 2, silica-based materials have been exploited in different sorptive extraction techniques. An example of silica functionalization is the study by Spelltini *et al.* [36], who thermally condensed humic acids in silica to extract glucocorticoids through SPE by allowing the sorbent to perform π stacking, H bond and dipole-dipole interactions. It should be highlighted that even when relatively high volumes (1000 mL of river water, 250 mL of effluent wastewater) were loaded in the 200 mg cartridges, no high matrix effects (less than 35% signal suppression) were observed. A different approach was presented in our previous study [37], in which silica was functionalized with quaternary amines, sulfonic groups and C_{18} chains to selectively extract acidic and basic pharmaceuticals through ion-exchange interactions. A washing step with MeOH was included in which the acidic pharmaceuticals were rinsed from the sorbent. The acidic compounds were thus retained through reversed-phase interactions, and the basic compounds were retained through cation-exchange interactions. Therefore, the sorbent was applied to extract the basic analytes with matrix effects lower than ±25%, as the

Table 1. Selected examples of the application of novel in-house polymeric materials and optimized extraction conditions.

Type of polymer	Monomer	Analytes	Extraction technique	Extraction procedure				Deter. tech.	%R	LOD	Ref
				Amount	Loading/ extraction	Washing	Elution/ desorption				
Hydroxyl-functionalized polymer	Luteolin	Neonicotinoid insecticides	SPE	30 mg	100 mL, pH 6	3 mL 10% MeOH in water	0.2 mL ACN	LC-UV	74 - 88	0.02 – 0.08 µg/L	[26]
Poly-2-HEMA	2 - HEMA	NSAIDs	SPE	0.465 mL (monolith precursor)	50 mL, pH 3.15	Water	1 mL ACN	LC-UV	42 - 53	1 – 2.6 µg/L	[27]
Hypercrosslinked DVB-VBC polymer	DVB and VBC chloride	MIX contaminants	SPE	200 mg	250 mL river, 100 mL effluent WW, pH 6	1 mL MeOH	5 mL 5% NH_4OH in MeOH	LC-MS/MS	52 - 105	0.03 - 2 ng/L	[18]
PS-DVB functionalized with carboxylic acid groups	Styrene and DVB	Illicit drugs	SPE	60 mg	50 mL, pH 6,	4 mL MeOH	4 mL, 4% TFA in MeOH	LC-MS/MS	83 - 116	0.17 – 1.67 ng/L	[19]
β-cyclodextrin polymer	β-cyclodextrin	Bisphenols	SPE	150 mg	8 mL	-	2 mL MeOH	LC-UV	95 - 102	0.3 µg/L	[16]
Amino functionalized triazine based HXL polymer MNPs	2,4-diamino-6-phenyl-1,3,5-triazine	EDCs	SPE	25 mg	100 mL	3 mL water	0.2 mL MeOH	LC-UV	84 – 107	20 – 60 ng/L	[28]
Polypyrrole MNPs	Pyrrole	β-lactams	MSPE	30 mg	40 mL, pH 4, 40 min stirring	-	2 mL 3% NH_4OH in MeOH, 20 min stirring	MEKC-UV	83 – 93	0.8 – 1 µg/L	[15]
Polydopamine functionalized with γ-cyclodextrin	Dopamine	Microcystins	MSPE	4 mg	20 mL, pH 7, 30 min stirring	-	0.5 mL 20% NH_4OH in MeOH, 7 min vortex	LC-MS/MS	87 - 102	0.8 - 2 ng/L	[23]
Polyethylene glycol functionalized with Schiff base	Ethylene glycol	Benzoylurea insecticides	MSPE	20 mg	10 mL, 2 min vortex	-	1 mL ACN, 1 min US, 1 min vortex	LC-UV	64 - 103	0.4 - 1 µg/L	[21]
PolyNVP-co-DVB MNPs	NVP and DVB	Pesticides	MSPE	50 mg	500 mL, 15 min USAE	-	3 mL 0.1 % HCOOH ACN	LC-MS/MS	63 - 117	0.13 – 0.42 ng/L	[17]
Polysaccharide chitosan MNPs	Chitosan	PCBs	MSPE	50 mg	30 mL, pH 7, 25% NaCl, 60 min stirring	-	1 mL hexane, 15 min USAE	GC-MS	93 - 106	20 ng/L	[25]

Table 1. Selected examples of the application of novel in-house polymeric materials and optimized extraction conditions (cont.).

o-hydroxyazobenzene polymer	2,6-diaminoanthraquinone	Hormones and insecticides	dSPE	17.5 mg	5 mL, pH 6.5, 36 min USAE	-	1 mL MeOH, 30 min vortex	LC-MS/MS	94 - 100	0.4 – 1.3 ng/L	[29]
Ionic liquid polymer	1-vinylbenzyl-3-hexadecylimidazoliumbis [(trifluoromethyl) sulfonyl] imide	UV filters	HS-SPME	-	10 mL, 100 ºC, 40 min,15% NaCl	-	6 min, 250ºC	GC-MS	72 - 122	2.8 - 26 ng/L	[30]
Combination of hydrophilic acrylic polymer and amylodextrine	Acrylic acid and amylodextrine	Triazole fungicides	PT-SPE	6 mg	20 mL, pH 7.5	-	1.1 mL MeOH	CD-IMS	93 - 96	0.1 – 0.2 µg/L	[24]
Azo-linked polymer	4,4'-diamonoterphenyl and phloroglucinol	Benzotriazoles	SBSE	-	10 mL (diluted with 45% of MeOH), 50 min stirring	-	0.15 mL 2 mM NaOH in acetone	LC-UV	96 - 118	0.12 – 0.33 µg/L	[31]
Polydopamine MNPs	Dopamine	Nitro musks	SBSDME	10 mg	25 mL, 15% NaCl, 10 min stirring	Water	10 min, 275 ºC	GC-MS	90 - 118	0.1 – 2.8 ng/L	[32]

Abbreviations: ACN: acetonitrile, CD-IMS: corona discharge-ion mobility spectrometry, dSPE: dispersive solid-phase extraction, DVB: divinylbenzene, EDCs: endocrine disruptor compounds, GC: gas chromatography, 2 – HEMA: 2-hydroxymethacrylate, HS-SPME: head-space solid-phase microextraction, LC: liquid chromatography, MEKC: micellar electrokinetic capillary chromatography MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, NSAIDs: non-steroidal anti-inflammatory drugs, NVP: N-vinylpyrrolidone, PCBs: polychlorinated bisphenols, PS: polystyrene, PT-SPE: pipette tip solid-phase extraction, SBSE: stir bar sorptive extraction, SBSDME: stir bar sorptive dispersive microextraction, SPE: solid-phase extraction, TFA: trifluoroacetic acid, USAE: ultrasound assisted extraction, UV: ultraviolet, VBC: vinylbenzene chloride, WW: wastewater

washing step had removed the compounds retained through non-specific interactions In another study, Yan *et al.* [38] functionalized silica with perfluorobenzene, allowing the material to perform π-hole bond interaction, which is a non-covalent interaction similar to a hydrogen bond. This material was applied to extract PAHs. Looking at the recoveries shown in Table 2, the results from these last two studies mentioned [37,38] seem to be low; however, in these studies the reported recoveries are "apparent recoveries", which show the yield of the extraction and not the accuracy of the method (relative recoveries) as in other studies.

Mesoporous silica is a type of silica characterized by having a more ordered porous structure in which the pores have size uniformity (2 – 50 nm). It has a higher surface area and more homogeneous structure, which is appropriate for being applied in extraction techniques [7,39]. There are several studies with different modifications to mesoporous silica; for example, Pellicer *et al.* [40] added Ti to extract phosphorous flame retardants because Ti enhances the interactions with P-containing compounds. As shown in Table 2, the elution was made with ethyl acetate (EtAOc), which was selected over hexane, ACN, MeOH and ethanol (EtOH), because a solvent with intermediate polarity was needed. Moreover, the performance of the new sorbent was compared with a commercial C_{18} material, and it showed higher recoveries than the commercial one. Zhang *et al.* [41] and Ri *et al.* [42] functionalized mesoporous silica with quaternary amines and carboxylic acid groups, respectively, to enable the ion-exchange interactions. In the study performed by Zhang *et al.* [41], it was observed that adding quaternary amine groups compared to the unmodified silica enhanced the retention of some of the EDCs determined (estriol, estradiol, estrone and bisphenol A), due to the contribution of positive charges in the sorbent. However, the analytes were eluted with ACN without any acidic additive, and therefore, despite the ion-exchange contribution, the compounds were mainly retained through π-π and hydrogen bonding interactions. EDCs were also extracted with a modified mesoporous silica material functionalized with aldehyde groups [43].

Other examples of functional groups were the amine groups and C_8 chains to extract phenoxyacid herbicides through ion-exchange interactions [44]. As it can be observed in Table 2, pH 5 was selected for the extraction since it ensured the ionization of both analytes and sorbent, which enhances the ion-exchange interactions. In another study, the possibility to immobilize an ionic liquid in mesoporous silica was also explored [45], when an imidazolium ionic liquid was used for extracting multi class pesticides (carbamates, OPPs and pyrethroids). Mesoporous silica has also been applied in stir bar supported micro-SPE (SB-μ-SPE) [46] when it was functionalized with N-sulfonyl-4-hydroxymethyl-1,2,3-triazole. This made it possible to perform hydrogen bonding and π-π stacking due to the hydroxyl groups and triazole groups, respectively, to extract phenolic compounds.

Table 2. Selected examples of the application of novel in-house silica-based materials and optimized extraction conditions.

Type	Analyte	Extraction technique	Extraction procedure				Deter. technique	%R	LOD	Ref
			Amount	Loading/ extraction	Washing	Elution/desorption				
Humic acids functionalized silica	Glucocorticoids	SPE	200 mg	1000 mL river, 250 mL effluent WW	-	2.5 mL MeOH	LC-MS/MS	79 - 123	0.02 – 0.9 ng/L	[36]
Amine, sulfonic and C_{18} functionalized silica	Pharmaceuticals	SPE	200 mg	100 mL river, 50 mL effluent WW	5 mL MeOH	5 mL 5% NH_4OH in MeOH	LC-MS/MS	40 - 85	1 - 5 ng/L	[37]
Mesoporous silica (UVM-7) doped with Ti	Flame retardants	SPE	200 mg	50 mL	-	0.25 mL EtOAc	GC-MS	66 - 109	0.019 – 0.21 µg/L	[40]
Perfluorobenzene functionalized silica	PAHs	SPE	200 mg	50 mL	-	2 mL DCM	LC-UV	32 - 89	2 - 80 ng/L	[38]
Carboxyl functionalized silica MNPs	Tetracyclines	On-line SPE	10 mg	pH 5	-	0.1 mL, MeOH, ACN and 0.02 M oxalic acid solution (10:20:70, v/v)	LC-MS/MS	97 - 111	12 – 74.1 ng/L	[42]
Aldehyde functionalized mesoporous silica MNPs	EDCs	MSPE	10g	100 mL, 5 min vortex	Water	3 mL ACN, 2 min stirring	LC-HRMS	82 - 96	0.2 - 3 ng/L	[43]
C_8 and amino functionalized mesoporous silica	Herbicides	MSPE	5 mg	10 mL, pH 5, 1 min	-	1 mL of 2% NH_4OH in acetone 1 min vortex	LC-MS/MS	88 - 115	5 – 20 ng/L	[44]
IL functionalized mesoporous silica (MCM-41)	Pesticides	MSPE	Not reported	10 min pH 6, 1 % NaCl	-	0.2 mL ACN, 3 min	GC-ECD	82 – 110	0.05 – 1.6 µg/L	[45]
Quaternary amine functionalized mesoporous silica (MCM-48)	EDCs	dSPE	15mg	20 mL, 0.1 g NaCl, pH 3, 30 min stirring	Water	0.8 mL ACN 10 min USAE	LC-UV	95 - 102	1.2 -2.6 ng/L	[41]
C_{18} functionalized silica	PAHs	IT-SPME	-	25 mL	-	0.2 mL hexane:acetone (9:1, v:v)	GC-FID	79 - 104	0.22 – 0.47 µg/L	[33]
N-sulfonyl-4-hydroxymethyl-1,2,3-triazole functionalized mesoporous silica (SBA-15)	Phenolic compounds	SB-µ-SPE	-	10mL, 2g NaCl, 30 min	2 mL 10% MeOH in water	0.3 mL MeOH 20 min USAE	GC-MS	85 - 109	0.03 – 0.3 µg/L	[46]

Abbreviations: ACN: acetonitrile, DCM: dichloromethane, dSPE: dispersive solid-phase extraction, ECD: electron capture detector, EDCs: endocrine disruptor compounds, EtOAc: ethyl acetate, FID: flame ionization detector, GC: gas chromatography, HRMS: high resolution mass spectrometry IT-SPME: in-tube solid-phase microextraction, LC: liquid chromatography, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, PAHs: polycyclic aromatic hydrocarbons, SB-µ-SPE: stir bar micro solid-phase extraction, SPE: solid-phase extraction, USAE: ultrasound assisted extraction, UV: ultraviolet, WW: wastewater

Although silica is a material that has been used extensively in sorptive extraction techniques, new silica-based materials, including novel functional groups, continue to appear. Moreover, the development of mesoporous silica materials offers another interesting research line.

4. Carbon-based materials

Carbon can be found in different allotropic forms, such as diamond, graphite, fullerenes, graphene and carbon nanotubes. The last two are currently the most commonly applied in extraction techniques, as can be seen in Table 3.

In recent years, graphene has attracted the attention of chemists from diverse fields, since it is characterized by properties like large delocalized π-density and high surface area. These properties make graphene (and graphene oxide) an interesting material to be applied in analytical chemistry for extraction techniques, mainly due to the π-π interactions that can perform with aromatic compounds [9,47]. The widest application of graphene is in combination with MNPs in MSPE [48–52]. As can be observed in Table 3, the MNPs with graphene (or graphene oxide) can be applied without modification [50,52], as Wang *et al.* [50] did when they applied it to extract imidazole antifungals. In this study, after a docking study to discern which type of interaction occurs (electrostatic attraction, cation-π interaction, hydrophobic interaction or π-π stacking), it was found that the major interactions between analytes and sorbent were, as expected, the π-π stacking. Moreover, they evaluated the matrix effect when river, effluent wastewater, and influent wastewater samples were analyzed. Low signal suppression was observed with river and effluent wastewater (less than 28%) but higher suppression with influent wastewater samples (from 23 to 43% signal suppression); however, this is acceptable considering the complexity of the matrix. This might be explained by the low specificity of this type of material.

The structure of the graphene (or graphene oxide) can be modified with an ionic liquid [48], a cyclodextrin [49], sulfur [53], or carboxylic acid groups [51], among others. The ionic liquid (1-butyl-3-methylimizadolium bromide) [48] made it possible to extract microcystins through a combination of ion-exchange interactions and π-π stacking; pH 4 was selected since it combined negative charges in the microcystins with positive charges in the sorbent. The addition of carboxylic acid groups [51] also made it possible to perform ion-exchange interactions to extract sulfonamides. Comparing the performance of the carboxylated materials with non-modified graphene, the recoveries were 23% higher on average. When a β-cyclodextrin was added to graphene MNPs [51], the combination of π-π interactions, due to the graphene, with the formation of host-guest complexes, due to the cyclodextrin, allowed PAHs to be extracted.

Regarding the conditions presented in Table 3, the amount of sorbent ranged from 10 to 80 mg and extraction times were similar, ranging from 10 to 20 min. In relation to the recoveries and detection limits, the recoveries were in all cases close to 100% and the detection limits depended on the detection used, ng/L (or less) with tandem MS detection and µg/L if UV or FID detection was applied.

Apart from dispersive techniques, graphene was also applied to SPE [54–56] and SBSE [57], although to a lesser extent. In the example of the SPE application [54], a graphene aerogel was created in combination with ethylenediamine for extracting organophosphorus pesticides (OPPs). In this case, three of the compounds (fenitrothion. parathion and fenthion) could be retained through π-π stacking, and the other OPPs (trichlorfon, ethoprophos and dimethoate) could possibly be retained through hydrophobic interactions since they do not have benzene rings. For SBSE [57], a nickel foam was covered with graphene for extracting benzotriazoles, and good extraction efficiencies were obtained. This can be seen in Figure 1 as they were over 50% for all compounds. Moreover, it can be seen in the figure that the developed stir bar performed better than the PDMS commercial stir bar. Both materials were compared under the same extraction conditions (50 min) and it was observed that the in-house stir bar had faster extraction kinetics because with the same time, the extraction yields were significantly higher.

Carbon nanotubes are the other allotropic form of carbon that is widely used in extraction techniques. Since carbon nanotubes are essentially a graphene sheet rolled into a cylinder, their properties are similar to graphene (high surface area, inertness, high π-density). They can appear as single walled carbon nanotubes (CNTs), which are similar to a single graphene layer, or multiwalled carbon nanotubes (MWCNTs), which consist of multiple concentric layers of graphene [47]. As can be seen in Table 3, carbon nanotubes have been mainly applied in dispersive techniques (dSPE and MSPE); however, some applications in other extraction techniques are reported, such as SPE [58].

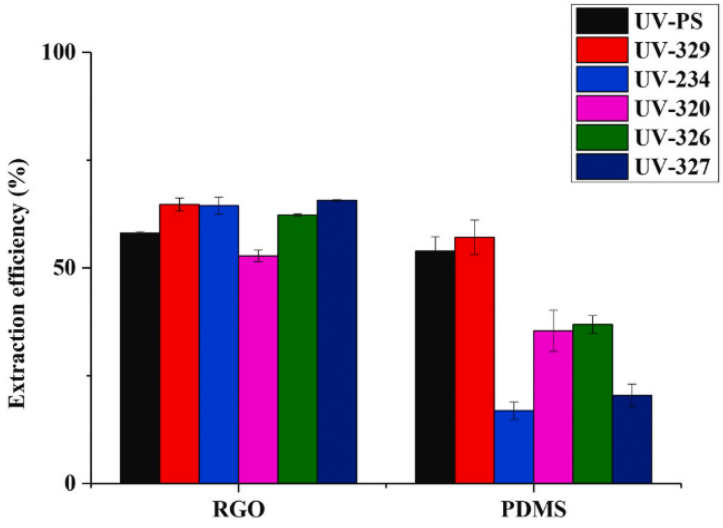

Figure 1. Comparison of the graphene stir-bar (RGO) with the commercial PDMS stir-bar (PDMS) for extracting UV filters. Reprinted from [57] of Elsevier

Table 3. Selected examples of the application of novel in-house carbon-based materials and optimized extraction conditions.

Type	Analyte	Extraction technique	Extraction procedure				Deter. technique	%R	LOD	Ref
			Amount	Loading/ extraction	Washing	Elution/desorption				
MNPs with graphene functionalized with ionic liquid	Microcystins	MSPE	10 mg	100 mL pH 4, 18 min stirring	-	1.8 mL 10% NH_4OH in MeOH, 1.5 min vortex	LC-MS/MS	89 - 99	0.22 - 0.41 ng/L	[48]
MNPs with graphene oxide functionalized with β-cyclodextrin	PAHs	MSPE	25 mg	20 mL 27% NaCl, 20 min stirring	-	0.3 mL toluene 6 min USAE	GC-FID	93 - 105	0.1 – 0.5 µg/L	[49]
MNPs with graphene	Imidazole fungicides	MSPE	80 mg	200 mL, 15 min stirring	Water	6 mL 2% NH_4OH in acetone, 2 min vortex	LC-MS/MS	70 - 92	0.11 – 0.32 ng/L	[50]
MNPs with graphene functionalized with carboxylic acid groups	Sulfonamides	MSPE	15 mg	200 mL, pH 4, 20 min stirring	-	2x2 mL 1% NH_4OH in MeOH, 1 min vortex	LC-MS/MS	73 - 90	0.49 – 1.59 ng/L	[51]
MNPs with graphene oxide	PAEs	MSPE	60 mg	25 mL, pH 6, 10 min stirring	-	4 mL DCM, 5 min stirring	LC-MS/MS	70 - 120	6 - 138 ng/L (LOQ)	[52]
Graphene aerogel	OPPs	SPE	-	40 mL, pH 6	-	5 mL THF	GC-MS	94 - 102	0.12 - 0.58 µg/L	[54]
Nickel foam covered with graphene	Benzotriazoles	SBSE	-	10 mL, 50 min,	Water	0.15 mL 30% EtOAc in acetone, 10 min USAE	LC-UV	83 - 112	0.33 - 0.50 µg/L	[57]
MNPs with MWCNTs	β-blockers	MSPE	200 mg	200 mL, 30 min stirring	-	6 mL 1% HCOOH in MeOH, 2 min vortex	LC-MS/MS	49 - 92	0.54-1.45 ng/L	[59]
MNPs with β-cyclodextrin modified CNTs	PAHs	MSPE	15 mg	10 mL, 20% NaCl, 20 min vortex	-	0.2 mL toluene, 10 min vortex	GC-FID	75 - 107	2 - 10 µg/L	[61]
MNPs with fluorinated CNTs	PFCAs and PFSAs	MSPE	30 mg	50 mL pH 4, 9 min stirring	-	0.5 mL 4% TFA in acetone, 2 min stirring	LC-MS/MS	79 -119	0.01-0.036 ng/L	[62]

Table 3. Selected examples of the application of novel in-house carbon-based materials and optimized extraction conditions (cont).

MNPs with amino modified MWCNTs	Phenoxy acid herbicides	MSPE	5 mg	10 mL pH 5.4, 1 min vortex	5 mL 15% MeOH in water	2x1 mL 2% NH_4OH in acetone, 1 min vortex	LC-MS/MS	86 - 109	0.01 – 0.02 µg/L	[63]
MWCNTs	Pharmaceuticals and metabolites	dSPE	4 mg	100 mL, pH 3, 30 min stirring	-	10 mL ACN:AcOH 3:7, 30 min stirring	LC-MS/MS	82 - 117	0.01 – 0.08 µg/L	[60]
MNPs with activated carbon	EDCs	MSPE	20 mg	0.75 mL, pH 10.5, 1 min, vortex	-	0.75 mL ACN:MeOH 1:1, 1 min vortex	LC-UV	56 - 81	0.10 mg/L	[64]
Carbon nanodots	OPPs and parabens	SPE	170 mg	50 mL pH 4.5	Water	6 mL 10% AcOH in ACN	LC-MS/MS	63 - 123	15 - 125 ng/L	[65]

Abbreviations: ACN: acetonitrile, AcOH, acetic acid, CNTs: carbon nanotubes, DCM: dichloromethane, dSPE: dispersive solid-phase extraction, ECD: electron capture detector, EtOAc: ethyl acetate FID: flame ionization detector, GC: gas chromatography, LC: liquid chromatography, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, MWCNTs: multiwalled carbon nanotubes, OPPs: organophosphorus pesticides, PAEs: phthalate esters, PAHs: polycyclic aromatic hydrocarbons PFCAs: perfluoroalkyl carboxylic acids, PFSAs: perfluoroalkyl sulfonic acids, SPE: solid-phase extraction, TFA: trifluoroacetic acid, THF: tetrahydrofuran, USAE: ultrasound assisted extraction, UV: ultraviolet, WW: wastewater

Table 3 shows that different amounts of sorbent have been used, ranging from 4 to 200 mg. As expected, the study in which 200 mg were applied matched with the highest volume (200 mL) [59]; however, it should be pointed out that the study that used 4 mg, was able to extract up to 100 mL [60]. Focusing on the applications, CNTs modified with β-cyclodextrin were applied to extract PAHs [61], as well as in the study that combined graphene and β-cyclodextrin [49]. The combination of π-π interactions with the formation of host-guest complexes due to the cyclodextrin enhanced the extraction of the PAHs in both studies [49,61]. CNTs can also be functionalized with fluorine, as Huang *et al.* [62] did to extract perfluoroalkyl carboxylic and perfluoroalkyl sulfonic acids. The presence of fluorine in the CNTs allowed F-F interactions, which increased the recoveries from 1.1 to 1.9 times compared to the non-fluorinated material. pH 4 was selected because it was observed that basic pH inhibited the F-F interactions. Focusing on MWCNTs, Wang *et al.* [59] applied non-modified MWCNTs to extract β-blockers, exploiting π-π stacking. The matrix effect was evaluated for river, effluent wastewater and influent wastewater samples. It was found to be less than -17% for river and effluent wastewater; however, influent wastewater samples had a significant matrix effect (ranging from -19 to -48%), which can be explained due to the high capacity of the material.

Jakubus *et al.* [60] presented an interesting study. They extracted pharmaceuticals and their metabolites with different types of MWCNTs: non-modified, OH-modified, COOH-modified and helical MWCNTs. It was observed that the modifications with -OH and -COOH groups decreased the π-π stacking. Comparing the non-modified and the helical MWCNTs, it was observed that the retention in the non-modified MWCNTs was more effective because the compounds were extracted completely with only 4 mg of the sorbent, whereas in the case of helical MWCNTs, the amount of sorbent was 10 mg, which is also a low amount of sorbent. In another study, amino groups were added to the MWCNTs [63] for determining phenoxy acid herbicides. This study is interesting since a washing step with 15% of MeOH was included to remove some interferences that were not retained through ion-exchange interactions, which was the mechanism for retaining the analytes. This washing step benefited the selectivity of the extraction.

In addition to graphene and carbon nanotubes, other formats of carbon have been reported, such as activated carbon [64] or carbon nanodots [65]. Activated carbon, which was widely used in the beginning of sorptive extraction techniques, was applied for the MSPE of EDCs, exploiting the hydrophobic and π-π interactions [64]. The application of nanodots in extraction techniques is interesting since they are not usually applied in these techniques [65], and are more applied in sensors [66]. Carbon nanodots were used for the SPE of pesticides (OPPs and parabens) from wastewater samples and the performance of carbon nanodots was compared with a commercial sorbent (Oasis HLB). They showed slightly lower recoveries than the commercial sorbent. The matrix effect was also evaluated,

showing values that are considerably high (ranging from -50 to +43%). This means that the sorbents had a high capacity but low specificity for the pesticides extracted.

As it has been pointed out, the current trends in the development of carbon-based for sorptive techniques are focused on graphene and carbon nanotubes. It is expected that in future years, the research lines will continue with these two materials, evaluating new applications and novel modifications to enhance the extraction procedure.

5. Metal organic frameworks

Metal organic frameworks (MOFs) are based on a metal ion surrounded by organic ligands, forming dimensional structures. Using different metal centers and organic ligands leads to a wide variety of MOFs with different properties [12]. The most applied MOFs are MIL-101 (either with Cr or Fe as metal ion), UiO-66 (Zr) and ZIF-8 (Zn). We will not cover them individually in this section, but rather they are present in some studies in which several MOFs are compared. Moreover, some applications of them will be discussed in the section of composite materials. Applications of these materials can be consulted in reviews focused on MOFs [12,67].

The applications presented in Table 4, show that MOFs have been more commonly applied in dispersive techniques (dSPE and MSPE) and SPME. The analytes were non-ionizable compounds (PAHs, PCBs, OCPs…) [68,69] or ionizable compounds in their non-charged form [70], since the most common interactions performed by MOFs are hydrophobic and π-π interactions.

In Table 4 there are some studies that compare different MOFs [69,71,72]. For instance, Gao *et al.* [71] compared MIL-101 (Cr), MIL-100 (Fe), MIL-53 (Al) and UiO-66 (Zr) for extracting estrogens and glucocorticoids by dSPE. These authors observed that MIL-53 (Al) showed the highest sorption capacity, which was due to the simple channel structure of the sorbent promoting the extraction of the analytes. In relation to the extraction conditions, it can be observed that pH 4 was used to prevent the ionization of the analytes to enhance the hydrophobic interactions. In another study, Rocío-Bautista *et al.* [72] also selected MIL-53 among other MOFs (HKUST-1 (Cu), MOF-5 (Zn), UiO-64 (Zr) and MOF-74 (Zn)) since as can be observed in Figure 2, it performed better for the extraction of a mixture of contaminants (one PAH, two hormones, two drugs and one disinfectant), with apparent recoveries (not relative recoveries) ranging from 13 to 51% in wastewater samples. Moreover, Zhang *et al.* [69] evaluated different MOFs to determine PCBs using SPME, and selected ZIF-90 over MOF-199 (Cu), MIL-101 (Cr) and MOF-5 (Zn). Its performance was also compared with commercial fibers (PDMS/DVB, PDMS and PA) and the in-house material was the one that performed best. 1000 (Zr) for the extraction of PAHs and OCPs, respectively, exploiting the π-π interactions occurring between the MOF and the analytes. The performance of NU-1000 was compared with commercial fibers (PDMS/DVB and PA), and the in-house material performed best.

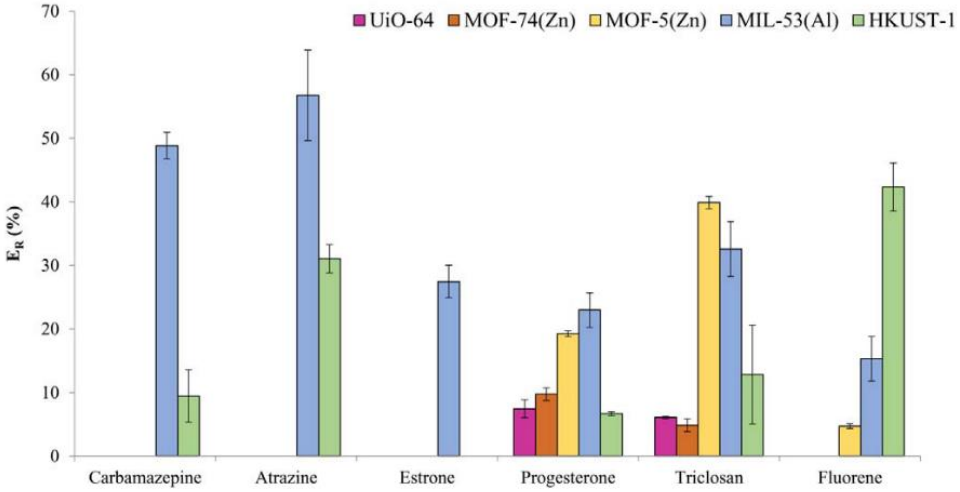

Figure 2. Comparison of the different MOFs for extracting the target compounds. Reprinted from [72] of Elsevier.

Liu *et al.* [68] developed MAF-66 (Zn) and Gong *et al.* [73] synthesized NU-. As was described previously, MOFs can be applied to extract ionizable analytes in their non-charged state, as Liu *et al.* [70] did when extracting non-steroidal anti-inflammatory drugs (NSAIDs), selecting a pH that prevented the deprotonation of these acidic analytes, and exploiting hydrogen bonding and π-π interactions. The best performing pH was 5, which is surprising considering that the pK_a of these analytes ranged from 3.7 to 5. However, pH 3 and pH 4 had lower recoveries than pH 5, which is difficult to explain considering the mentioned pK_a.

To extract ionizable compounds in their charged form, MOFs can be modified to add functional groups, and the amine groups are the most commonly used for this [74,75]. For instance, Lian *et al.* [75] developed MIL-125 (Ti) with amine groups to promote the extraction of fluoroquinolones, combining the ion-exchange interactions with the π-π interactions. Moreover, cysteine was included in the structure of the material to increase the shell thickness and improve the extraction.

The MOFs can be subjected to high temperatures to carbonize the material, creating a porous carbon material [76,77]. The properties of these materials can be controlled by the structure of the precursor MOF and the temperature of carbonization. For instance, Akbar *et al.* [77] subjected MOF-71 (Co) to 600 ºC during 2 h to obtain a carbonaceous material that was used to extract PAHs thanks to the combination of hydrophobic and π-π interactions.

It is interesting to consider the use of MOF-on-MOF structures, which are obtained by growing a MOF on the surface of another MOF. Abad *et al.* [78] combined a MOF based on Co with another based on Fe for extracting benzodiazepines.

Table 4. Selected examples of the application of novel in-house metal organic framework (MOF) materials and optimized extraction conditions.

Material	Analyte	Extraction technique	Extraction procedure			Deter. technique	%R	LOD	Ref
			Amount	Loading/extraction	Elution/desorption				
MIL-101(Cr), MIL-100(Fe), MIL-53(Al) applied, and UiO-66(Zr)	Estrogens and glucocorticoids	dSPE	8 mg	10 mL pH 4, 15% NaCl, 30 min vortex	1 mL MeOH, 20 min USAE	LC-MS/MS	79-107	0.0015 - 1 µg/L	[71]
HKUST-1, MOF-5(Zn), MIL-53(Al) applied, UiO-64 and MOF-74(Zn)	MIX contaminants	dSPE	5 mg	10 mL, 5 min vortex	3x0.2 mL ACN 5 min vortex	LC-TOF	13-51	0.013 – 0.04 µg/L	[72]
Na-Bi MOF	PAEs and adipate esters	dSPE	5 mg	5 mL, 5 min vortex	0,5 mL 2,2-dimethoxypropane	GC-FID	52 – 80	0.66 – 1.2 µg/L	[79]
MOF on MOF (Co-MOF and Fe-MOF)	Benzodiazepines	dSPE	40 mg	20 mL, 1% NaCl, 5 min US	0,137 mL MeOH, USAE	LC-UV	88 – 98	0.06 – 0.09 µg/L	[78]
Carbonized MOF-71 (Co)	PAHs	MSPE	28 mg	25 mL, 10% NaCl, 15 min stirring	0.13 mL ACN, 2 min vortex	LC-UV	52-82	0.06 – 0.18 µg/L	[77]
MIL-100 (Fe)-NH_2	NSAIDs	MSPE	25 mg	50 mL pH 5, 45 min stirring	1 mL 1% HCOOH in ACN, 15 min USAE	LC-MS/MS	75-105	0.02 – 0.09 µg/L	[70]
MIL-125 (Ti)-NH_2	Fluoroquinolones	MSPE	25 mg	50 mL, pH 7, 30 min stirring	2 mL 30% NH_4OH MeOH, 20 min stirring	LC-UV	85-109	0.05 - 0.2 µg/L	[75]
ZIF-90 (Zn), MOF-199 (Cu), MIL-101 (Cr), MOF-5 (Zn)	PCBs	SPME	-	20 mL, 50 °C, 40 min	230 °C 13 min	GC-MS	87-99	0.0013 – 0.053 ng/L	[69]
NU-1000 (Zr)	Organochlorine pesticides	SPME	-	10 mL, 40 °C, 40 min	260 °C, 6 min	GC-MS	83-122	0.011 – 0.058 ng/L	[73]
MAF-66 (Zn)	PAHs	HS-SPME	-	10 mL, 25% NaCl, 25 °C, 40 min	250 °C, 5 min	GC-FID	90-108	0.1 – 7.5 ng/L	[68]

Abbreviations: ACN: acetonitrile, dSPE: dispersive solid-phase extraction, FID: flame ionization detector, GC: gas chromatography, HS-SPME: head-space solid-phase microextraction, LC: liquid chromatography, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, NSAIDs: non-steroidal anti-inflammatory drugs, PAEs: phthalate esters, PCBs: polychlorinated biphenyls, SPME: solid-phase microextraction, TOF: time of flight, USAE: ultrasound assisted extraction, UV: ultraviolet.

Another interesting study is the one performed by Pezhhanfar *et al.* [79], who applied a heterometallic MOF based on Na and Bi. It should be highlighted that 2,2-dimethoxypropane (DMP) was used as the desorption solvent in that study. This solvent is transformed to methanol and acetone in the presence of water. Therefore, the inherent moisture of the MOF particles resulting from vortexing them in the aqueous solution led to the partial conversion of DMP to methanol and acetone. The in-situ-generating ternary solvent (methanol, acetone, and unreacted DMP) led to the desorption of the target compounds from the MOF via vortexing for 5 min.

Other MOF materials apart from ZIF-8, MIL-101 and UiO-66 have also been developed and applied, and it is expected that even more materials will be developed and applied. Moreover, the possibility to functionalize or carbonize them expands the diversity of MOFs.

6. Covalent organic frameworks

Covalent organic frameworks (COFs) are one of the new trends in recent years in the field of extraction techniques. Table 5 shows some relevant examples of their applications. They are based on monomers formed by light elements (C, B, O, N…) that are connected through strong covalent bonds to form a network. The wide diversity of monomers available and the capability to predict how the monomers will assemble make them an interesting material to be applied in analytical chemistry. Despite the diversity among them, all of them are characterized by low density, large surface areas and good chemical stability [11,80]. The monomers employed usually have benzene rings (at least one of the monomers presented in Table 5 for each study), so one of the most common mechanisms to extract the analytes is π-π stacking; for instance, both 1,3,5-tris(4-aminophenyl)benzene and terephtaldehyde [81] have aromatic rings in their structures.

In terms of the dispersive techniques, Table 5 shows that the amount of sorbent ranges from 10 to 30 mg and that the limits of detection are around µg/L when UV detection is used and around ng/L when MS detection is applied. As has been highlighted, most of the studies exploit the π-π interactions to extract the analytes. For instance, when a COF was applied for extracting disinfection byproducts [82], the pH used for the extraction of the compounds was very acidic (pH 0.5) compared to the pH usually applied in extraction techniques, because when pH was higher than 2, then negative charges appeared in the analytes and the COF. Therefore, electrostatic repulsions were generated and the π-π stacking was hindered. However, in some cases, the presence of hydroxyl groups or amines allowed the materials to perform hydrogen bonds [83,84]. Moreover, there are some examples where COFs have been functionalized with amine groups to exploit ion-exchange interactions, for instance to extract perfluoro alkyl acid substances (PFAAs) [81]. Their synthetic and extraction procedures are shown in Figure 3. The extraction procedure was based on using an acidic pH (pH 4) for the extraction to promote the ion-exchange interactions and an elution step with ammonium hydroxide to disrupt these interactions. The addition of the amines was compared with the unmodified sorbent, showing better

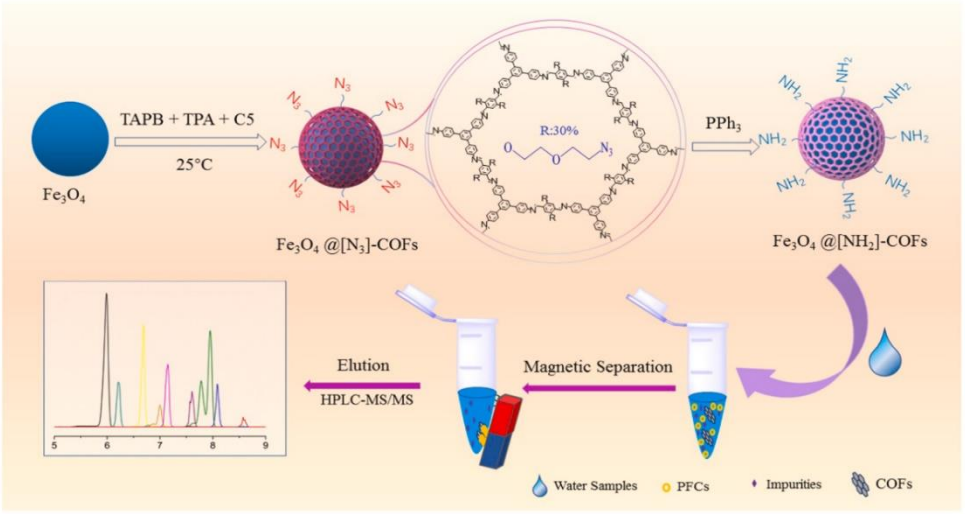

Figure 3. Preparation and application of the MNPs modified with aminated COF. Reprinted from [81] of Elsevier.

extraction yields, especially for the smaller PFAAs. Moreover, Li *et al.* [85] also exploited the ion-exchange interactions to extract NSAIDs, using a molecular imprinted COF (MICOF) that used ibuprofen as template to promote the selectivity of the extraction.

In the case of SPME applications, when this technique was combined with GC/MS determination, significantly low detection limits were achieved (Table 6), even with liquid desorption [86]. It should be noted that some COFs applied in SPME had high surface areas, reaching values such as 1254 [86] or 1560 m^2/g [87]. Similarly to the sorbents applied in dispersive techniques, the π-π interactions and the hydrogen bonding were the mechanisms exploited to extract the analytes [86–89]. The fibers developed were compared with the commercial fibers most commonly used, such as DVB/CAR/PDMS, PA, PDMS and PDMS/DVB [87–89]. The in-house material was the one that performed best in all cases. In another comparison with commercial fibers, the COF based on the combination of 1,3,5-triformylphloroglucinol (Tp) and 2,5-dichloro-1,4-phenylenediamine (2,5-DCA) [88], also showed a better performance than PDMS and PDMS/DVB fibers for extracting PCBs. The commercial fibers had lower extraction yields as the chlorine atoms of the PCBs increased. This behavior was not observed in the COF developed.

Although COFs are a relatively new material in the field of sorptive extraction techniques, interesting applications have already been demonstrated. More applications of these materials with new combinations of monomers are expected to be reported in the future with successful results.

Table 5. Selected examples of the application of novel in-house metal organic framework (MOF) materials and optimized extraction conditions.

Monomers	Analyte	Extraction technique	Extraction procedure			Deter. technique	%R	LOD	Ref
			Sorbent amount	Loading/ Extraction	Elution/ desorption				
N, N, N', N'-Tetrakis (4-aminophenyl)-1, 4-benzenediamine (TPDA) and 2, 6-pyridinedicarboxaldehyde (PCBA)	Parabens	MSPE	20 mg	10 mL pH 7, 20 min US	2x1 mL ACN, 5 min USAE	LC-UV	82-110	0.15 - 0.20 µg/L	[84]
1,3,5-tris(4-aminophenyl) benzene (TAPB) and terephthalaldehyde (TPA)	PFAAs	MSPE	30 mg	25 mL pH 4, 15 min stirring	2 mL 5% NH_4OH in MeOH, US 3 min	LC-MS/MS	72-115	0.05 – 0.38 ng/L	[81]
3,3' -diaminobenzidine (DAB) and 1,3,5-triformylphloroglucinol (Tp)	Phenyl urea herbicides	MSPE	30 mg	120 mL, 5% NaCl, 25 min stirring	1 mL ACN, 1 min vortex	LC-UV	84-105	0.3 – 0.5 µg/L	[83]
1,3,5-triformylphloroglucinol (Tp) and benzidine (BD)	Aromatic disinfection byproducts	MSPE	10 mg	50 mL pH 0.5, 6 min vortex	1 mL 4% HCOOH in MeOH, 1 min vortex 0.5 mL 1% NH4OH in MeOH, 10 min USAE	LC-MS/MS	78-115	0.07 – 1.81 ng/L	[82]
1,3,5-triformylbenzene (TFB) and 4,4'-diaminobiphenyl (BD)	NSAIDs	dSPE	15 mg	10 mL pH 2, 5 min USAE	1 mL 0.6% TFA in MeOH, 10 min	LC-UV	78-112	0.2 – 1.4 µg/L	[85]
2,3,5,6-tetrafluoro-4-pyridinecarbonitrile (TFPC) with 2,3,6,7,10,11-hexahydroxy triphenylene (HHTP)	PFAs	SPME	-	10 mL, pH 7, 25 min		LC-MS/MS	90-105	0.002 - 0.0045 ng/L	[86]
1,3,5-tri-(4-aminophenyl) benzene (TAPB) with 2,5-dimethoxyterephthalaldehyde (DMTA)	Polar phenols	SPME	-	10 mL pH 6, 30% NaCl, 50 °C, 60min	280 °C, 5 min	GC-MS/MS	81-112	0.0048 - 0.015 ng/L	[87]
1,3,5-Tris(4-aminophenyl) benzene (TPB) and 2,5-dimethoxyterephthalaldehyde (DMTP)	OCPs	HS-SPME	-	10 mL, 80 °C, 40 min	280 °C, 6 min	GC-MS/MS	83-107	0.001 - 14 ng/L	[89]
1,3,5-triformylphloroglucinol (Tp) and 2,5-dichloro-1,4-phenylenediamine (2,5-DCA)	PCBs	HS-SPME	-	10 mL, 60 °C, 15 min	260C, 5 min	GC-MS	79-124	0.0015 - 0.0088 ng/L	[88]

Abbreviations: ACN: acetonitrile, dSPE: dispersive solid-phase extraction, GC: gas chromatography, HS-SPME: head-space solid-phase microextraction, LC: liquid chromatography, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, NSAIDs: non-steroidal anti-inflammatory drugs, OCPs: organochlorine pesticides, PCBs: polychlorinated biphenyls, PFAAs: perfluoroalkyl acid substances, PFAs: perfluoroalkyl substances, SPME: solid-phase microextraction, TFA: trifluoroacetic acid, USAE: ultrasound assisted extraction, UV: ultraviolet,

7. Combination of materials

The described materials can be combined to exploit the properties of both in a synergistic way. As seen in Table 6, the material that had the most combinations was polymers, mainly with carbonaceous materials and MOFs. Focusing on the combinations between carbonaceous and polymer materials, similarly to the trend with carbon-based materials, most of the applications are with dispersive techniques. Graphene and graphene oxide have been combined with polystyrene [90,91], polycaprolactone [92] and polyaniline [93], among others. For instance, Song *et al.* [90] combined polystyrene microsphere with graphene oxide to extract bisphenols. They compared the recoveries of the components separately and observed that the performance was better when the components were combined. The authors attributed this improvement to the synergistic effects of the polymeric microspheres that increased the surface available to interact with the analytes while graphene enhanced the π-stacking with their high π density. In terms of the methodology of this study (Table 6), the elution volume used (6 mL) is surprising compared to the low amount of sorbent (10 mg). The study does not report which elution volumes were tested, so this volume might be reduced without reducing the recoveries. Another application combined polystyrene and graphene to generate an aerogel [91]. After the generation of the aerogel, the polymer was removed and the final product was applied in the in-syringe SPE for pyrethroids, which allowed on-site sampling. It stands out that in another study [93], an elution volume as low as 0.15 mL of ACN was used when 2.5 mg of sorbent based on the combination of polyaniline and graphene oxide was applied to extract aromatic compounds through π-π interactions. Moving to the combination with carbon nanotubes, Amiri *et al.* [94] combined polyethylene glycol with CNTs to extract OPPs. The polymer provided a 3D network while the CNTs enhanced the π-π interactions. Moreover, Zhou *et al.* [95] also exploited the structure of the polymer, in their case polyamidoamine, and the π density of MWCNTs to extract

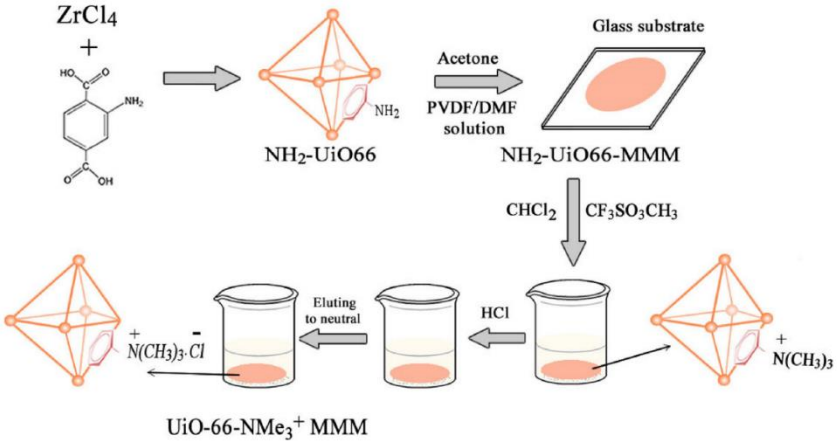

Figure 4. Illustration for the synthesis of UiO-66-NMe_3^+ membranes. Reprinted from [97] of Elsevier.

Table 6. Selected examples of the application of novel in-house composite materials and optimized extraction conditions.

Type	Description	Analytes	Extraction technique	Extraction procedure			Deter. technique	%R	LOD	Ref
				Amount	Loading	Elution				
Polymer/ Carbon	Alginate, polyvinylpyrrolidone and activated carbon	Nevirapine and zidovudine	dSPE	100 mg	20 mL, pH 6.5, 15 min US	5 mL ACN, 10 min USAE	LC-UV	91-99	0.20 – 0.23 µg/L	[96]
	Polyethylene glycol and CNTs	OPPs	SPE	-	70 mL	0.8 mL ACN	GC-FID	95-99	0.01 – 0.03 µg/L	[94]
	Polystyrene and GO	Bisphenols	dSPE	10 mg	10 mL, 30 min vortex, pH 6	6 mL MeOH	LC-MS/MS	71-88	0.02 – 0.11 µg/L	[90]
	Polystyrene and graphene (aerogel)	Pyrethroids	In syringe SPE	-	10 mL	0.5 mL EtOAc	GC-MS	66-106	0.012 - 0.11 µg/L	[91]
	Polyamidoamine and MWCNTs (MNPs)	Heterocyclic aromatic hydrocarbons	MSPE	50 mg	60 mL pH 7, 15% NaCl, 50 min stirring	7.5 mL ACN, 12 min	GC-MS/MS	87-115	0.001 - 20 µg/L	[95]
	Polyaniline and GO (MNPs)	PAHs and NitroPAHs	MSPE	2.5 mg	20 mL, 15 min USAE	0.15 mL ACN, 5 min USAE	GC-MS	92-113	0.04 – 0.05 µg/L	[93]
	Polycaprolactone and graphene	Contaminants	RDSE	-	150 mL, 2h stirring	1 mL MeOH, 1 min shaking	LC-UV	52-120	3 – 7.6 µg/L	[92]
Polymer/ MOF	HKUST-1 (Cu)-polyMAA	Fluoroquinolones	Online SPE	0.1 mg	pH 7	2% HCOOH in MeOH	CE-UV	92-100	30 ng/L	[103]
	TMU-4 (Zn) and polyethersulfone	OPPs	HS-SPME	-	10 mL, 30% NaCl, 75° C, 40 min	220 °C, 6 min	GC-NPD	91-108	5 - 8 µg/L	[102]
	Polyvinylidene fluoride UiO-66 (Zr)-NMe3+	Phenoxy acid herbicides	DME	-	50 mL, 30 min stirring	2x2.5 mL 1% NH4OH in MeOH	LC-MS/MS	80-117	0.03 – 0.59 ng/L	[97]
	Chitosan + MIL-53 (Al) USED, MIL-53 (Fe), MIL-101 (Cr), MIL-101 (Fe), UiO-66 (Zr) and MIL-100 (Fe)	Parabens	dSPE	-	10 mL pH 5, 20 min US	1 mL EtOAc, 15 min vortex	LC-MS/MS	79 - 102	0.09 – 0.45 µg/L	[98]
	MIL-101 (Cr), MIL-100(Fe), ZIF-8 (Zn), MOF-199 (Cu), MIL-53 (Al) and PVA	NSAIDs	SPE	-	10 mL, pH 6, 1 h vortex	1 mL ACN USAE	LC-MS/MS	78-106	7 - 37 ng/L	[99]

Table 6. Selected examples of the application of novel in-house composite materials and optimized extraction conditions.

Polymer/ MOF	UiO66 (Zr)-NH2 and poly-4 vinylpyridine	Sulfonylurea herbicides	SBSE	-	5mL, pH 5, 30 min	0.2 mL 10% AcOH in MeOH, 30 min	LC-UV	69-98	0.04 – 0.84 µg/L	[100]
	Polydopamine and MIL-101 (Fe) (MNPs)	Sulfonylurea herbicides	MSPE	60 mg	25 mL pH 5, 3 min vortex	7 mL MeOH, 3min USAE	LC-UV	91-109	0.12 – 0.34 µg/L	[101]
Polymer/ COF	Polyacrylonitrile - COF (terephthalaldehyde and 1,3,5-tris (4-aminophenylbenzene) poly (styrene-divinyl benzene-glycidyl-methacrylate) - COF (1,3,5-triformylbenzene and 4,4'-diamino-biphenyl)	OCPs	SPME	-	20 mL pH 6, 10 % NaCl, 30 min 40 °C	250 °C, 5 min	GC-ECD	79-116	0.05 - 20 ng/L	[105]
		NSAIDs	In syringe SPE	20 mg	3x10 mL, pH 4	3x0.5 mL EtOH	LC-UV	93-100	0.54 – 2.74 µg/L	[104]
MOF/Carbon	MIL-101 (Cr) and GO (MNPs)	Neonicotinoid insecticides	MSPE	20 mg	10 mL pH 7, 4% NaCl, 20 min USAE	1 mL 30% 3-ethylamine in EtOH, 6 min USAE	LC-UV	89-102	19 - 22 ng/L	[106]
Silica/ Polymer	Mesoporous silica and polydopamine (MNPs)	Amphetamines	MSPE	50 mg	50 mL, pH 11, 15 min stirring	5 mL 5% HCOOH in ACN, 3 min vortex	LC-MS/MS	95-107	0.5 – 2.5 ng/L	[107]
Silica/ Carbon	Silica and graphene (MNPs)	Benzothiazoles, benzotriazoles and benzenesulfonamides	SPE	200 mg	100 mL river, 50 mL effluent WW and influent WW	5 mL MeOH	LC-HRMS	48-85	10 - 455 ng/L	[108]
Silica/ MOF	Cu/Ni MOF and silica MNPs	Aflatoxins	MSPE	39 mg	15 mL, pH 7.7, 0.3 g NaCl, 6 min USAE	0.265 mL MeOH, 7 min USAE	LC-FLD	94 - 96	0.01 – 0.04 ug/L	[109]

Abbreviations: ACN: acetonitrile, AcOH: acetic acid, dSPE: dispersive solid-phase extraction, ECD: electron capture detector, EtOAc: ethyl acetate EtOH: ethanol, FLD: fluorescence detector, GC: gas chromatography, HRMS: high resolution mass spectrometry, HS-SPME: head-space solid-phase microextraction, LC: liquid chromatography, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, NPD: nitrogen-phosphorous detector, NSAIDs: non-steroidal anti-inflammatory drugs, OCPs: organochlorine pesticides, OPPs: organophosphorus pesticides, PAHs: polycyclic aromatic compounds, SBSE: stir bar sorptive extraction, SPE: solid-phase extraction, USAE: ultrasound assisted extraction, UV: ultraviolet, WW: wastewater

heterocyclic aromatic hydrocarbons. Apart from CNTs and graphene, activated carbon has been combined with polyvinylpyrrolidone and alginate to extract neviparine and zidovudine [96], it can be highlighted that alginate is a natural biopolymer, so using it follows the trends of Green Analytical Chemistry.

The combination of polymer and MOF has been also deeply investigated some examples are shown in Table 6. The structural possibilities that allow working with polymers combined with MOFs created some interesting formats, such as membranes [97], foams [98] and cryogels [99]. For example, Wu *et al.* [97] combined UiO-66 (Zr) – NMe_3^+ with polyvinyl fluoride to create membranes for extracting phenoxy carboxylic acid pesticides through dispersive membrane extraction (DME), as can be observed in Figure 4. Since the extraction is based on ion-exchange interactions, it is surprising that there was no pH adjustment to control the ionization of the analytes. On a different note, Li *et al.* [98] created foams by combining chitosan (a biopolymer) with several MOFs: (MIL-53(Al), MIL-53 (Fe), MIL-100(Fe), MIL-101(Cr), MIL-101 (Fe) and UiO-66(Zr). It was observed that MIL-53 (Al) performed the best for extracting parabens through a combination of hydrophobic and π-π stacking because the extraction pH was 5 (analytes in neutral form) and ethyl acetate was used for the elution. It stands out that a low matrix effect was observed, being lower than ±20%. Moreover, Wang *et al.* [99] also tested different MOFs (MIL-101 (Cr), MIL-100(Fe), ZIF-8 (Zn), MOF-199 (Cu), MIL-53 (Al)) in combination with polyvinyl alcohol (PVA) to produce cryogels for extracting NSAIDs. The best performing material was MIL-101 (Cr) and the adsorption mechanism discussed was a combination of H-bonding (hydroxyl groups in PVA) and π-π stacking due to the π density on the MOF. These three applications [97–99] are examples in which the structure of polymers makes it possible to create formats to exploit MOFs in an easier way in comparison to powder. Moreover, Yang *et al.* [100] and Deng *et al.* [101] combined UiO-66 (Zr) and poly-4-vinylpyridine and MIL-101 (Fe) and dopamine, respectively, to extract sulfonyl urea herbicides. As can be seen in Table 6, the two studies used pH 5 for the extraction since the interactions exploited were π-π stacking and H-bonding, preventing the analysis from being charged. In addition, Bagheri *et al.* [102] developed a new fiber for HS-SPME by combining TMU-4 (Zn) and polyethersulfone to extract OPPs. In this case, the MOF contributes with high surface area (although it is not reported) and the polymer with a high π density.

Table 6 shows that other combinations have been reported, such as polymer with COF [104,105], MOF with graphene [106] and silica with polymer [107], graphene [108] or MOF [109]. In most cases, one of the materials acted as a substrate (due to their structure) to promote the interactions of the other material. This is the case of the combinations of polymer and COF [104,105] and the combination of silica and graphene [108], in which the extraction was based on the π-π interactions due to the inclusion of COFs or graphene in the silica structure. In the case of combining MOF and graphene [106], the combination of MIL-101 (Cr)-NH_2 and graphene oxide made it possible to perform H-bonds due to the amine

groups in the MOF, as well as π-stacking due to the graphene for extracting neonicotinoid insecticides. Silica also acted as the substrate to enhance the retention of the analytes when it was combined with polydopamine [107] and a bimetallic MOF (Cu-Ni) to extract amphetamines and aflatoxins through π-stacking and H-bonding [109].

Although different combinations have been presented in this review, it is expected that in the future more and more composite materials will be developed, exploiting the properties of each component synergistically.

8. Other materials

In this section, we discuss some relevant applications that did not fit in the previous sections. As can be seen in Table 7, most of these applications are based on modified MNPs with different functionalities. The use of materials with a similar structure to graphene was evaluated by combining MNPs with MoS_2 [110] and hexagonal boron nitride [111]. Regarding the similarity of these compounds with graphene, the π-stacking is the governing interaction between the S-Mo-S rings and the extracted neonicotinoid pesticides [110] or between the BN rings and the phenoxy acid herbicides [111]. Materials that are regularly used in other fields of chemistry were evaluated for functionalized MNPs, such as a surfactant (DC193C) [112], hydroxyapatite [113] and a zeolite (ZSM-5) [114]. In addition to MNPs, using a ZnAl layered double hydroxide (LDH) in SPME can be highlighted for extracting UV filters [115]. In this study, the performance of the in-house fiber was compared with commercial fibers (PA and PDMS) and showed a better performance than both the commercial fibers. This improved performance was related to the higher surface area that LDH allowed (although the surface area was not reported) and also to the capability of the sorbent to perform cation-π interactions with the aromatic compounds.

Some interesting new formats have also been reported that follow the principles of Green Chemistry, such as paper [116], cork [117] and fabric [118]. Paper is very interesting and promising due its hydroxyl groups and also because its porosity can be impregnated with liquids, for instance using a deep eutectic solvent (thymol-vanillin) to extract triazine herbicides [116]. Cork also has an interesting structure with good hydrophobicity and a large number of aromatic rings due to the presence of lignin and suberin. These were exploited for extracting multiclass contaminants through rotating stir disk extraction (RSDE) [117]. The easy modification of fabric through sol-gel reactions make it possible to introduce several modifications. For instance, Ferracane *et al.* [118] modified fabric with Carbowax 20M, a mixture of poly(caprolactone) and poly(dimethylsiloxane) and poly(tetrahydrofuran), to extract up to seven different families of pesticides (carbamates, morpholines, nitrosamines, OCPs, OPPs, pyrethroids, and triazines). It was observed that combining Carbowax 20M and poly(tetrahydrofuran) provided the best results. Since green chemistry is becoming increasingly relevant nowadays, it is expected that the application of these sustainable formats will increase significantly in future years.

Table 7. Selected examples of the application of novel in-house miscellaneous materials and optimized extraction conditions.

Material	Analytes	Extraction Technique	Extraction procedure			Deter. technique	%R	LOD	Ref.
			Amount	Loading	Elution				
ZSM-5 (Zeolite) MNPs	NSAIDs	MSPE	40 mg	20 mL pH 2.2, 2.5% NaCl, 2 min vortex	0.424 mL MeOH, 2 min USAE	LC-UV	86 – 107	0.5 – 3 µg/L	[114]
Boron nitride MNPs	Phenoxy carboxylic acid herbicides	MSPE	7 mg	10 mL pH 3, 10 min vortex	3 x 0.5 mL 5% NH_4OH in ACN, 3 min vortex	LC-MS/MS	77 – 107	5.6 – 10.3 ng/L	[111]
Hydroxypatite MNPs	Parabens	MSPE	2 mg	50 mL pH 3, 7 min USAE	0.1 mL ACN, 5 min USAE	GC-MS	95 - 104	5 – 10 ng/L	[113]
MoS_2 MNPs	Neonicotinioid insecticides	MSPE	40 mg	30 mL pH 7, 10min stirring	3x1 mL acetone: EtOAc (1:1) 30 s vortex	LC-UV	66 - 91	0.48 – 0.67 µg/L	[110]
Surfactant modified MNPs	Parabens	MSPE	20 mg	80 mL, pH 7, 10 min stirring	0.28 mL MeOH, 15 min USAE	LC-UV	87 – 116	2.4 – 6.3 µg/L	[112]
Paper modified with deep eutectic solvent	Triazine herbicides	dSPE	-	25 mL, 60 min stirring	1 mL MeOH, 5 min stirring	GC-MS	89 - 106	0.4 – 0.6 µg/L	[116]
Fabric	Pesticides	FPSE	-	100 mL 5% NaCl, 50 min stirring	0.25 mL acetone, 2 min vortex	GC-MS	20 - 100	3 - 160 ng/L	[118]
ZnAl-LDH	UV filters	SPME	-	15 mL pH 9.5, 3% NaCl, 35 min, 35 °C	Liquid desorption	LC-UV	80 – 109	17 – 52 ng/L	[115]
Cork	Contaminants	RDSE		35 mL	1 mL MeOH: EtOAc (1:1)	GC-MS	80 - 120	0.11 - 1.44 µg/L	[117]

Abbreviations: ACN: acetonitrile, dSPE: dispersive solid-phase extraction, EtOAc: ethyl acetate, FPSE: fabric-phase solid extraction, GC: gas chromatography, LC: liquid chromatography, LDH: layered double hydroxide, MeOH: methanol, MNPs: magnetic nanoparticles, MS: mass spectrometry, MS/MS: tandem mass spectrometry, MSPE: magnetic solid-phase extraction, NSAIDs: non-steroidal anti-inflammatory drugs, RDSE: rotating disc solid extraction, SPE: solid-phase extraction, USAE: ultrasound assisted extraction, UV: ultraviolet,

Conclusions

In this review we have discussed that during the last five years several different materials have been developed to be applied in sorptive extraction techniques for determining contaminants in water samples. A wide variety of materials have been applied through the diverse extraction techniques, exploiting the properties of each material to extract the compounds and improve the extraction features.

It is expected that this area of analytical research will never stop evolving and improving the analytical procedures. The most reported applications were when different materials were used combining their properties synergically, and it is expected that most new materials will follow this trend. It is also expected that the growing relevance of green chemistry will influence the development of novel materials and the search for more sustainable substrates will also increase in future years.

Acknowledgements

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1.3.2. Other novel materials applied in sorptive extraction techniques

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

In section 1.3.1. the most relevant materials that have been applied in sorptive extraction techniques have been presented, moreover, there are other materials that deserve to be mentioned. For this reason, in this section several materials who could not be discussed or where mentioned briefly in previous section are going to be presented.

Aptamers are another material with enhanced selectivity like MIPs or immunosorbents. They are synthetic oligonucleotides developed by a method called SELEX (Systematic Evolution of Ligands by EXponential enrichment). This method is presented in Figure 16, consist of the exposition of a sequence of oligonucleotides to the target molecule. Then, the unbound sequences are removed in the washing step. After that, the target analyte is removed and the potential aptamers are eluted and amplified through polymerase chain reaction (PCR) to obtain the aptamers [18,19,66]. They have a high affinity and specificity for the target molecules obtained thanks to the appropriate three-dimensional structure [67].

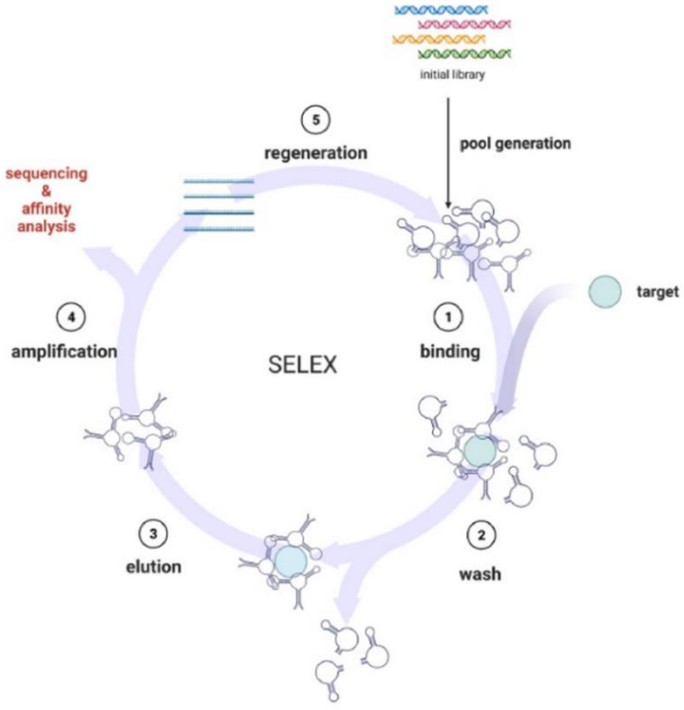

Figure 16. Scheme of SELEX [66].

They are sometimes called “chemical antibodies”, however, they have some advantages over antibodies such as lower cost, easier modification or higher stability. Aptamers can be bonded to silica, MOFs, MNPs, gold nanoparticles, carbon-based materials,…[66]. For instance, Xu *et al.* [68] functionalized a MOF composite (polyacrylonitrile nanofibers modified with UiO-66-NH_2) with an aptamer to extract microcystin-LR through SPME from

river water samples. The specificity was assessed comparing the performance of the material with or without the aptamer to extract the microcystins LR, RR and YR. It was observed that the material modified with the aptamer extracted the microcystin-LR with a recovery 95%, meanwhile the two other microcystins presented recoveries lower than 10%. The unmodified fiber presented low recoveries for the three microcystins. In another study, Khodadadi *et al.* [69] functionalized MNPs with an aptamer capable to selectively extract aflatoxin M1 from milk samples through MSPE. The specificity was also evaluated, with two other toxins with similar structure (ochrtoxin and zeralenone). It was observed that the presence of these toxins did not affect the binding of aflatoxin M1.

Polyoxometalates (POMs) are polyatomic anions based on the combination of oxygen and metals of the groups V and VI at their highest oxidation state (W, Ta, Mo, Ni, V) that can be combined with trace amounts of heteroatoms such as As, P, Si or Ge. They are characterized by high negative charges, high stability and strong acidity. For these reasons, they are specially interesting for the extraction of positively charged compounds like metal ions or basic analytes. Moreover, π-stacking interactions can be performed since POM present π density due to the d-p π bond in the POM framework (bond between the d-orbitals of the metal and the p-orbital of oxygen) [70]. POMs are commonly used in combination with other materials, like carbon nanotubes for the extraction of OPPs in juices and water samples through dSPE [71]. The synergistic effect of the nanotubes that contributed with its π-stacking and high surface area, meanwhile the POM contributed with its π-stacking and hydrogen bonding. In addition, POMs can be also combined with polymers, for example, polyoxomolybdate was combined with polyaniline to develop a fiber for SPME [72], which was used to extract antidepressants drugs from urine and blood samples. It was observed that when the fibers were functionalized with pure polyaniline or POM, the recoveries were lower than 68%, meanwhile the combined material achieved recoveries higher than 92%. The polyaniline made it possible to perform π-stacking whereas the POM also presented high delocalized electrons, causing high affinity toward conjugated compounds like antidepressants (tricyclic compounds).

Layered double hydroxides (LDHs) are an interesting inorganic material characterized by positively charged metal hydroxide sheets neutralized with layers of hydrated negative anions as depicts Figure 17. The LDHs can be characterized by the following formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{n-})_{x/n}.mH_2O$. Examples of the divalent cation are: Ca^{2+}, Zn^{2+} or Ni^{2+}; examples of the trivalent cation are: Fe^{3+}, Al^{3+} or Cr^{3+}; and examples of the anion are: Cl^-, CO_3^{2-} or NO_3^-. LDHs are interesting for its low cost, high surface area, two-dimensional tunable structure and high anion-exchange capacity and good efficiency in cation adsorption. Regarding these properties, they are usually applied to extract ions or charged compounds [73,74]. For instance, a Mg/Al LDH with Cl^- as the counter anion was applied for the determination of tetracyclines in milk samples through SPE [75]. To maximize the extraction efficiencies, the pH of the milk samples was adjusted to pH 10, since the tetracyclines were negatively charged at pH higher than 7 leading to ion-exchange interactions with the positively charged LDH. At higher pH, the presence of OH^- competed with analytes, reducing the recoveries. In addition, it should be noted that despite presenting limited extraction efficiencies for organic uncharged analytes, it is possible to intercalate organic anions in the structure enabling hydrophobic interactions for instance, with sodium dodecyl

sulfate (SDS) [73,74]. An example of an application was the modification of a Mg/Al LDH with different surfactants (sodium dodecylbenzene sulfonate (SDBS), SDS, sodium 1-nonane sulfonate (SNS), and sodium 1-hexane sulfonate monohydrate (SHS)) to extract benzoylurea insecticides from soft drinks through dSPE [76]. The surfactants were added to enable the extraction of hydrophobic compounds such as benzoylurea insecticides, it was observed that SBDS was the best additive due to its highest hydrophobicity since SDBS because of owing the longest chain and the presence of benzene rings in the surfactant that made it possible to perform π-π interactions with the analytes.

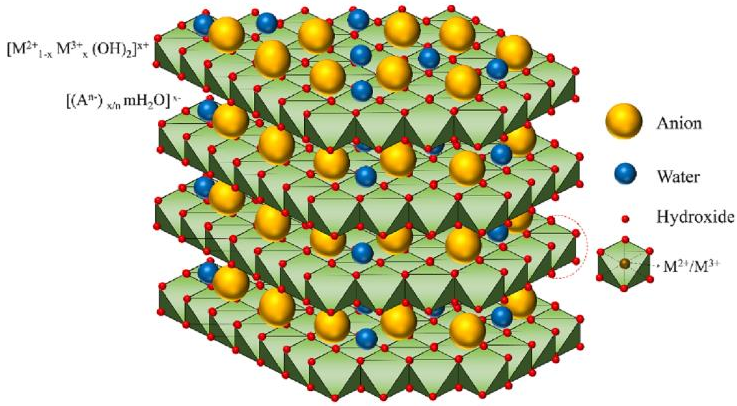

Figure 17. Scheme of the structure of a LDH [74].

MXenes are a relatively new category of 2D materials, being discovered in 2011 [77]. They are composed by transition metal carbides and carbon nitrides, being their general formula $M_{n+1}X_nT_x$ where M refers to early transition metals such as Ti, Sc or V; X refers to C and/or N, and T refers to functional groups such as -O, -OH or -F. MXenes can be applied in sorptive extraction techniques thanks to their layered high surface area, hydrophilicity and tunable surface selecting different functional groups. Despite being an interesting material, there are not yet many studies where they are applied. For instance, a $Ti_3C_2T_x$ MXene was developed for the extraction of PAHs through SPME [78]. Apart from evaluating the MXene-modified fiber for the extraction of PAHs, the extraction of melamine (which is a polar analyte) was successfully performed, demonstrating the potential of this type of materials to extract analytes with different properties. In addition, MXenes can be modified, for instance a $Ti_3C_2T_x$ MXene was modified with boronic acid to extract catecholamines from urine samples [79]. The laminar structure from MXene combined with the boronic acid allowed the performance of van der Waals forces, hydrogen bonding and π stacking interactions to extract the analytes through MSPE.

Following the trends of GAC, the application of sustainable materials has gained relevance in the recent years. For example, in the field of polymers, the use biopolymers has been evaluated. The most applied biopolymers are chitosan, cellulose, starch agarose and alginate (Figure 18). Chitosan is obtained after the deacetylation of chitin which is a

polysaccharide present in many living organisms, the primary source of chitin and chitosan is crab and shrimp shell waste. Chitosan is interesting thanks to the amino groups present in their structure (Figure 18), which allows the easy modification of the biopolymer. Moreover, the amino groups allow chitosan to perform ion-exchange interactions [80,81]. Most of the applications of chitosan are modifying the biopolymer, for instance, it can be modified with polyaniline [82]. The functionalization with polyaniline added additional binding sites to extract phthalate esters from milk samples. Another modification that can be performed is the introduction of a MOF [83]. In the mentioned study, a bimetallic MOF (Fe/Ni-MIL-53) was combined with chitosan for the extraction of aflatoxins in several samples (herbal distillate, tea, corn and water). Chitosan increased the mechanical stability of the sorbent meanwhile the MOF was the main contributor to the retention of the analytes through π stacking interactions.

Agarose and alginate are polysaccharides obtained from algae, and their most interesting property is their gelling ability, being capable of entrap particles of solid sorbents. The applications of these biopolymers consist of modifications of them [80,81,84]. For instance, agarose can be modified with MWCNTs like Sanagi *et al.* [85] did to extract triazine herbicides from water samples. Agarose gels are a format easier to handle than dust in dSPE, since the fragments of agarose gel can be easily separated from the solution meanwhile when dust is used, centrifugation is needed.

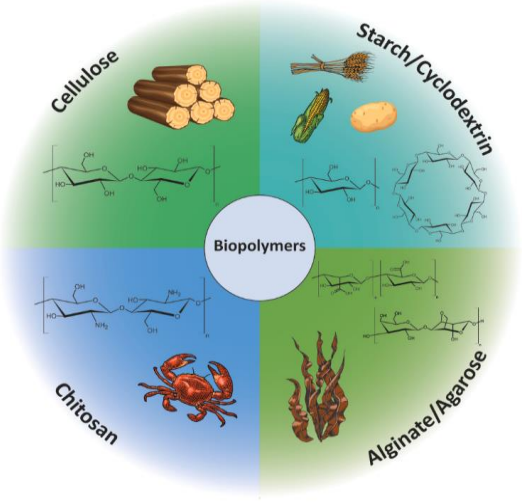

Figure 18. Most popular biopolymers applied in sorptive extraction techniques [81].

Starch is a plant polysaccharide present in cereal grains and tubers. Its low specific surface area is a significant drawback for its application. However, as well as cellulose, has hydroxyl groups in its structure, enabling the introduction of functional groups [80,81]. The applications of starch are few, for instance, it was combined with a Mg/Al LDH for the extraction of NSAIDs through SPE [86]. Starch molecules are integrated between the layers

of LDH, the presence of hydroxyl groups in both starch and LDH allowed the retention of the NSAIDs through hydrogen bonding, meanwhile the alkyl chains of starch enabled the retention through hydrophobic interactions. In addition, one of the most interesting applications of starch is its transformation into cyclodextrins. Cyclodextrins are cyclic compounds formed by sugar units, being the most commonly used α-, β- and γ- which are the ones containing six, seven and eight sugar units respectively. Cyclodextrins are characterized for presenting a hydrophobic cavity that can form host-guest complexes with compounds [80,81]. Cyclodextrins can be crosslinked to generate cyclodextrin-based polymers [87] or can be attached to other substrates as functional groups, for instance, to silica [88], MOFs [89] or COFs [90].

Cellulose is a polysaccharide, the most abundant biopolymer on Earth that can be obtained from plants. As can be observed in Figure 18, has hydroxyl groups in the surface, which make cellulose hydrophilic and easy to functionalize. Moreover, its low cost and large surface area make this biopolymer a great option [80,81]. The most common use of cellulose is in the format of filter paper coated with polymers, such as polydopamine [91] or polyvinyl chloride [92] for thin film microextraction in both cases. The functionalization was very simple: through dip-coating in both cases, moreover in the study of polyvinyl chloride another modification was performed to introduce phenyl groups using diphenylamine. Figure 19 presents a scheme of the functionalization procedure. The polyvinyl chloride modified cellulose material was applied for the extraction of opioids in saliva through cation-π and π-π interactions. Despite not being the most common application, unmodified cellulose filter paper can be used, as Díaz-Liñán *et al.* [93] did when extracted biogenic amines from beer samples. The main interaction was hydrogen bonding between the hydroxyl groups present in cellulose and the amines present in the analytes. Another format to exploit cellulose is cotton; for instance, Su *et al.* [94] developed a composite of cotton and the MOF UiO-66 to extract phenoxy acid herbicides in water, soil and cucumber samples. The fiber-based structure of cotton increased the efficiency of the extraction, being needed less amount of MOF than without combining with cotton.

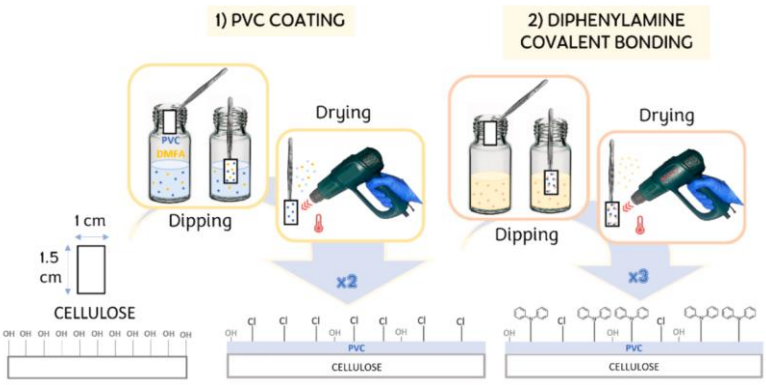

Figure 19. Functionalization of a cellulose material with polymers [92].

Another sustainable material is biochar, which is obtained through pyrolysis processes of biomass at temperatures between 500 and 700 ºC. The biomass is commonly obtained from agricultural waste and wood [95]. Examples of biochar sources can be corn straw [96], pomelo peel [97] or peanut shell [98]. The materials derived from corn-straw and pomelo peel were successfully applied to the extraction of OPPs and NSAIDs, respectively, from water samples. Moreover, in the study where peanut shell was applied, apart from obtaining biochar, the obtention of activated carbon was also evaluated. It was observed that the extraction performance of the activated carbon was better for all analytes (ethyl paraben, diclofenac, triclosan, bisphenol A, and 17-α-ethinylestradiol) than the biochar. However, the performance of the activated carbon was compared with Oasis HLB, obtaining lower recoveries than the commercial material for all analytes excepting bisphenol A [98].

Other sustainable approaches that are being evaluated in the recent years are materials as common as cork or wooden toothpicks. Cork is a material mainly formed by suberin, lignin, cellulose and hemicellulose. Lignin and suberin are organic polymers which are formed by an aliphatic and an aromatic domain, meanwhile, cellulose and hemicellulose are polysaccharides with hydroxyl groups. Therefore, the components of cork make it an interesting material to extract non-polar compounds through hydrophobic and π-π interactions, in addition, the hydroxyl groups enable the retention of polar analytes through hydrogen bonding [81]. Cork has been exploited in many formats such as SPE [99], SPME [100] or PT-SPE [101], for the extraction, exploiting π-π interactions and hydrogen bonding, of fungicides, OCPs and emerging contaminants, respectively. Regarding wooden toothpicks, the components are similar to cork, being cellulose, lignin and hemicellulose the major components. Therefore, the major retention mechanisms that are exploited are hydrogen bonding and π-π interactions. One of the most interesting properties of wooden toothpicks is the direct coupling to the mass spectrometer, improving significantly the preconcentration of analytes [102]. Apart from exploiting the interactions that can perform the wooden toothpicks by themselves like it was done for the extraction of drugs from saliva [103], they can be modified with polyamide [102] or octanol [104] through dip coating. It was observed that when the octanol was immobilized in the wooden toothpicks, the retention of the analytes (NSAIDs) increased significantly. The presence of octanol enhanced the ability to extract the NSAIDs through hydrogen bonding.

As has been shown, several novel materials with different properties have been applied for the extraction of compounds, demonstrating that this is a field that possesses practically unlimited development potential, more and more materials are expected to be developed and applied in sorptive extraction techniques. Moreover, the impact of Green Chemistry is leading to the development of more sustainable and environmentally friendly approaches.

1.4. References

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IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
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2. OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

The main aim of the present Doctoral Thesis is to develop in-house mixed-mode ion-exchange sorbents with improved features so that they can be applied in solid-phase extraction (SPE). The new sorbents have been evaluated for the selective determination of organic contaminants in environmental samples by SPE followed by liquid chromatography-mass spectrometry.

This general aim can be divided into the following specific aims:

- To evaluate various new mixed-mode zwitterionic silica-based sorbents, some of which have been modified with activated carbon and graphene.
- To synthesise and evaluate various in-house mixed-mode ion-exchange polymer-based sorbents that use novel approaches to introduce the ionic groups and enhance the morphology.
- To apply the sorbents developed to the SPE of contaminants of emerging concern in a variety of environmental samples.

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3. EXPERIMENTAL, RESULTS AND DISCUSSION

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IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
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This section contains the experimental part, results and discussion of the studies developed during this Doctoral Thesis. The main aim of this Doctoral Thesis was the development and evaluation of novel mixed-mode ion-exchange materials to be applied to the solid-phase extraction of organic contaminants from environmental water samples. The section is divided in two chapters, the first of which is devoted to the evaluation of silica-based materials meanwhile the second one covers polymer-based materials. Both chapters have similar structure, owning a brief introduction to present what will be addressed, the studies and a brief discussion the results of each chapter. The studies presented in the first chapter that have been published or submitted for its publication are presented in article format, meanwhile the studies in the second chapter are not ready yet for its publication and a preliminary version is presented. In both chapters, the developed materials have been successfully applied to the determination of organic pollutants in environmental samples through solid-phase extraction followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) or high-resolution mass spectrometry (LC-HRMS).

The first chapter is focused on the evaluation of several silica-based mixed-mode zwitterionic ion-exchange sorbents. All these sorbents have been in collaboration with Dr. A. Kabir and Prof. K.G. Furton (Florida International University). This chapter presents five different studies were eight different silica-based sorbents are evaluated for the extraction of pharmaceuticals, high volume production chemicals and illicit drugs and its metabolites. Most of the studies were focused on applying the in-house sorbents in the determination of these compounds in environmental water samples, although in one study one of the sorbents was applied as clean-up for organic extracts from dust and fish samples.

The second chapter is focused on the synthesis, characterization and evaluation of polymer-based mixed-mode ion-exchange sorbents. The synthesis and characterization of the sorbents was carried out during my stay in Strathclyde University in Glasgow in the group of Prof. P. A. G. Cormack. Two different sorbents are presented in this chapter: mixed-mode strong cation-exchange hypercrosslinked core-shells functionalized with sulfonic acid groups and mixed-mode weak anion- and weak cation-exchange microparticles functionalized with primary amines and carboxylic acid groups. The sulfonated core-shells were evaluated for the extraction of basic pharmaceuticals meanwhile the amphoteric microparticles were evaluated for the extraction of acidic and basic pharmaceuticals.

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3.1. Evaluation of sol-gel silica-based sorbents

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This chapter is focused on the evaluation of several silica-based sorbents. As it was discussed in the introduction, silica-based sorbents have been widely used in extraction sorptive techniques due to its low cost and ease to functionalize. Initially, the most common functional groups bonded to silica were reversed phase ones (C_{18}, C_8, phenyl or cyclohexyl, among others) or normal phase ones (CN, OH among others), then, the ion-exchange groups were introduced to extract ionic and ionizable compounds. Later, materials that combined both interactions were developed, appearing the mixed-mode ion-exchange sorbents [1]. In the recent years, our group has been interested in the development and evaluation of mixed-mode ion-exchange sorbents. Previous studies have developed new polymer-based mixed-mode ion-exchange sorbents, having different moieties such as strong cation-exchange (SCX) [2], weak cation-exchange (WCX) [3] or strong anion-exchange (SAX) [4]. It was not until 2020 when a silica-based mixed-mode ion-exchange material was first evaluated in our group.

The sorbents described in this chapter were synthesized in collaboration with Dr. A. Kabir and Prof. K. G. Furton in Florida International University (FIU). As was pointed out in the introduction, Kabir and Furton are known for the development of fabric phase sorptive extraction (FPSE) [5] and capsule phase microextraction (CPME) [6]. They have developed several new sorbents by applying sol-gel approaches to be applied in sorptive extraction techniques. Sol-gel allows to modify the structure of fabric or silica introducing a wide variety of functionalities such as polymers like polytetrahydrofuran [7] or polyethylene glycol [8] which were used for the determination of antihistamines and bisphenols, respectively.

The first collaboration between the research groups was the evaluation of fabric functionalized with different coatings integrated through sol-gel approach for the extraction of personal care products and pharmaceuticals (PCPPs) [9]. The coatings integrated in the fabric were polymeric: poly(dimethyldiphenylsiloxane) (PDMDPS), poly (tetrahydrofuran) (PTHF) poly(ethylene glycol)–block-poly(propylene glycol)–block-poly(ethylene glycol) (PEG–PPG–PEG triblock) and Carbowax 20M (a polymer rich in hydroxyl groups), being the last the best performing one. Sol-gel materials were also evaluated through capsule phase microextraction (CPME) [10]. CPME as was described in the introduction, it is technique related with SBSE, where two porous capsules are used to perform the extraction, the first is packed with a sorbent meanwhile the second contains a magnet. In this study, silica particles were modified with different polymers through sol-gel approach, being Carbowax 20M the best performing one. The Carbowax 20M capsules were successfully applied to the extraction of PPCPs from environmental water samples.

After these collaborations evaluating polymeric modifications on fabric or silica-based materials, mixed-mode ion-exchange moieties were integrated on silica, being the first time that silica-based mixed-mode ion-exchange sorbents were evaluated by our research group. In that study [11], two silica materials were developed, one of them was functionalized with C_{18} chains and sulfonic acid groups being a SCX material meanwhile the second one had C_{18} chains and quaternary amines to create a SAX material. The silica-based sorbents were packed in CPME and evaluated for the extraction of basic and

acidic compounds in environmental samples. The SAX sorbent provided good results for the extraction of acidic analytes meanwhile the SCX sorbent did it for the extraction of the basic ones. It can be highlighted that low matrix effects were obtained, being lower than ±33% when analyzing river and effluent wastewater samples.

Regarding the good results with the SCX and SAX materials separately, we were interested in developing silica-based sorbents that combined both moieties. Previously, polymer-based sorbents with WCX/WAX had already been developed [12]. Later, sorbents with SCX/WAX and WCX/SAX moieties were also developed [13]. Taking into account the good results obtained with these polymeric sorbents, we were interested in the evaluation of silica sorbents with similar features. In this sense, this chapter presents several studies where silica-based mixed-mode zwitterionic ion-exchange materials were developed in collaboration with FIU, which synthesized and characterized the sorbents and were then evaluated and applied by our research group. In contrast to the previous collaborations with FIU where CPME or FPSE were used, the novel sorbents are in particle format and were applied to solid-phase extraction (SPE). Once the SPE extraction method was optimized, the method was applied to the determination of the compounds by liquid chromatography with tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS).

The functionalized materials were able to perform reversed-phase, cation-exchange and anion-exchange interactions, so they can retain a wide variety of compounds. Another feature of these sorbents that combine reversed-phase and ion-exchange interactions can be the use of a washing step based on an organic solvent. This washing step removes the compounds retained through reversed-phase interactions, increasing the selectivity for the determinations of the compounds that were retained through ion-exchange interactions.

The sorbents can be divided in two parts, in the first one an initial series of sorbents were functionalized with C_{18} chains, quaternary amines and sulfonic acid groups, being able to perform SCX, SAX and reversed-phase interactions. Apart from evaluating the combination of SCX and SAX moieties, the influence of adding carbon-based materials was also tested. This group of sorbents was composed of five different ones, four of them were evaluated in the first study (section 3.1.1) being silica-based or silica-based modified with activated carbon microparticles, where they were evaluated for the extraction of acidic and basic pharmaceuticals. There was another sorbent which was silica-based with graphene microparticles embedded in the structure and was evaluated for the extraction of benzothiazoles, benzotriazoles and benzenesulfonamides (sections 3.1.2 and 3.1.3). The last study of this part (section 3.1.4) compares the sorbent modified or not with graphene (which was the best performing one from section 3.1.1) for the extraction of pharmaceuticals, drugs of abuse and metabolites.

The second part includes one study (section 3.1.5) where a new series of silica-based mixed-mode zwitterionic ion-exchange sorbents were evaluated. In this case the functional groups included were zwitterionic additives with the quaternary amines and the sulfonic acid groups in the same moiety, instead of including these groups individually. This sorbent was also evaluated for the extraction of acidic and basic pharmaceuticals.

The first four studies of this chapter have been published in different journals (list Anex II) and the last one has been submitted for its publication in Journal of Chromatography A. All of them are presented in the following sections in article format. Moreover, in the last section of the chapter there is an overall discussion of the results presented.

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3.1.1. Development of sol-gel silica-based mixed-mode zwitterionic sorbents for determining drugs in environmental water samples

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Development of sol-gel silica-based mixed-mode zwitterionic sorbents for determining drugs in environmental water samples

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Abstract

Four novel mixed-mode zwitterionic silica-based functionalized with strong moieties sorbents were synthesized and evaluated through solid-phase extraction (SPE) to determine acidic and basic drugs in environmental water samples. All sorbents had the same functionalization: quaternary amine and sulfonic groups and C_{18} chains so that hydrophobic and SCX/SAX interactions could be exploited, in addition, two of them had carbon microparticles embedded.

All sorbents retained both acidic and basic compounds in the preliminary assays but only the basic compounds were retained selectively through ionic exchange interactions when a clean-up step was introduced. The SPE method was therefore optimized to promote the selective retention of the basic compounds, initially with the two best-performing sorbents.

After optimization of the SPE protocol, these sorbents were evaluated for the analysis of environmental water samples using LC-MS/MS. The method with the best-performing sorbent was then validated with 100 mL of river samples and 50 mL of effluent wastewater samples in terms of apparent recoveries (%Rapp) spiking samples at 50 ng/L (river) and 200 ng/L (river and effluent), matrix effect, linear range, method quantification and detection limits, repeatability, and reproducibility. It should be highlighted that %Rapp ranged from 40 to 85% and matrix effects ranged from -17 to -4% for spiked river samples. When the method was applied to river and effluent wastewater samples, most compounds were found in the range from 24 to 1233 ng/L with detection limits from 1 to 5 ng/L.

Keywords: mixed-mode zwitterionic sorbents; silica sorbents; environmental samples; solid-phase extraction; basic compounds

1. Introduction

Complex samples require selective sample treatments to separate the analytes from the interferences that may cause matrix effect, mainly in liquid chromatography-mass spectrometry (LC-MS). One way to achieve this is to use selective materials in sorptive extraction techniques, the most representative of which is solid phase extraction (SPE) [1,2]. Variants of this technique are also used, such as microsolid-phase extraction (µSPE) [3], dispersive solid-phase extraction (dSPE) [4], on-line SPE [5] and pipette tip solid-phase extraction (PT-SPE) [6], as well as other sorptive extraction techniques such as stir bar solid extraction (SBSE) [7] or fabric phase solid extraction (FPSE) [8].

In recent years, research has focused on developing new sorbents [9] that can improve the sensitivity and selectivity of the methods in which they are applied, through the decrease of the interferences and the matrix effect.

Mixed-mode ion-exchange sorbents are an example of these new types of sorbents [10,11]. These sorbents can retain non-charged compounds through hydrophobic interactions and charged compounds through ion-exchange interactions, thus enabling them to interact with a wide range of compounds. The compounds retained by hydrophobic interactions are eluted with an organic eluent. Those retained by ion-exchange interactions, on the other hand, require an acidic or basic eluent to disrupt the interactions with the sorbent. This duality affords great flexibility. For instance, if the target compounds are in the ionic state (e.g. acidic or basic compounds), a clean-up step with an organic solvent can remove the hydrophobic compounds attached to the sorbent. The acidic or basic compounds can then be eluted selectively with an acidic or basic solvent [10-12], which neutralizes the compounds and enables the ionic interactions to be disrupted.

These sorbents can be classified according to the type of ion exchange interaction established. On the one hand, the sorbents are anionic exchangers if they retain anionic compounds, being strong exchangers (SAX) or weak exchangers (WAX) depending on the functionalization. On the other hand, the sorbents are cationic exchangers if they retain cationic compounds. In this case, they can also be strong (SCX) or weak (WCX) depending on the functionalization.

The pH in the different steps of the extraction protocol is therefore the key parameter when this kind of sorbent is used. To select the pH to promote high retention of the analytes, the pKa of each compound must be taken into account to ensure that, at that pH, the analytes are charged and can interact with the sorbent, which is also charged at the working pH.

The most common mixed-mode ion-exchange sorbents are polymeric sorbents, and they are available commercially as, for example, Oasis (from Waters) or Strata X (from Phenomenex). Another interesting group are silica-based sorbents, though these are less stable at extreme pH than polymeric sorbents. They also usually present low retention of polar compounds, though this may be beneficial since they have fewer

unspecific interactions than polymeric-based sorbents [12]. However, silica-based sorbents have high organic resistance and good mechanical stability. Moreover, the silanol groups present in the silica network are easy to modify, which enables a wide range of functionalization [13–19]. Liu et al. [14], for example, developed two sorbents when functionalizing mesoporous silica with octadecylsilane or octylsilane and sulfonic acid to obtain a mixed-mode sorbent based on reversed-phase and SCX interactions. These sorbents were satisfactorily evaluated for determining veterinary drug residues.

One of the main problems with mixed-mode ion-exchange sorbents is that most of them are only based on one type of ionic interaction (as occurs with the commercial sorbents [12]), which means that they are selective for only one type of compound (basic or acidic). One approach to extract both acidic and basic compounds could be the combination of commercial polymeric anionic and cationic mixed-mode ion-exchange sorbents in a single cartridge [20] or in series [21,22] to determine acidic and basic compounds in one extraction. For instance, commercial anionic and cationic Oasis sorbents were combined in a single cartridge to selectively extract acidic and basic compounds from water samples [20]. Another approach is the development of sorbents that combine anionic and cationic interactions, i.e. zwitterionic exchangers. One of the developments in the field of new sorbents is the study of materials that can simultaneously retain cationic and anionic compounds through zwitterionic-exchange interactions. One example is the microporous polymer developed by Nadal et al.[23], which was used to determine a mixture of drugs, pharmaceuticals and sweeteners with acidic and basic character in water. In this study, polymeric-based microspheres were developed for SPE based on weak anionic and cationic interactions that were controlled using the pH of the loading solution. By loading samples at pH 6, it was possible to retain acidic and basic compounds to determine those compounds in river and effluent wastewater samples through liquid chromatography-mass spectrometry in tandem LC-MS/MS.

Some silica-based [16,18] and polymer-based [23–25] zwitterionic sorbents have already been developed, though research is still needed. The silica-based sorbents reported [16,18] are based on weak ionic interactions since they are functionalized with carboxylic groups and primary amines, in both cases the chargeability of the sorbents depended on the pH along the SPE protocol.

In our study, we present a series of zwitterionic silica-based sorbents based on the functionalization of a silica network. Two of these sorbents were based on silica without modification and two were based on silica with carbon microparticles embedded. All sorbents were functionalized with quaternary amines and sulfonic acid groups, therefore the novelty of the sorbents arise in the functionalization of silica with strong ionic moieties, so that, the sorbent will be always charged at any pH. Once the sorbents were synthesized, they were evaluated using SPE and the best-performing sorbent was used to selectively determine basic drugs in river

and effluent wastewater water samples through LC-MS/MS.

2. Experimental

2.1. Reagents and standards

Chemicals and reagents for sol-gel mixed-mode zwitterionic sorbents include methyl trimethoxysilane (MTMS), tetramethyl orthosilicate (TMOS), activated carbon, trifluoroacetic acid (TFA), isopropanol (IPA), methylene chloride, methanol (MeOH), and ammonium hydroxide purchased from Sigma-Aldrich (St. Louis, MO, USA). Octadecyl trimethoxysilane (C_{18}-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-Trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride and 4-(Trimethoxysilylethyl) benzyltrimethyl ammonium chloride were obtained from Gelest Inc. (Morrisville, WI, USA).

Thirteen drugs were selected for the sorbent evaluation. Six of these were basic, atenolol (ATE), trimethoprim (TRI), metoprolol (MTO), venlafaxine (VEN), ranitidine (RAN) and propranolol, while seven were acidic, bezafibrate (BEZ), clofibric acid (CLO), diclofenac (DICLO), fenoprofen (FEN), flurbiprofen (FLB), naproxen (NPX) and valsartan (VAL). All these drugs were purchased as pure standards from Sigma-Aldrich (purity >96%).

Stock solutions of individual standards were prepared in methanol (MeOH) at a concentration of 1000 mg/L and stored at −20 °C. Working solutions of a mixture of all compounds were prepared weekly in a mixture of ultrapure water and MeOH (80/20 v/v) and stored at 4°C in brown bottles in the dark. Ultrapure water was provided by a water purification system (Millipore, Burlington, United States), while “HPLC grade” MeOH and acetonitrile (ACN) were purchased from J. T. Baker (Deventer, The Netherlands). “MS grade” ACN and water were purchased from Scharlab (Barcelona, Spain). Formic acid (HCOOH), acetic acid (AcOH) and HCl were acquired from Sigma-Aldrich.

2.2 Synthesis of sol-gel mixed-mode zwitterionic sorbents

Sol solutions to create the sol-gel mixed-mode zwitterionic sorbents were obtained by sequential addition and subsequent vortexing of methyl trimethoxysilane (MTMS), tetramethyl orthosilicate (TMOS), octadecyl trimethoxysilane (C_{18}-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride (N-TMPTMAC), isopropanol (IPA) and trifluoroacetic acid (TFA, 0.1M) in a 50 mL centrifuge tube. The relative ratios of the various ingredients (MTMS, TMOS, C_{18}-TMS, 3-MPTMS, N-TMPTMAC, IPA, and TFA were 1: 1: 0.1: 0.1: 0.2: 3.8: 3, respectively. To introduce phenylethyl linker connected to trimethyl ammonium chloride, N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride was replaced with 4-(Trimethoxysilylethyl) benzyl trimethyl ammonium chloride in another set of sol-gel sorbents. The mixture was vortexed for 5 min and then sonicated for 15 min to remove any trapped air bubbles from the sol solution. The sol solution was kept at room temperature for 8h to allow the sol-gel precursors to be hydrolysed. Freshly prepared ammonium hydroxide (1 M) was then added in droplets to the sol solution under continuous stirring in a

magnetic stirrer. The solution slowly became viscous before turning into solid gel. To produce activated carbon impregnated sol-gel sorbent, 0.5 g of activated carbon was added to the sol solution before ammonium hydroxide solution was added.

The solid gel was thermally conditioned and aged at 60°C for 48h. The monolithic bed of the sol-gel network was then crushed and dried at 80°C for 24 h and the sol-gel sorbent was crushed into fine particles in a ball mill and rinsed with MeOH: methylene chloride (50:50, v/v) under sonication for 30 min. The particles were air-dried and treated with 30% H_2O_2 (with 0.1 M sulphuric acid) for 4 h. The particles were rinsed with deionized water several times and then dried at 80°C for 12 h. The sol-gel mixed-mode zwitterionic sorbents were then ready for loading into the SPE cartridges.

2.3. Structure of sol-gel mixed-mode zwitterionic sorbents

The characterization of the sorbents was performed with a Cary 670 FTIR, Agilent Technologies Cary 600 Series FTIR Spectrometer (Agilent Technologies, Santa Clara, CA, USA) for the Fourier Transform Infrared Spectroscopy (FT-IR) and with a JEOL JSM 5900LV Scanning Electron Microscope (SEM) equipped with EDS-UTW detector, JEOL USA, Inc. (Peabody, MA, USA) for recording SEM images.

The four sorbents (Figure 1) tested in this study were based on a silica skeleton functionalized with C_{18} to perform hydrophobic interactions; quaternary amines to perform strong anionic exchange (SAX) interactions; and sulfonic groups to perform strong cationic exchange (SCX) interactions.

All sorbents were functionalized with the same groups to perform SAX and SCX interactions. Two of them (SiO_2-SAX/SCX - SiO_2-SAX/SCX(Ph)) were based on a silica network (S-type) and

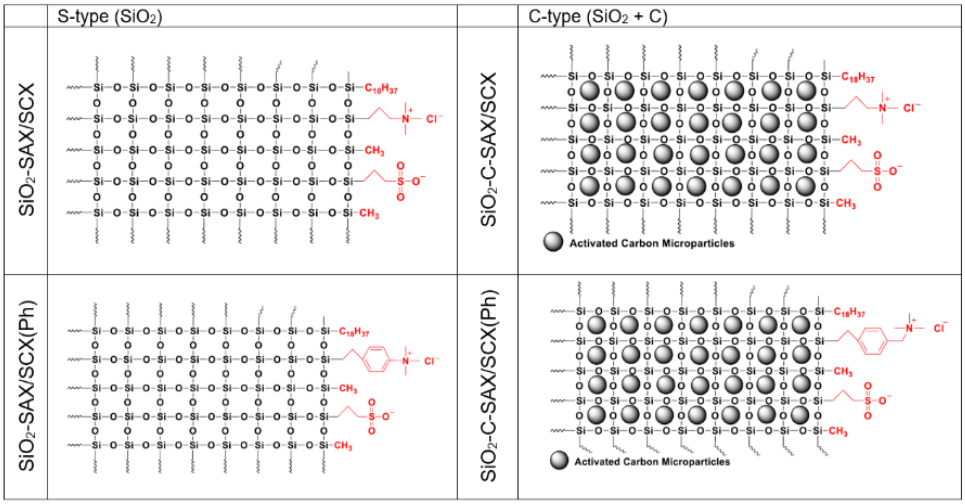

Figure 1. Structure of the sol-gel mixed-mode zwitterionic sorbents.

two (SiO_2-C-SAX/SCX - SiO_2-C-SAX/SCX(Ph)) were based on a silica network with activated carbon embedded (C-type). Figure 1 shows the structure of the four sorbents tested. SiO_2-SAX/SCX and SiO_2-C-SAX/SCX had the same functionalization, with propyl groups between the network and the quaternary amine. SiO_2-SAX/SCX(Ph) and SiO_2-C-SAX/SCX(Ph) also had the same functionalization, though in this case with a phenylethyl group in the anionic exchange chain.

2.4. Solid-phase extraction procedure

An empty 6 mL SPE cartridge (Symta, Madrid, Spain) was fitted with a 10 µm polyethylene frit (Symta) and filled with 200 mg of sorbents. A 10 µm polyethylene frit was then placed above the sorbent bed.

The SPE procedure was performed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. The first step was to condition the sorbents with 5 mL of MeOH and 5 mL of ultrapure water adjusted at pH 3. 100 mL of sample adjusted at pH 3 with HCl were loaded into the cartridge. For the effluent wastewater samples, the volume was 50 mL. After the loading step, the washing step was performed with 5 mL of MeOH. Finally, the elution step involved 5 mL of MeOH containing 5% of NH_4OH. The eluted volume was evaporated with a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to complete dryness and then reconstituted with 1 mL of initial mobile phase solution (H_2O/ACN, 95/5, v/v). The reconstituted extracts were filtered using 0.45 µm polytetrafluoroethylene (PTFE) syringe filters (Scharlab) before analysis. To reuse the SPE cartridges a washing step with MeOH was performed and then, it was completely dried by applying vacuum for 10 minutes.

Samples from river and effluent wastewater treatment plants were filtered through a 0.45 µm Nylon membrane filter (Scharlab). The effluent samples were previously filtered using a 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK).

2.5. Instrumentation and chromatographic conditions

The initial tests and the optimization of the SPE conditions were performed with an Agilent 1200 UHPLC equipped with a binary pump, an autosampler, an automatic injector, and a diode array detector (DAD) (Agilent, Waldbronn, Germany). The chromatographic column used was a Luna® Omega 5 µm Polar C18 100 (150 x 3.0 mm, 5 µm particle size) supplied by Phenomenex (Torrance, CA, United States). The mobile phase was a mixture of ultrapure water adjusted to pH 3 with HCl (solvent A) and ACN (solvent B). The gradient profile began with 5% of B. The % of B was then increased to 40% within 10 min, then to 45% within 4 min, and finally to 100% within 1 min. It was then held at 100% for 3 min before returning to the initial conditions in 1 min, where it was held for 3 min to stabilize the column. The column temperature was 30ºC and the flow rate was 0.4 mL/min. The injection volume was 20 µL. ATE, TRI, MTO, PRO, BEZ, VAL, FEN, FLB and CLO were measured at 210 nm, while RAN, VEN, DICLO, and NPX were measured at 230 nm.

Once the SPE conditions were optimized, the method was validated for the basic compounds analysing real samples with LC-MS/MS using an Agilent 1260 Infinity 2 connected to a triple quadrupole mass detector Agilent 6460 an electrospray ionization (ESI) interface. The chromatographic conditions were the same as in LC-DAD, except that the injection volume was 10 µL and the pH of solvent A was adjusted with HCOOH rather than HCl. The optimized parameters in the (ESI) MS/MS were gas temperature 320 ºC, gas flow rate 10 mL/min, nebulizer pressure 35 psi and the capillary voltage 3000 V. The fragmentor potential for all transitions was 100 V. For each compound, the diagnostic ion was $[M+H]^+$. One of the transitions was used as quantifier and at least one more was used as qualifier. Table S1 shows the MRM transitions selected and their collision energies.

2.6. Validation parameters

The method was validated in terms of recovery, matrix effect, linear range, method quantification and detection limits, repeatability and reproducibility.

Recovery (%R) and apparent recovery ($\%R_{app}$) were used to evaluate the yield of the extraction. %R was obtained with LC-DAD, being the ratio of the concentration obtained after the SPE of a spiked sample and the concentration expected. $\%R_{app}$ was obtained in the same way that %R but the analysis was performed with LC-MS/MS, and it considers the extraction recovery and the matrix effect.

The matrix effect (%ME) was calculated from the formula: $\%ME = (C_{Exp}/C_{Theo} \times 100) - 100$, where "$C_{Exp}$" is the concentration obtained by spiking a blank sample after SPE and "C_{theo}" is the expected concentration. A negative value indicates suppression of the signal, while a positive value indicates enhancement.

The instrumental linear range was evaluated with external calibration curves analysing in triplicate seven solutions with different concentrations. Matrix matched calibration curves were obtained spiking river samples at seven different concentrations.

Method quantification limit (MQL) was obtained from the matrix-matched calibration curves, being the lowest concentration from the curve and method detection limit (MDL) was calculated as the concentration that provided a signal-to-noise ratio of 3.

Repeatability was obtained as the %RSD intra-day (n=3) analysing by triplicate samples spiked at the same concentration the same day. The reproducibility between days was obtained as the %RSD inter-day (n=3) analysing samples (n=3) spiked at the same concentration during different days (n=3).

3. Results and discussion

3.1. Synthesis of the sol-gel mixed-mode zwitterionic sorbents

Many environmental and biological samples simultaneously contain neutral, acidic and basic analytes. If all the analytes are of interest, the separation and preconcentration of these compounds pose serious analytical challenges. One way to solve this analytical challenge is to create a mixed-mode zwitterionic sorbent by

incorporating a neutral carbon chain, a cation exchanger, and an anion exchanger into a single sorbent. To maintain the cations and anions in their charged state at full pH range, the cation exchanger and anion exchanger should be strong so that they maintain their ionic state at all pH levels. Octadecyl silane is the most prevalent sorbent in SPE. Octadecyl trimethoxysilane was therefore chosen as the neutral sorbent. To include a SCX in the sorbents, 3-mercaptopropyl trimethoxysilane, which generates propyl sulfonic acid after oxidation, was used. N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride and 4-(trimethoxysilylethyl) benzyl trimethyl ammonium chloride were used as SAX. To incorporate these functional groups into the silica network, sol-gel synthesis, which is considered a popular, environment-friendly and facile synthesis approach, was used. Sol-gel synthesis can be performed under acidic or basic catalysis or acidic hydrolysis followed by condensation in basic environment. Acidic hydrolysis followed by basic condensation renders the sol-gel network stronger and more porous [26]. Moreover, to facilitate synthesis, the sol-gel process enables the creation of sol-gel sorbent particles or surface coating in situ at room temperature. Propyl sulfonic acid was obtained after post-gelation treatment of the sorbent with 30% hydrogen peroxide (impregnated with 0.1 M sulphuric acid). The creation of sol-gel mixed-mode zwitterionic sorbents is a new milestone in separation science.

3.2. Characterization of sol-gel silica based mixed mode zwitterionic sorbents

All the sorbents were subjected to characterization using Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). However, as the results provided were quite similar, we only present the results of the tests performed with SiO_2-SAX/SCX. FT-IR spectra reveal valuable information regarding the functional composition of the building blocks and their successful integration into the final composite material. SEM images, on the other hand, shed light on the surface morphology of the composite material.

3.2.1. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the individual building blocks, methyl trimethoxysilane (MTMS), octadecyl trimethoxysilane (C_{18}-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-trimethoxysilyl N,N,N-trimethyl ammonium chloride (TMTAMC) and the sol-gel mixed mode zwitterionic sorbent are presented in Figure 2 (a-e), respectively. All FT-IR spectra were collected over a range between 3000 cm^{-1} and 700 cm^{-1} at a resolution 4 cm^{-1}.

FT-IR spectra of MTMS (Fig. 2 a) displays several signature bands at 1266 cm^{-1} and 789 cm^{-1} which are attributed to the vibration of CH_3 group connected to Si on the precursor molecule. The peaks at 1077 cm^{-1} and 1189 cm^{-1} are attributed to C-O stretching vibration Si-O-CH_3. The peaks at 2842 cm^{-1} and 1464 cm^{-1} are attributed to C-H stretching and bending vibration of Si-O-CH_3, respectively [27]. The noteworthy peaks in the C18-TMS spectra 2922 cm^{-1} and 2852 cm^{-1} which

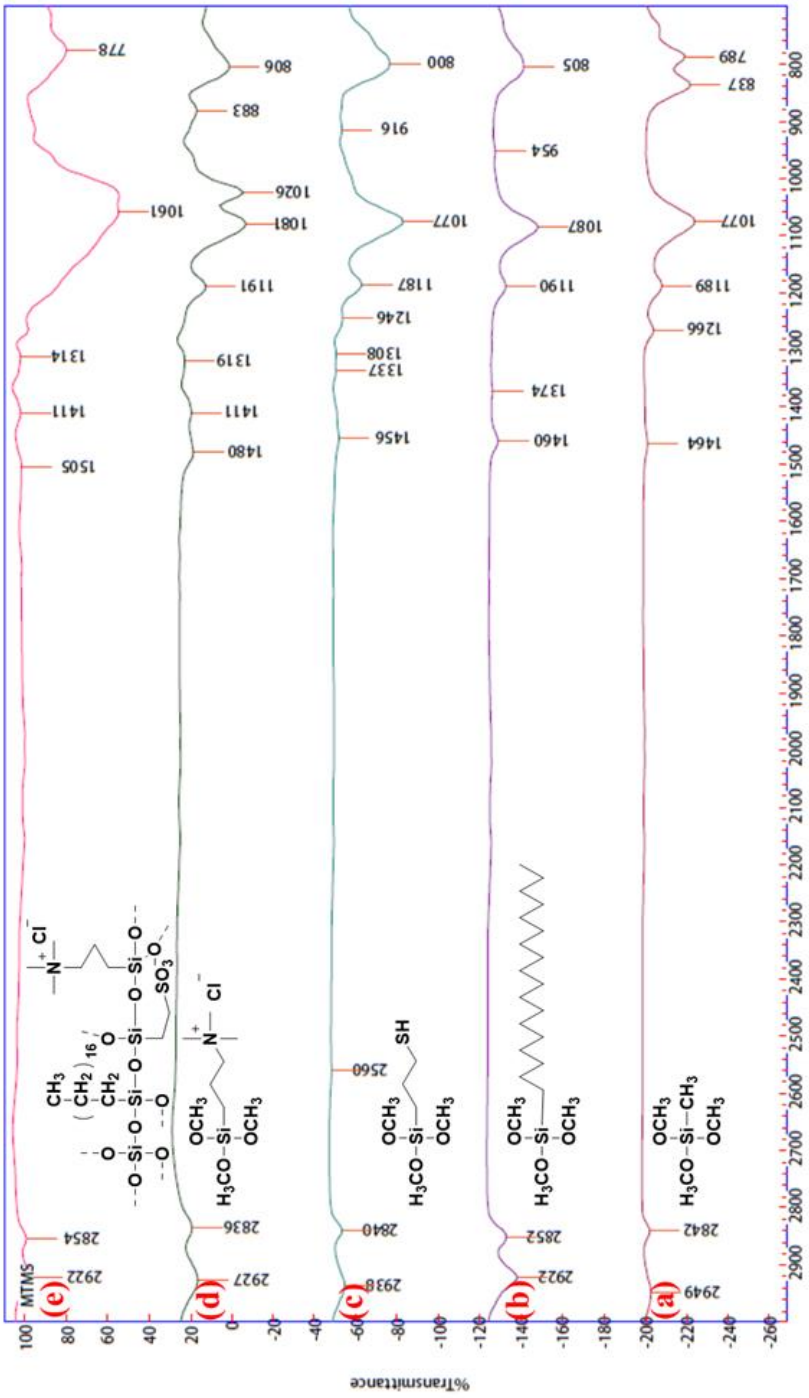

Figure 2. FT-IR spectra of (a) methyl trimethoxysilane; (b) octadecyl trimethoxysilane; (c) 3-mercaptopropyl trimethoxysilane; (d) N-trimethoxysilyl N,N,N-trimethyl ammonium chloride; (e) sol-gel mixed-mode zwitterionic sorbent.

can be assigned to antisymmetric [ν_a (CH_2)] and symmetric [ν_s (CH_2)] bands for the alkene chains of C_{18}-TMS. The FT-IR spectra of 3-mercaptopropyl trimethoxysilane demonstrate signature band at 2560 cm^{-1} that can be attributed to S-H stretching [28]. The bands at 1187 cm^{-1} and 1080 cm^{-1} are related to –CH_3OCH_3 [28]. The signature band in N-trimethoxysilyl-N,N,N-trimethyl ammonium chloride FT-IR spectra includes 1480 cm^{-1} that can be attributed to N-CH_3 bending vibration [29]. It is important to note that all precursors have a common end consisting of -Si (CH_3)$_3$. As a result, many spectral bands are common. The FT-IR spectra of sol-gel SiO_2-SAX/SCX include many bands such as 1505 cm^{-1}, 1441 cm^{-1}, 1314 cm^{-1}, 1061 cm^{-1}, and 778 cm^{-1} that also appeared in the FT-IR spectra of individual building blocks which manifests successful integration of the building blocks into sol-gel SiO_2-SAX/SCX. 3.2.2. Scanning Electron Microscopy (SEM)

The surface morphology of the sol-gel SiO_2-SAX/SCX was investigated using a Scanning Electron Microscope. The SEM images are presented in Figure 3 (a, b) at 100x and 1,000x magnifications, respectively. The SEM images revealed that the particle sizes are not homogeneously distributed and possess irregular shapes. Some particles of the SiO_2-SAX/SCX are in sub-micron size while others are bigger, in the range of 50-60 micron (gross estimation). The surface of the particles apparently look rough that should enhance the interaction between the particles and the analytes during the extraction process. The broad range of particle size distribution also helps reducing the void volume due to the close packing of the sorbents.

3.3. Optimization of the SPE procedure

The SPE procedure was optimized using a mixture solution of standards prepared in ultrapure water. The analysis was performed using LC-DAD.

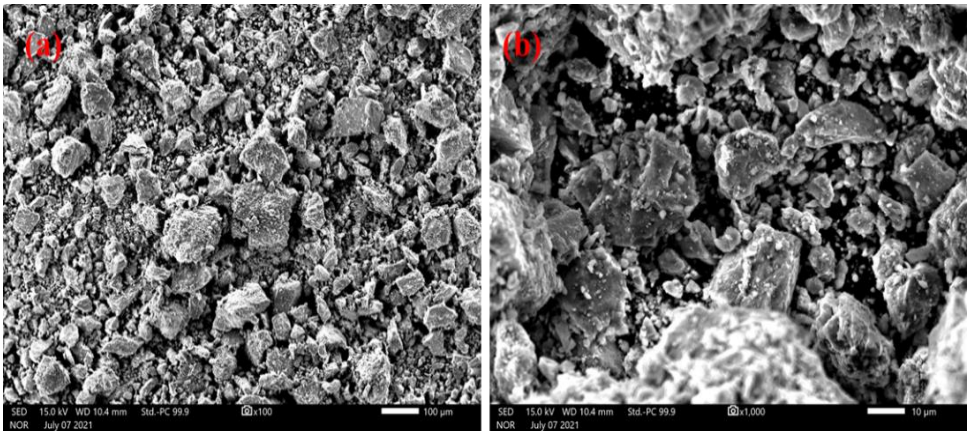

Figure 3. Scanning electron microscopy of sol-gel Silica SAX/SCX sorbent at (a) magnifications at 100x; (b) magnifications at 1,000x.

3.3.1. Extraction performance evaluation of the sorbents

Since the functionalization of the sorbents evaluated was based on strong ionic interactions, they will always be charged at any pH. To select the initial pH, the pK_a of the compounds was therefore considered (Table 1) and it was set at 5 (pH at which the acidic and basic compounds were charged). The conditions of loading volume and elution were based on a previous study reported by our group [23] that analysed acidic and basic compounds using a weak zwitterionic sorbent. These conditions were: 25 mL of loading volume and an elution step with 5 mL of 5% AcOH in MeOH to elute the acidic compounds; and 5 mL of 5% NH_4OH in MeOH to elute the basic compounds.

The four sorbents were initially tested to discern which ones provided the highest recoveries. The four sorbents had the same functionalization, with sulfonic groups to perform SCX interactions and quaternary amines to perform SAX interactions. The difference between these sorbents was the support since some were based on the silica network (S-type), while for others the silica network was embedded with activated carbon microparticles (C-type). Each group had two variants: one in which the SAX groups were bonded through a propyl group to the silica network (SiO_2-SAX/SCX and SiO_2-C-SAX/SCX), and another which had a phenylethyl group between the silica network and the SAX groups (SiO_2-SAX/SCX(Ph) and SiO_2-C-SAX/SCX(Ph)).

As Table 1 shows, the sorbents that provided the greatest recoveries were SiO_2-SAX/SCX and SiO_2-C-SAX/SCX. The recoveries of sorbents SiO_2-SAX/SCX(Ph) and SiO_2-C-SAX/SCX(Ph) were significantly lower than those of SiO_2-SAX/SCX and SiO_2-C-SAX/SCX.

Table 1. pK_a of the compounds and recoveries performed by each sorbent at initial conditions.

			S type		C-type	
		pK_a	SiO_2-SAX/SCX	SiO_2-SAX/SCX(Ph)	SiO_2-C-SAX/SCX	SiO_2-C-SAX/SCX(Ph)
Basic	ATE	9.6	94	72	96	79
	RAN	8.2	93	87	90	54
	TRI	7.1	97	82	89	68
	MTO	9.7	96	81	87	50
	VEN	10.1	92	84	83	38
	PRO	9.4	98	70	94	57
Acidic	BEZ	3.8	98	86	80	20
	VAL	3.6	87	90	74	30
	FEN	4.5	100	74	88	37
	FLB	4.4	101	45	92	0
	CLO	3.2	91	71	82	29
	DICLO	4.1	82	43	53	8
	NPX	4.1	103	89	86	16

*RSD (%) < 10%. (n = 3)

[a] pK_a values obtained from PubChem for all compounds except for BEZ, FLB and CLO (values obtained from Drugbank)

Adding the aromatic ring seemed to hamper interactions between the compounds and the ionic exchange groups, thus resulting in lower recoveries. Sorbents SiO_2-SAX/SCX(Ph) and SiO_2-C-SAX/SCX(Ph) were therefore discarded, and the subsequent tests were performed with SiO_2-SAX/SCX and SiO_2-C-SAX/SCX.

Moreover, by comparing the S-type and C-type sorbents it can be observed that the S-type sorbents presented higher recoveries than the C-type sorbents. For example, DICLO presented a %R of 82% with SiO_2-SAX/SCX and 53% with SiO_2-C-SAX/SCX.

3.3.2. Optimization of the loading pH

As we explained in the Introduction, the control of pH is important when evaluating these sorbents, thus, the first parameter to be evaluated was the pH of the loading solution, which governs the retention of the compounds. Since the sorbents were based on strong ion-exchange interactions, they were charged at any pH. The loading pH was therefore used to control the chargeability of the analytes.

As has been highlighted in section 3.3.1., pH 5 was initially selected since in this range all compounds were charged considering the pK_a of the analytes. Moreover, a cleaning step of 2 mL was also introduced to check whether the compounds were being retained through ionic interactions. As can be observed in Figure 4, where results of SiO_2-SAX/SCX are presented, good recoveries are obtained for basic compounds, only ATE and RAN provided %R below 80%. However, the acidic compounds provided low recoveries.

Then, pH 3 and 9 were evaluated to promote the specific ionic interactions in each range; at pH 3, the cationic interactions displayed by the basic compounds and at pH 9, the anionic interactions by the acidic compounds. Attending to Figure 4, it can be observed that the recoveries of the basic compounds improved with pH 3, achieving recoveries higher than 80% for the six compounds. However, at pH 9, the recoveries of the acidic compounds did not improve.

pH 4 was evaluated since the best results were obtained at pH 3 and we considered interesting to test this pH. As can be observed in Figure 4, the results for basic compounds were slightly better than pH 5 and slightly worse than pH 3. The good recoveries were explained since at these pHs, the analytes were protonated and therefore able to interact with the sorbent through ionic interactions. The low recoveries obtained for the acidic compounds suggested that retention occurred only via hydrophobic interactions since these compounds were eluted from the sorbent when MeOH was applied, meaning that the SAX interactions did not work.

The optimization of the pH was performed with SiO_2-SAX/SCX and SiO_2-C-SAX/SCX. Although Figure 4 shows the results obtained from the pH evaluation with SiO_2-SAX/SCX both sorbents provided similar results, being the %R of SiO_2-SAX/SCX slightly higher. Jin *et al.* [16] also observed that only basic compounds were retained via ionic interactions. These authors evaluated a homemade mixed-mode zwitterionic sorbent based on weak interactions grounded in carboxylic acids and

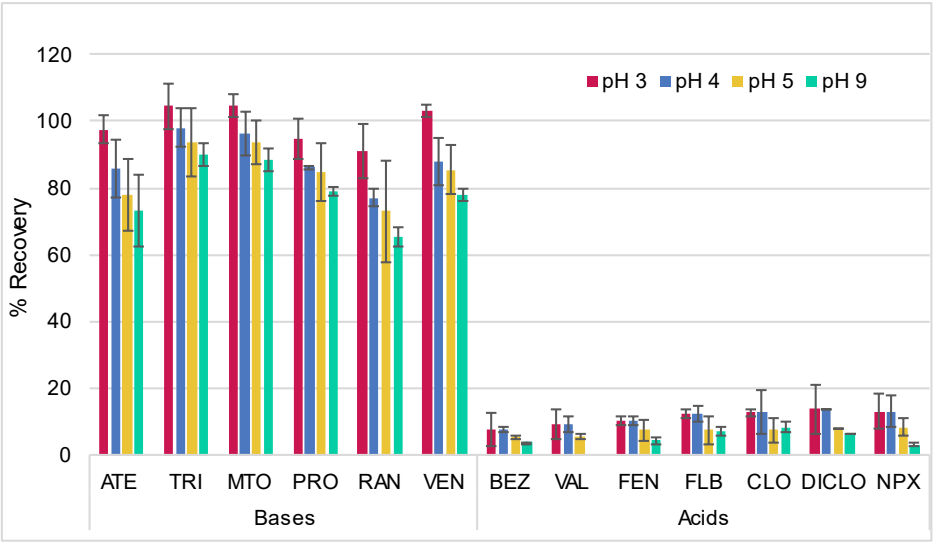

Figure 4. Comparison of the recoveries obtained at pH 3, 4, 5 and 9 with the SiO_2- SAX/SCX sorbent.

secondary amines to determine a group of acidic, basic and neutral compounds with a loading pH of 6.

Given the zwitterionic nature of the sorbents, the loading pH should have been closer to the neutral pH used by Jin *et al.* [16], who chose a loading pH of 6 to determine basic antidepressants in aquatic products using a homemade zwitterionic mixed-mode sorbent functionalized with carboxylic acids and secondary amines. The above authors observed that the acidic compounds were not retained through ionic exchange interactions [16]. A similar explanation can be adapted in our study, in which all the acidic compounds presented aromatic rings that tended to interact with the C_{18} chains through hydrophobic interactions.

When the clean-up step was included, the behaviour of the sorbents was therefore closer to a cationic exchanger than to a zwitterionic exchanger. As occurred in previous studies [30,31] that evaluated SCX sorbents to selectively determine basic compounds from aqueous samples and selected a pH in the acidic range, the loading pH for our study was acid.

3.3.3. Optimization of the clean-up step

A clean-up step is needed to remove the interferences and to increase the selectivity of the method. In the previous section, we introduced a clean-up with 2 mL of MeOH. We then used 5 mL of MeOH to test whether the cleaning volume could be increased without the recoveries being affected, thereby enhancing the selectivity of the extraction. Table 2 shows results when 25 mL of sample was loaded at pH 3 with or without a clean-up step (2 or 5 mL). When this clean-up step (2 or 5 mL of MeOH) was applied, both sorbents

showed the same performance, with recoveries for the basic compounds above 80% and those for the acidic compounds below 10%. for acidic compounds proved that these compounds were retained through hydrophobic interactions since they were removed from the sorbent with MeOH. On the other hand, the basic compounds were retained through ionic exchange interactions since they were not eluted during the clean-up step.

The clean-up step was set at 5 mL of MeOH since there was no evident decrease in the recoveries when the volume was increased from 2 mL to 5 mL. Moreover, this increase would help to increase selectivity. It is common to use MeOH to perform the clean-up step when working with mixed-mode ion-exchange sorbents to disrupt the hydrophobic interactions and promote selectivity. Using 5 mL has been reported in a study with a homemade mixed-mode SCX sorbent [32]. In another study [23], the volume was set at 1 mL to reduce the loss of analytes in the determination of illicit drugs, sweeteners and pharmaceuticals using a homemade mixed-mode ion-exchange zwitterionic sorbent based on weak interactions.

Other studies, on the other hand, have reported a clean-up step not fully based on MeOH. Hu *et al*. [33], for example, performed this step with 2 mL of a mixture of water/MeOH (95/5, v/v) when using a modified silica sorbent with a triazine to determine anthraquinones in urine, which could not be enough to produce a remarkable clean-up effect.. Therefore, an aqueous clean-up was not evaluated in this study and a clean-up step with 5 mL of MeOH was selected.

3.3.4. Optimization of the elution

Initially, the elution was conducted in two steps: an acidic step (5% AcOH in MeOH) to elute the acidic compounds and a basic step (5% NH_4OH in MeOH) to elute the basic compounds. Since the acidic compounds are eluted just with MeOH, the AcOH was not needed, and the acidic step was then removed.

After testing 5% NH_4OH in MeOH in a previous section, the two options tested

Table 2. Recoveries obtained when 100 mL of ultrapure water were loaded without cleaning and cleaning with 2 and 5 mL of MeOH clean.

		SiO_2-SAX/SCX			**SiO_2-C-SAX/SCX**		
		No clean	2 mL	5 mL	No clean	2 mL	5 mL
Basic	ATE	94	95	99	96	95	98
	RAN	93	84	84	90	100	86
	TRI	97	99	99	89	104	100
	MTO	96	104	101	87	92	100
	VEN	92	94	92	83	84	88
	PRO	98	92	89	94	94	86
Acidic	BEZ	98	7	2	80	2	0
	VAL	87	3	4	74	1	0
	FEN	100	6	2	88	3	2
	FLB	101	8	4	92	6	5
	CLO	91	8	7	82	7	7
	DICLO	82	11	2	53	0	0
	NPX	103	10	4	86	4	2

*RSD (%) < 10%. (n = 3)

were 5 mL of 10% NH_4OH in MeOH and 10 mL of 5% NH_4OH in MeOH. All three options provided similar results: 85-100% for the SiO_2-C-SAX/SCX sorbent and 90-105% for the SiO_2-SAX/SCX sorbent. The first option was therefore chosen since it is greener and generates a lower volume to evaporate.

This elution has previously been used in some studies to elute basic compounds [20,23,31] from mixed-mode ion-exchange sorbents. Moreover, when Salas *et al.* [20] studied combinations of commercial cation and anionic exchangers, the authors also began with elution in two steps, i.e. an acidic step based on 5% AcOH in MeOH and a basic step with 5% NH_4OH in MeOH. However, during the optimization process it was observed that all compounds were eluted in the basic step.

3.3.5. Optimization of the loading volume

The final step in the optimization process was the loading volume. The larger the loading volume, the higher the preconcentration factor, though the breakthrough volume should also be taken into account.

The initial volume was 25 mL, while 100, 250 and 500 mL were also tested with standard solutions. Every volume showed good recoveries for both sorbents (80–100% for SiO_2-C-SAX/SCX and 85–105% for SiO_2-SAX/SCX). The results were therefore good even with 500 mL with standard solutions.

Then, 100 and 250 mL were evaluated with spiked river samples to select the loading volume with river samples. As Figure S1 shows, a significant decrease in the recoveries occurred when the volume was increased from 100 to 250 mL. The volume selected with river samples was therefore 100 mL. Klan *et al.* [30] tested 100, 200, 500 and 1000 mL and obtained satisfactory results for most of their analytes when analysing river water samples. However, a significant decrease in %R was observed in the most polar compounds. The authors considered the increase in time inherent to the increase in volume. They also considered the possibility that the cartridge would get clogged and decided to select 200 mL as the loading volume.

When working with effluent wastewater samples, recoveries were low with 100 mL. The loading volume was therefore reduced to 50 mL, which led to satisfactory recoveries (44–78%). Gilart *et al.* [32] also evaluated the loading volume and found that 500 mL presented good recoveries in standard solutions. For effluent wastewater samples, however, they also had to reduce the volume to 50 mL.

3.4. Validation of the method

The method was validated for river water samples and effluent wastewater from treatment plants using LC-ESI-MS/MS to improve sensitivity and selectivity. The chromatographic method was transferred to LC-MS/MS, which enabled work at lower concentrations. The parameters of gas temperature, gas flow rate, nebulizer pressure and capillary voltage were optimized experimentally. Gas temperature was evaluated between 200 and 400 ºC; gas flow rate between 6 and 14 mL/min; nebulizer pressure between 20 and 60 psi; and capillary voltage between 2500

and 5000 V. For each compound, the fragmentor potential was also evaluated between 50 and 200 V. The collision energy (CE) was evaluated between 0 and 30 eV. The conditions selected are shown in section 2.5 and Table S1. For all fragments, the CE ranged from 15 to 25 eV, except for VEN, which ranged from 7 to 5 eV.

The instrumental linear range was 0.5–250 µg/L for most compounds. The R^2 was above 0.995 for all compounds except MTO, whose R^2 was 0.992. The instrumental LOD and LOQ were 0.1 µg/L and 0.5 µg/L respectively for all compounds except VEN, whose limits were 0.05 and 0.1 µg/L, respectively.

Before validating the method, the best performing sorbent was selected. Since both sorbents provided good results during the optimization of the SPE procedure, to select one of them, they were tested in terms of apparent recovery ($\%R_{app}$), when river samples were spiked at 200 ng/L. To calculate the apparent recovery correctly, a blank was measured to subtract the signal of the analytes naturally present from the signal of the spiked samples. As Table 3 shows (and as we highlighted during the optimization procedure), the SiO_2-SAX/SCX sorbent showed higher $\%R_{app}$ for the spiked river samples (with values ranging from 60 to 78%), while the results for the SiO_2-C-SAX/SCX sorbent ranged from 47 to 62 % (except for ATE, whose $\%R_{app}$ were 40 and 34%, respectively). The sorbent chosen to validate the method was therefore SiO_2-SAX/SCX. The addition of carbonaceous particles into the sol-gel composite sorbent increased the overall surface area of the composite sorbent but decreased the absolute loading of the sol-gel silica sorbent, and consequently, the overall interaction sites. It is evident from the recovery data that the sorption feature of the carbonaceous particles in the composite sol-gel sorbent played no role in the extraction process. In a future project, we intend to investigate the impact of carbonaceous particles on other type of molecules.

After selecting the best sorbent, river samples were analyzed to perform the validation, according to the parameters described in section 2.6, in terms of recovery at two concentrations (50 ng/L and 200 ng/L), matrix effect, linear range, method quantification and detection limits (MQL and MDL), repeatability (% RSD, n = 3) and reproducibility between days (% RSD, n = 3). As Table 4 shows, $\%R_{app}$ spiking at 50 ng/L were good, i.e. 60–85% for all compounds except ATE, whose $\%R_{app}$ were 40%, being similar results to the $\%R_{app}$ spiking at 200 ng/L presented in Table 3. These recoveries were comparable to those obtained by Nadal *et al.* [23] (58%–87%) when determining TRI, MTO and PRO in 100 mL of river samples using a homemade mixed-mode zwitterionic sorbent based on weak ionic interactions. Zhu *et al.* [13], whose values ranged from 75 to 98%, also obtained slightly higher recoveries

Table 3. $\%R_{app}$ obtained with each sorbent when 100 mL of river samples spiked at 200 ng/L was extracted.

	SiO_2-SAX/SCX	SiO_2-C-SAX/SCX
ATE	40	34
RAN	78	62
TRI	71	58
MTO	66	55
VEN	73	36
PRO	60	47

*RSD (%) < 15%. (n = 3)

when analysing aromatic amines in environmental water samples with a WCX mixed-mode silica-based sorbent. Moreover, Afonso-Olivares *et al.* [34] obtained recoveries ranging from 78 to 98% when determining pharmaceuticals (atenolol among others) in seawater with a commercial sorbent (Oasis HLB).

The %MEs (Table 4) were remarkably low (ranging from -17 to -4 %), which indicates low ion suppression due to the inclusion of a clean-up step with 5 mL of MeOH. These results are lower than those found in other studies, e.g. Krizman *et al.* [31], who used a commercial sorbent (Oasis MCX) and obtained matrix effects for opioids and their metabolites ranging from -38 to -7%. For their part, Nadal *et al*. [24] obtained matrix effects ranging from -30 to +5 when using a mixed-mode SAX/WCX sorbent to determine, for example, TRI, MTO, RAN, ATE and PRO.

The linear range was obtained from matrix-matched calibration curves and river samples were spiked from 1 to 500 ng/L. In all cases, determination coefficient (R^2) was above 0.99.

Attending to the method quantification and method detection limits (Table 4), in both cases, the values were in the ng/L range, which were comparable to those found in developed methods based on determining those analytes in river samples [23,30,32], whose limits were also in the ng/L range.

The values for repeatability (intra-day precision, n=3) and reproducibility (inter-day precision, n=3) were acceptable (as Table 4 shows, in all cases they were below 16%).

The method was also evaluated in terms of $\%R_{app}$, %ME and %RSD with effluent wastewater samples. Since the analytes were present at high concentrations in the effluent wastewater samples, no matrix-matched calibration curves were done. To quantify the analytes in real samples, external calibration curves and apparent recoveries were used. The $\%R_{app}$ obtained spiking at 200 ng/L ranged from 40 to 71%. lower concentrations were not evaluated due to the presence of the compounds in the sample. Moreover, spiking at higher concentration was neither evaluated since similar recoveries in river samples were obtained when spiking the samples both at 50 and 200 ng/L.These results are comparable to others reported ,e.g. a combination of commercial cationic and anionic exchangers (where the $\%R_{app}$ ranged from 50 to 73% [20]), and with a novel SCX sorbent (where the $\%R_{app}$ ranged from 39 to 84% [32]). In both

Table 4. Validation parameters for SiO2-SAX/SCX sorbent with river samples.

	$\%R_{app}$ (50 ng/L)	%ME	Linear range (ng/L)	MQL (ng/L)	MDL (ng/L)	% RSD intra-day (n = 3)	% RSD inter-day (n = 3)
ATE	48	-16	2 – 500	2	1	8	13
RAN	72	-9	5 – 500	5	2	9	11
TRI	60	-14	2 – 500	2	1	7	9
MTO	66	-14	2 – 500	2	1	10	15
VEN	85	-17	2 – 500	2	1	9	13
PRO	63	-4	10 - 500	10	5	11	16

cases similar compounds were found. The %ME obtained ranged from -25% to -18%. These results are comparable to those obtained by Gilart *et al.* [32] (ranging from -12 and +21%), who used a novel SCX sorbent when determining similar compounds (ATE, PRO, MET, RAN and TRI among others). Moreover, Jaukovic et *al.* [35] determined cardiovascular drugs (MTO among others) in effluent wastewater samples using a commercial sorbent (Oasis HLB), and obtained higher recoveries ranging from 84 to 106% and higher %ME ranging from -28 to +23%. In all cases, %RSD intraday (n=3) was below 14%.

3.5. Analysis of real samples

Samples from river Ebro and samples from effluent wastewater treatment plants from Reus and Tarragona, Spain were analyzed.

Table 5 shows the occurrence of the compounds in river and effluent wastewater samples. As can be observed, the concentrations of ATE and PRO were below the MQL in the river samples, while those of RAN, TRI, MTO and VEN were found at similar levels of concentration ranging from 24 to 72 ng/L. Nadal *et al.* [36] also analysed Ebro river samples and found that the concentrations of ATE and PRO (ranging from 0.7 to 9.5 ng/L) were above their MQL. On the other hand, the concentration of TRI was below its MQL. The concentration of MTO found in the present study was one order of magnitude higher than that found in the study by Nadal *et al.*: 29–67 ng/L compared to 3–7 ng/L. When Klan *et al.* [30] determined MTO, PRO, RAN and VEN in river water samples from Slovenia, all compounds were below the MQL except for VEN, whose concentration ranged from 0.08 to 3.01 ng/L.

Regarding the effluent wastewater samples (Figure 5), all compounds were quantified. RAN and VEN presented the highest concentrations (653–1233 ng/L), while the concentrations of PRO and MTO were lower (29-113–ng/L). These levels were comparable to those quantified previously with similar samples. When Gilart *et al.* [32] measured ATE, RAN, TRI, MTO and PRO from effluent wastewater treatment plant samples, obtaining concentrations values similar to the ones presented in Table 5. PRO, for example, was found between 50 and 100 ng/L while RAN was found at around 1000 ng/L. Nadal *et al.* [36], for their part, measured MTO, ATE, wastewater treatment plant samples, obtaining concentration values similar to the ones presented in Table 5. PRO, for example, was found between 50 and 100 ng/L while RAN was found at around 1000 ng/L. Nadal *et al.* [36], for their part, measured MTO, ATE, PRO and TRI (among others) in effluent wastewater treatment plants and also found similar concentrations to those in Table 5. Moreover, when Iancu *et al.* [37]

Table 5. Range of concentrations (ng/L) obtained after the analysis of river and effluent wastewater samples through SPE-LC-MS/MS method based on the SiO_2-SAX/SCX sorbent.

	River	Effuent wastewater
ATE	<MQL	234– 282
RAN	24 – 32	848 – 1233
TRI	31 – 39	59 – 642
MTO	29 – 67	29 – 113
VEN	32 – 72	653 – 970
PRO	<MQL	48 – 82

Figure 5. Chromatogram of an effluent wastewater sample when it was analyzed using the developed method.

quantified ATE and PRO (among others) in effluent wastewater samples from Romania, the concentrations of PRO ranged from 5 to 40 ng/L, which are close to those found in our study (48–82 ng/L). On the other hand, the average PRO and TRI (among others) in effluent concentrations of ATE (94.6 ng/L) was lower than ours (234–282 ng/L).

4. Conclusions

In this study, we developed and evaluated four novel mixed-mode zwitterionic-exchange sorbents to determine pharmaceuticals in environmental water samples through SPE followed by LC-MS/MS. It has been shown that the sol-gel approach successfully works for the preparation of a series of mixed-mode zwitterionic silica-based sorbents.

When applying a clean-up step to achieve selective extraction, only basic compounds were retained through ion-exchange interactions, whereas acidic compounds were mainly retained by reversed-phase interactions. A method based on the best-performing sorbent was successfully developed to selectively determine the basic drugs in environmental samples. The application of this method in environmental samples is therefore interesting due to the low

matrix effect achieved thanks to the clean-up step.

The results obtained in this study, especially when it comes to the low matrix effect, are encouraging for the determination of basic compounds in complex samples. Moreover, the sorbents used could be interesting for extracting other compounds in the future and applying them to other matrices.

Acknowledgements

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Supplementary material

Table S1. Mass transitions, collision energy and fragmentor potential selected for each compound.

Drug	MRM	Collision Energy (eV)
Atenolol	**267>145**	15
	267>190	25
Ranitidine	**315>176**	15
	315>130	15
	315>124	15
Trimethoprim	**291>230**	20
	291>261	25
	291>123	25
Metoprolol	**268>116**	15
	268>191	15
	268>133	25
Venlafaxine	**278>58**	7
	278>260	5
Propranolol	**260>116**	15
	260>155	20

*Bold denotes quantification transition.

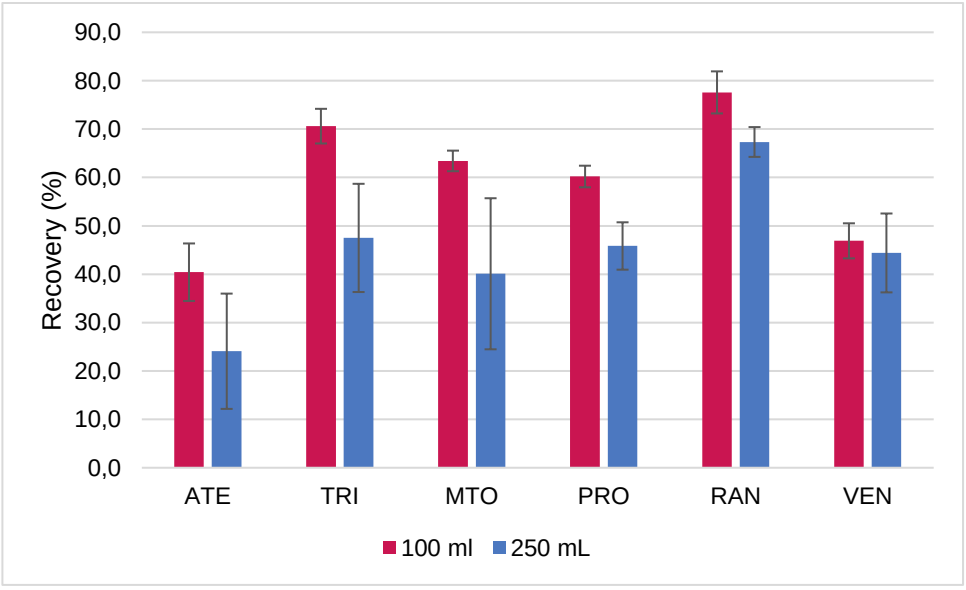

Figure S1. Optimization of the loading volume with river samples for the SiO_2-C-SAX/SCX sorbent.

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

3.1.2. Extraction of selected benzothiazoles, benzotriazoles and benzene-sulfonamides from environmental water samples using a home-made sol-gel silica-based mixed-mode zwitterionic sorbent modified with graphene

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Extraction of selected benzothiazoles, benzotriazoles and benzene-sulfonamides from environmental water samples using a home-made sol-gel silica-based mixed-mode zwitterionic sorbent modified with graphene

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Abstract

A novel sol-gel silica-based mixed-mode zwitterionic sorbent modified with graphene microparticles was synthesized. Thanks to the inclusion of multiple functional groups and graphene microparticles to exert a wide range of intermolecular/interionic interactions including dipole-dipole interactions, ion-exchange interactions and π-π interactions, the sorbent showed high retention in the solid-phase extraction (SPE) of benzothiazoles, benzotriazoles and benzenesulfonamides.

The SPE protocol was optimized in terms of pH, sample loading volume and elution conditions using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). The method based on SPE followed by LC-HRMS was validated. Apparent recoveries at two levels of concentration were in the range from 48 to 85% (in most cases) in matrices such as influent wastewater, matrix effect was lower than ±30% in most cases, method detection and quantification limits being lower than 20 ng/L and repeatability and reproducibility between days were lower than 18% (n=4).

River, effluent and influent wastewaters samples were analyzed, obtaining concentrations ranging from 3 to 175 ng/L in river samples, from 12 to 499 ng/L in effluent samples and from 15 to 632 ng/L in influent samples, when the compounds were above the method quantification limits.

Keywords: mixed-mode zwitterionic sorbents; silica sorbents; environmental samples; solid-phase extraction; basic compounds

1. Introduction

In recent years, benzotriazoles (BTRs), benzothiazoles (BTs) and benzenesulfonamides (BSAs) have been categorized as high production volume chemicals. BTRs are mainly used as UV blockers [1] or as corrosion inhibitors [2,3], BTs have a wide variety of industrial applications (vulcanization accelerators, fungicides, corrosion inhibitors or UV stabilizers) [4,5], and BSAs have also many applications such as plasticizers or intermediates in the synthesis of diverse products such as dyes or pesticides [4,6]. Their widespread use has compelled to consider them as emerging pollutants. These compounds have been determined not only in samples of environmental interest such as water [7–11], sludge [12] or particle matter [13,14] but also in fish [15], urine [16], clothes [17,18] and tea beverages [19] among others. Their ubiquitous occurrence has led to evaluate the toxicity effects, for instance, the effect of the exposition to BTs and BTRs has been studied in several vertebrates, mainly fishes [20,21] but also in humans [14,22,23]. Meanwhile, there are less studies about the toxicity of the BSAs [24–26].

From the structural point of view, BTRs, BTs and BSAs are highly heterogeneous groups of compounds. BTRs and BTs are based on a benzene ring fused with a five-member heterocycle which for the BTRs is a 1,2,3-triazole ring and for the BTs is a 1,3-thiazole ring. BSAs are based on a benzene ring with a sulfonamide group. Depending on the functionalization, the physicochemical characteristics of these compounds are broadly dispersed and far apart from each other, for instance, benzothiazole has a pK_a of 0.9, meanwhile their “amino” and the “hydroxy” derivatives have pK_a values of 3.9 and 10.4, respectively.

One way to determine these compounds in liquid samples is to use solid-phase extraction (SPE) followed by chromatographic techniques with mass spectrometry detection, which allow low levels to be quantified in analyzed samples [6,7,9,27,28]. One of the most common option is to use polymer reversed-phase sorbents in SPE, such as the Oasis HLB sorbent commercialized by Waters [6,7]. However, the search for more selective methods and the ability to perform ionic interactions have led to the use of commercial mixed-mode ion-exchange sorbents because they combine reversed-phase and ionic interactions [11,29] or the combination of mixed-mode ion-exchange sorbents (Strata X-AW and X-CW) with reversed phase sorbents (Isolute ENV+) [28]. Moreover, the development of home-made sorbents with high π delocalized density to perform π-π interactions has been also explored [9].

As have been mentioned, the group of BTs, BTRs and BSAs cluster a wide variety of compounds. Ideally, they can be retained through cationic, anionic, and reversed-phase interactions [11], so a sorbent which combines these three interactions could be of interest. Mixed-mode zwitterionic sorbents combine reversed-phase interactions with both ionic interactions. Currently, this kind of sorbents cannot be acquired commercially, so the sorbents reported in literature are homemade. They can be polymer- [30,31] or silica-based [32,33]. Silica is an interesting substrate for sorbents because the reactivity of the

silanol groups in the network allows for a wide variety of functional moieties to be introduced. And, in the recent years, the introduction of carbonaceous nanomaterials has been explored [34]. In this regard, the use of fullerenes, carbon nanotubes or graphene has been evaluated [35]. Graphene has a rich π delocalized density, high surface area, excellent thermal stability, and corrosion resistance, which make it particularly suitable to be used in novel sorbent development.

This study evaluates a novel mixed-mode zwitterionic sorbent. It is based on a sol-gel silica derived network where graphene microparticles are embedded and functionalized to simultaneously perform anionic, cationic, reversed-phase and π-π interactions. The sorbent was evaluated for the retention of a group of BTs, BTRs and BSAs, since they form a heterogeneous group having compounds that can be retained through cationic, anionic, reversed phase and π-π interactions. After being optimized for SPE, the sorbent was used to extract these compounds in river, effluent wastewater, and influent wastewayter samples followed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) determination.

2. Experimental

2.1. Reagents and standards

The reagents used for sol-gel mixed-mode zwitterionic sorbent were tetramethyl orthosilicate (TMOS), methyl trimethoxysilane (MTMS), isopropanol (IPA), trifluoroacetic acid (TFA), hydrogen peroxide, methylene chloride, ammonium hydroxide and sulphuric acid. All of them were purchased from Sigma Aldrich (St. Louis, MO, USA).

3-mercaptopropyl trimethoxysilane (3-MPTMS), octadecyl trimethoxysilane (C_{18}-TMS), and N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride (N-TMPTMAC) were acquired from Gelest Inc. (Morrisville, WI, USA).

Ultrapure water was provided by a water purification system (Millipore, Burlington, USA) and HPLC grade methanol (MeOH) and acetonitrile (ACN), and MS grade MeOH, ACN and water were purchased from Carlo Erba (Val de Reuil, France). Acetic acid (HAc), formic acid and hydrochloric acid were purchased from Sigma-Aldrich.

Solid standards of 1-H-benzotriazole (BTR), 4-methyl-1-benzotriazole (4TTR), 5-methyl-1-benzotriazole (5TTR), 5,6-dimethyl-1-benzotriazole (XTR), 5-chloro-1-H-benzotriazole (ClBTR), benzothiazole (BT), 2-hydroxybenzothiazole (OHBT), 2-(methylthio)-benzothiazole (MeSBT), benzenesulfonamide (BSA), o-toluenesulfonamide (o-TSA), p-toluenesulfonamide (p-TSA), N-methyl-p-toluenesulfonamide (Me-p-TSA) and N-ethyl-p-toluenesulfonamide (Et-p-TSA) were purchased from Sigma Aldrich. Stock solutions of individual standards were prepared in MeOH at a concentration of 1000 mg/L and stored at −20 °C. The working solutions were prepared weekly in a mixture of ultrapure water and methanol (80/20, v/v) and were stored at 4°C in brown bottles in the dark.

2.2 Synthesis of sol-gel mixed-mode zwitterionic sorbent with encapsulated graphene particles

The sol-gel mixed-mode zwitterionic sorbent was prepared using a facile sol-

gel synthesis protocol developed in our laboratory. The sol solution for the sorbent was created by sequentially adding and subsequently vortexing of the building blocks tetramethyl orthosilicate (TMOS), methyl trimethoxysilane (MT MS), octadecyl trimethoxysilane (C_{18}-TMS), N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride (N-TMP TMAC), 3-mercaptopropyl trimethoxy-silane (3-MPTMS), isopropanol (IPA) and trifluoroacetic acid (TFA, 0.1M) in a 50 mL centrifuge tube. The relative ratios of the ingredients (TMOS, MTMS, 3-MPTMS, C_{18}-TMS, N-TMPTMAC, IPA, and TFA) were maintained at 1: 1: 0.1: 0.1: 0.2: 3.8: 3, respectively. The mixture was vortexed for 5 min and subsequently sonicated for 15 min in order to eliminate any trapped air bubbles potentially present in the sol solution. The sol solution was kept at room temperature for 8 h to allow the sol-gel precursors to be completely hydrolyzed.

In a batch of approximately 65 mL sol solution, 0.1 g of graphene micro particles (5-10 µm) were added, and solution was sonicated for 30 min to obtain a near-homogenous dispersion of graphene particles.

Freshly prepared ammonium hydroxide (1 M) was then added in droplets to the hydrolyzed sol solution containing dispersed graphene microparticles under continuous stirring in a magnetic stirrer. The solution was seen to become viscous before turning into solid gel. The solid gel was aged and thermally conditioned at 60 °C for 48 h. The monolithic bed of the sol-gel network embedded with graphene micro particles was then pulverized and dried at 80 °C for 24 h and the sol-gel sorbent was broken into fine particles in a ball mill and rinsed with MeOH:methylene chloride (50:50, v/v) under sonication for 30 min. The particles were dried with air and treated with 30% H_2O_2 (with 0.1 M sulphuric acid) for 4 h. The sorbent particles were subsequently washed with deionized water several times and finally dried at 80 °C for 12 h. A detail process

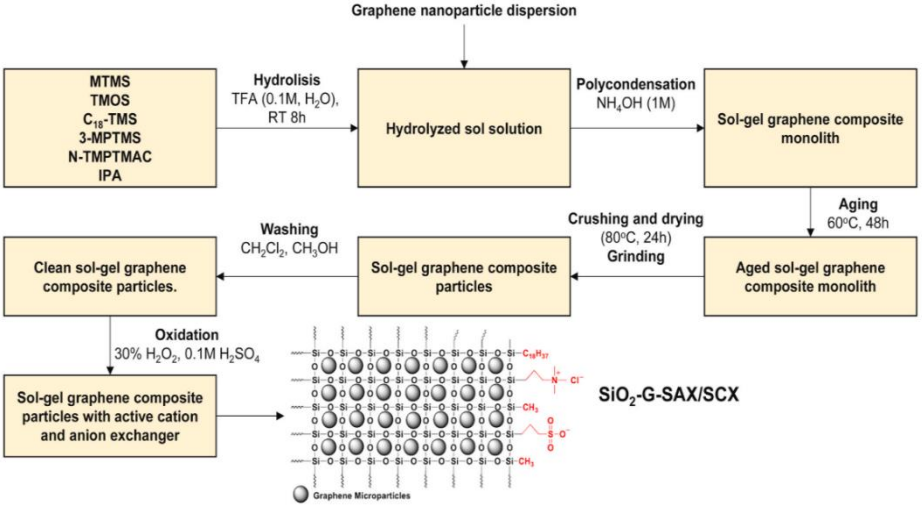

Figure 1. Process flow diagram for synthesizing sol-gel multi-mode graphene composite sorbent.

flow diagram for synthesizing sol-gel zwitterionic multi-mode sorbent with embedded graphene particles is presented in Figure 1. The sol-gel zwitterionic mixed-mode sorbent with embedded graphene particles were then ready for using it for SPE.

2.3. Structure of sol-gel mixed-mode zwitterionic sorbent

The characterization of the sorbent was performed with a JEOL JSM 5900LV Scanning Electron Microscope (SEM) equipped with EDS-UTW detector, JEOL USA, Inc. (Peabody, MA, USA) for recording SEM images and a Cary 670 FTIR Spectrometer (Agilent Technologies, Santa Clara, CA, USA) for the Fourier Transform Infrared Spectroscopy (FT-IR).

The sorbent evaluated was named SiO_2-G-SAX/SCX (Figure 1). It is based on silica modified with graphene microparticles and functionalized with C_{18} chains (reversed phase interactions), quaternary amines (strong anionic interactions, SAX) and sulfonic groups (strong cationic interactions, SCX). Moreover, the graphene microparticles allow to perform π-π interactions

2.4. Solid-phase extraction procedure

A 10 µm polyethylene frit (Symta, Madrid, Spain) was fitted in an empty 6 mL SPE cartridge (Symta), to which 200 mg of the sorbent was added. Another 10 µm polyethylene frit was placed above the sorbent.

The SPE procedure was carried out in a SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. The sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3, and then 100 mL of river water or 50 mL of influent and effluent wastewater adjusted to pH 3 with HCl were loaded into the cartridge at an aproximately flow rate of 10 mL/min. Elution was carried out with 5 mL of MeOH. The eluted volume was evaporated with a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to an approximate volume of 150 µL and reconstituted to 1 mL with initial mobile phase solution. The reconstituted extracts were filtered with 0.45 µm PTFE (polytetrafluoroethylene) syringe filters (Scharlab) before the analysis. To reuse the SPE cartridges, they were washed with 5 mL of MeOH and then they were completely dried by applying vacuum for 10 minutes. The reusability of the cartridges was evaluated up to 20 times, showing good precision.

Before analysis, all samples were filtered through a 0.45 µm nylon membrane filter (Scharlab). Effluent and influent samples were previously filtered with a 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK).

2.5. Liquid chromatography – high resolution mass spectrometry

Chromatographic analysis was carried out with an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) equipped with an automatic injector (Accela Autosampler) and a quaternary pump. The LC system was coupled to an Exactive Orbitrap mass spectrometer from Thermo Scientific equipped with a heated electrospray ionisation (HESI) source and a high-energy collision dissociation (HCD) cell that fragmented the analytes for their confirmation. The chromatographic column used was Acquity UPLC HSS T3

(100 mm x 2.1 mm, 1.8 µm particle size) acquired from Waters (Milford, MA, USA).

The mobile phase was a mixture of 0.1% HCOOH in H_2O/ACN 98/2, v/v (solvent A) and MeOH (solvent B). The gradient profile started with 0% of B, and was increased to 20% in 8 min, when %B was held for 2 min before being increased to 25% in the next 5 min. Finally, %B was increased to 100% in the next 6 min, held for 3 min and then returned to the initial conditions in 1 min where it was held for 3 min. The flow rate was 400 µL/min, the column oven temperature was 50 ºC and the injection volume was 20 µL.

The optimal parameters in positive ionisation mode were: sheath gas flow rate, 40 arbitrary units (AU); auxiliary gas flow rate, 20 AU; sweep gas, 0 AU; spray voltage, 4 kV; capillary voltage, 37.5 V; tube lens voltage, 85 V; and skimmer voltage 20 V. The optimal parameters in negative ionisation mode were: sheath gas flow rate, 45 AU; auxiliary gas flow rate, 15 AU; sweep gas, 0 AU; spray voltage, 4 kV; capillary voltage, -25 V; tube lens voltage, -90 V; and skimmer voltage -25 V. In both cases, the capillary temperature was 350 ºC and the heater temperature was 400 ºC. The compounds measured in positive mode were: BT, OHBT, MeSBT, BTR, 5TTR, 4TTR, ClBTR, XTR, Me-p-TSA and Et-p-TSA. The compounds measured in negative mode were BSA and TSA

Four windows were used for data acquisition, two in negative mode (0 – 5.75 min and 8.50 – 10.10) and two in positive mode (5.75 – 8.50 and 10.10 – 22). In every window, two alternative scan events were set with a range of 50 – 250 m/z. The first event was a full scan at 50,000 FWHM with an injection time of 250 ms. The second scan was a fragmentation scan at 10,000 FWHM with an injection time of 50 ms and a collision voltage of 30 eV in the HCD in all windows. For quantification, the molecular ions were measured (with a mass extraction window of 5 ppm) and the selected fragments were used for confirmation. Table S1 shows the mass of the selected ions and their fragments.

2.6. Validation parameters

The method was validated in terms of recovery, matrix effect, linear range, method detection and quantification limits, repeatability and reproducibility between days.

SPE recovery ($\%R_{SPE}$) and apparent recovery ($\%R_{app}$) were used to evaluate the yield of the extraction. $\%R_{SPE}$ was obtained by analyzing standard solutions and was the recovery of the extraction. $\%R_{app}$ was obtained by analyzing spiked environmental water samples and was the recovery of the extraction considering the matrix effect. In both cases, the recovery was calculated as the ratio of the theoretical concentration and the concentration in LC-HRMS. In the case of the environmental water samples, the blank concentration was subtracted.

The matrix effect (%ME) was obtained using the formula: $\%ME = (C_e/C_t \times 100) - 100$, where "$C_e$" is the concentration obtained by spiking a blank sample after SPE and "C_t" is the expected concentration. A positive value indicates that the signal is enhanced while a negative value indicates that it is suppressed.

The linear range was tested with external calibration curves which analyzed seven solutions with different concentrations in triplicate. The method detection limit (MDL) was obtained as the concentration that had a signal-to-noise ratio higher than 3 with one of the fragments providing a signal higher than 10^3, and the method quantification limit (MQL) was the lowest point from the calibration curves, accomplishing also a signal-to-noise ratio higher than 10.

Repeatability was obtained as the %RSD (relative standard deviation) (n=4) by analysing spiked samples on the same day. The reproducibility between days was obtained as the %RSD by analysing spiked samples (n=4) on different days.

3. Results and discussion

3.1. Synthesis of the sol-gel mixed-mode zwitterionic graphene embedded sorbents

Sustainability and healthy progress of sorptive extraction and microextraction techniques primarily depend on the continuous influx of novel sorbents with improved selectivity, chemical and thermal stability. Carbonaceous particles including graphene oxide, graphene, activated carbon, carbon nanotubes have been found very promising in developing new sorbent materials. Graphene and graphene oxide are frequently used in both the extraction (e.g., solid-phase extraction) and microextraction (e.g., solid-phase microextraction) techniques [36]. Although graphene oxide, due to its possession of abundant hydroxide and carboxylic acid functional groups, are favoured in many applications, in the current project we used pristine graphene particles to increase the overall surface area so that the sample matrix can easily permeates through the composite sorbents and the analytes interact easily with different functional moieties implanted into the sol-gel matrix, resulting in faster extraction kinetic and higher extraction recovery. Graphene complements the other ingredients of the sol solution, methyl trimethoxysilane, octadecyl trimethoxysilane, 4-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride and 3-mercaptopropyl trimethoxysilane to end with a sol-gel mixed-mode zwitterionic graphene embedded sorbent named SiO_2-G-SAX/SCX.

3.2. Characterization of sol-gel zwitterionic mixed-mode graphene embedded sorbents

Sol-gel zwitterionic mixed-mode graphene embedded sorbents were characterized using FT-IR and SEM-EDS to understand the chemical makeup, surface morphology and the elemental chemical composition. FT-IR spectra provides important information regarding the functional composition of the building blocks and their successful integration into the final sol-gel composite material. SEM images as well as the data obtained from Energy Dispersive X-ray Spectroscopy provide information regarding the surface morphology, apparent particle sizes and their distribution as well the elemental composition of the composite material.

3.2.1. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the major component of graphene, and the sol-gel

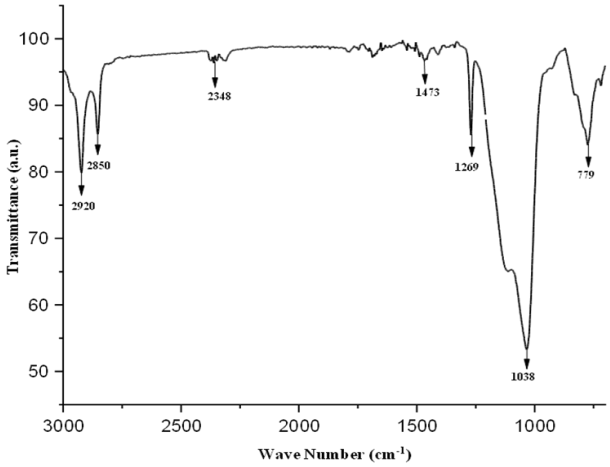

Figure 2. FT-IR spectra of sol-gel graphene SAX/SCX sorbent.

sorbent with embedded graphene are presented in Supplementary Figure S1 and Figure 2, respectively. All FT-IR spectra were presented over a range between 3000 and 700 cm $^{-1}$ at a resolution 4 cm^{-1}. Unlike graphene oxide, graphene does not possess different functional sites and therefore not many distinct features. FT-IR spectra of pristine graphene FT-IR spectra (Fig. 1S) has a broad and weak peak at ~1608 cm^{-1} that can be assigned to C=C stretching. The peak at ~2343 cm^{-1} corresponds to CO_2, suggesting incomplete reduction of graphene oxide during the manufacturing process of graphene [37]. The peak at ~ 1470 cm^{-1} may be attributed to C-H bending vibration. The FT-IR spectra of SiO_2-G-SAX/SCX (Fig. 2) maintained the signature peaks that appeared in pristine graphene spectra (Fig. S1). In addition, the peak at ~1037 cm^{-1} can be assigned to CO stretching vibration of Si-O-CH_3. Due to the very high content of graphene in the sol-gel matrix, the spectral contributions of other two major ingredients, 3-mercaptopropyl trimethoxysilane (3-MPTMS) and 4-(trimethoxysilylethyl) benzyl trimethyl ammonium chloride (4-TMSEBTMAC) were not recognized.

3.2.2. Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy

The surface morphology and the elemental composition of the sorbent was investigated using a SEM-EDS. The SEM images are presented in Figure 3 (a, b) at 500x and 1,000x magnifications, respectively. The EDS images are presented in Figure 3 (c, d). The SEM images showed that the particle sizes are not homogeneously distributed and have irregular shapes. Majority of the particles are in the range of 1-10 µm and of irregular shape. The broad distribution of particles helps them packing well for SPE with minimal void volume. The surface of the particles apparently looks rough that may augment the interaction between the particles and the analytes during the

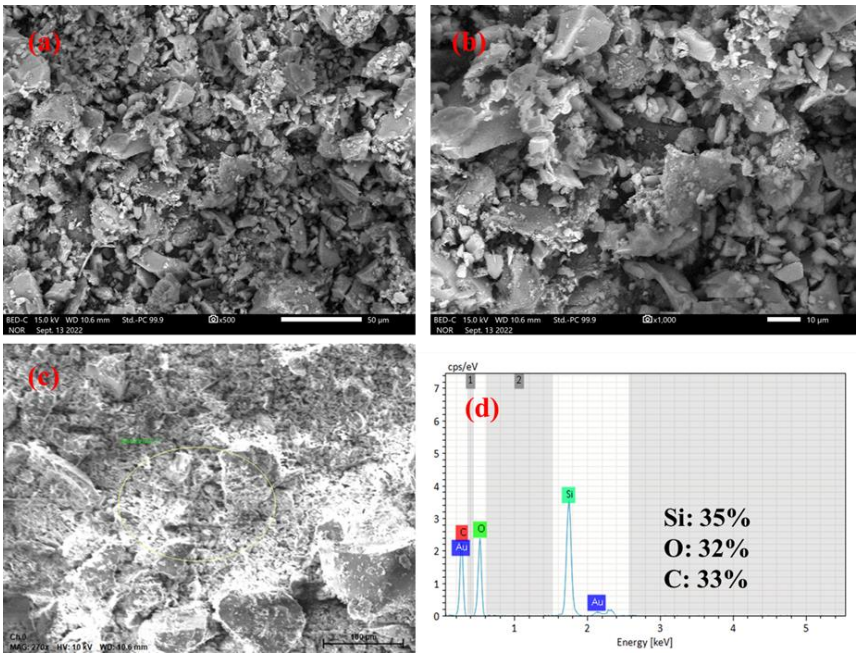

Figure 3.- SEM images of SiO2-G-SAX/SCX particles (a) at 500x magnification; (b) at 1,000x magnification; (c) circled area used for EDS; (d) individual elemental spectra obtained from EDS.

extraction process. The EDS data revealed the elemental composition of the sorbent with 35% Si, 32% O and 33% C. Due to the two sources of carbon, C_{18}-TMS and graphene in the sol solution, carbon appears to be a dominant component in the composite sorbent.

3.3. LC-HRMS conditions

Before evaluating the sorbent towards the selected group of compounds, the LC-HRMS conditions were settled. The challenge of the chromatographic optimization was the separation of two pairs of isomers: 4TTR and 5TTR and o-TSA and p-TSA. In order to separate 4TTR and 5TTR, solvent A initially consisted of 0.1% HCOOH in H_2O, but the compounds were not completely separated. Therefore, 2% of ACN was added [27], which made the separation satisfactory. However, the separation of the TSA isomers was not achieved under optimal conditions for the other compounds. So, they were determined as a mixture named TSA. This has also been reported in other studies [7,11,27].

In the HRMS, the signals were acquired in both positive and negative mode to optimize the signal of each analyte. This optimization was performed in full scan at high resolution (50,000 FWHM) in a mass range of 50 – 500 m/z. All the ions were quantified with positive ionization except BSA and TSA (Table S1), which, in agreement with previous studies, were quantified with negative ionization to obtain the highest signal possible [7,11,27]. Calibration curves were obtained for each analyte, with the highest limit being 500 µg/L. The lowest

limit, which was also the instrumental quantification limit (IQL), ranged between 0.5 and 1 µg/L, except for BSA and TSA, which showed low ionization yields that caused low signal and IQL values of 20 µg/L. The instrumental detection limits (IDL) ranged between 0.1 and 0.5 µg/L except for BSA and TSA (10 µg/L).

3.4. Solid-phase extraction

Initially, the sorbent SiO_2-G-SAX/SCX was evaluated with standard solutions prepared in ultrapure water with all compounds at a concentration of 5 µg/L (except for BSA and TSA which was prepared at a concentration of 50 µg/L). The initial conditions were based on a previous study [32] which evaluated other home-made sol-gel silica-based mixed-mode zwitterionic sorbents: the loading volume was 25mL of ultrapure water at pH 6, since there were a variety of pK_a compounds, and the elution step was 5 mL of 5% NH_4OH in MeOH and 5 mL of 5% HAc in MeOH.

As can be observed in Figure 4, SiO_2-G-SAX/SCX presented SPE recoveries higher than 80% for all BTs and BTRs, and higher than 70% for the BSAs except for BSA whose $\%R_{SPE}$ was 40%. The sorbent was compared with two sorbents developed in a previous study [32] which had the same functionalization with C_{18} chains, quaternary amines, and sulfonic acids, but a silica network that was unmodified (SiO_2-SAX/SCX) or modified with carbon microparticles (SiO_2-C-SAX/SCX). As can be seen in Figure 4, for most compounds the SPE recoveries of SiO_2-SAX/SCX and SiO_2-C-SAX/SCX are close to the ones obtained with SiO_2-G-SAX/SCX. However, the SPE recoveries for BTR, BSA and TSA were considerably higher and SiO_2-G-SAX/SCX showed that the inclusion of

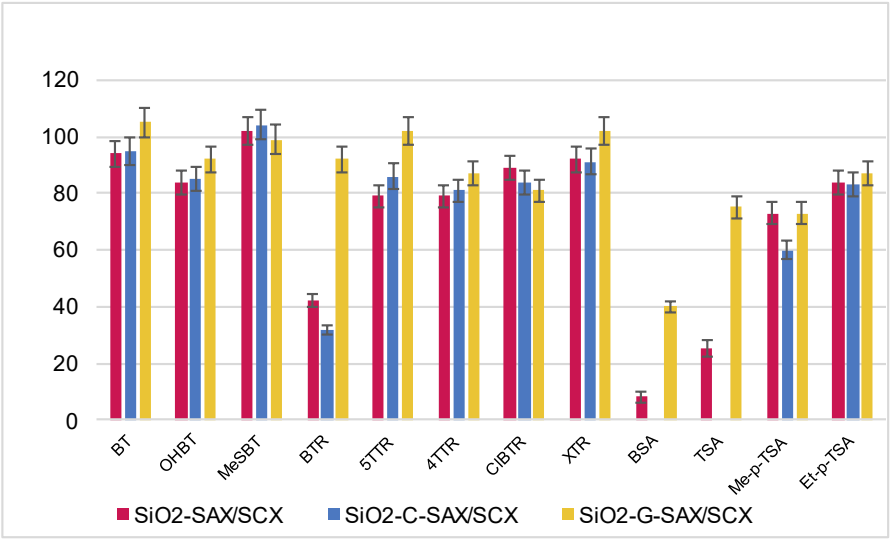

Figure 4. Recoveries obtained when 25 mL of ultrapure water spiked at 5 µg/L were percolated with the different sorbents.

graphene microparticles improved performance.

In a previous study, Salas *et al.* [11] proposed that the delocalization of charge between the fused rings in the BTs and the BTRs allows these compounds to interact through ionic exchange interactions. In our case, we exploited this electronic delocalization to interact with the graphene high π delocalized density, allowing SiO_2-G-SAX/SCX to present π-π interactions with these analytes. Moreover, BSAs have only one aromatic ring in their structures, so the delocalization is lower and there are fewer π-π interactions. Therefore, the SPE recoveries for these compounds were slightly lower than for BTs and BTRs.

To further improve the retention of the compounds, the pH was optimized by testing and comparing pH 3 and pH 6. As can be observed in Table 1, the SPE recoveries were slightly better at pH 3 for compounds like Me-p-TSA, so this pH was selected for the following tests. Some studies [11,27] that determined similar compounds using polymer-based sorbents such as Oasis HLB and Polyclean polymeric reported that the loading pH did not affect the $\%R_{SPE}$ of their compounds, whereas other studies [6,7,38] selected pH 3 for the loading solution.

Table 1. Recoveries obtained when 25 mL of ultrapure water adjusted at pH 3 and 6 were percolated through SiO2-G-SAX/SCX.

		pH 3	pH 6
BT	BT	107	105
	OHBT	91	92
	MeSBT	97	89
BTR	BTR	92	85
	5TTR	99	97
	4TTR	90	87
	ClBTR	85	81
	XTR	102	102
BSA	BSA	42	40
	TSA	78	75
	Me-p-TSA	95	73
	Et-p-TSA	90	87

*RSD (%) < 15%. (n = 3)

To confirm that the compounds were retained through π-π interactions, a clean-up step was used to try to separate the compounds retained through hydrophobic and π-π interactions from the compounds retained through ionic interactions. The use of 1 mL of MeOH and 1 mL of a mixture of water and MeOH (1:1) was evaluated. In both cases, the compounds washed out in the clean-up step. In greater detail, the % of analytes eluted with the MeOH ranged from 35% (BSA) to 90% (Me-p-TSA). In the case of the mixture water:MeOH, the % lost in the clean-up step was slightly lower but was still too high, between 24% (XTR) and 75% (Et-p-TSA). These results confirmed that the compounds were mostly retained through hydrophobic and π-π interactions since they were rinsed with MeOH. Nonetheless, some studies did not include a clean-up when determining similar compounds [7,9,27].

Since the compounds were partially eluted with 1 mL of MeOH, the use of 5 mL of MeOH instead of a solution of NH_4OH in MeOH was evaluated. The $\%R_{SPE}$ eluting with 5 mL of MeOH ranged from 72% (TSA) to 98% (OHBT), except for BSA (54%) whose low retention has been mentioned above (Table 1). Therefore, this elution was selected. The use of MeOH to elute similar compounds has been reported in previous studies [7,9,27]. Of particular note is the study by Spelltini *et al.* [9] who modified silica with humic acids to determine BTs and BTRs.

Humic acids made it possible for the sorbent to perform π-π interactions, as graphene did in the present study. They used 4 mL of MeOH to elute the compounds, which disrupted these interactions.

The increase in loading volume from 25 to 100 mL was evaluated with ultrapure water and no significant decrease was observed in the $\%R_{SPE}$. For 25 mL, values were between 77% and 92% (except BSA, 31%) and for 100 mL they were between 75% and 92% (except BSA, 33%) (Table S2). Thus, the optimized SPE procedure consisted of 100 mL of water adjusted to pH 3 for the loading step and 5 mL of MeOH for the elution step.

With these parameters, the $\%R_{SPE}$ was calculated by spiking ultrapure water at 1 and 5 µg/L, as can be observed in Table S2. Only BSA provided low recoveries (lower than 40%), whereas, the $\%R_{SPE}$ of the other compounds ranged from 71% to 92%.

After the procedure had been optimized using ultrapure water, the sorbent was tested with environmental water samples (river water, effluent wastewater, and influent wastewater). The apparent recoveries ($\%R_{app}$) obtained for 100 mL of river water adjusted to pH 3 and spiked at 5 µg/L, ranged from 58% to 78%. However, for effluent and influent wastewater samples, the $\%R_{app}$ for 100 mL ranged from 19% to 60%. The use of 50 mL was then tested, and the results were acceptable ($\%R_{app}$ between 49% and 76%). Thus, 100 mL was the volume selected for river samples and 50 mL for effluent and influent samples. Various loading volumes were selected for determining similar analytes: Asimakopoulos *et al.* selected 20 mL [38], Ajibola *et al.* 50 mL (effluent and influent) [6] and Hidalgo *et al.* 1 L (river and sea water) [7], although in this latter study the amount of sorbent was 500 mg. We considered that increasing of the loading volume was not needed due to the high sensitivity of LC-HRMS which provided low limits of detection. Moreover, if the loading volume had been increased, the amount of sorbent would have also been increased.

The elution volume of 5 mL of MeOH was evaporated to improve the sensitivity to around 150 µL and reconstituted to 1 mL of mobile phase. Evaporation to complete dryness was tested, but some compounds like the BTRs or Me-p-TSA suffered losses of about 30%, so evaporation was performed to about 150 µL to prevent analytes from being lost.

3.5. Validation of the method

Once the SPE procedure had been optimized, the method was validated according to the parameters described in section 2.6

that are apparent recovery calculated at two concentrations for river, effluent and influent wastewater samples, matrix effect, method detection (MDL) and quantification limits (MQL), repeatability and reproducibility between days. To calculate these parameters when necessary, the concentration of the blank was measured and subtracted from the signal of the spiked samples. The $\%R_{app}$ was calculated after the river samples had been spiked at 1 and 5 µg/L (except for BSA and TSA which were spiked at 10 and 50 µg/L), and effluent and influent wastewater samples at 2 and 10 µg/L

Table 2. Apparent recoveries ($\%R_{app}$) when river, effluent and influent wastewater samples were spiked at two levels of concentration.

		River (100 mL)		Effluent WW (50 mL)		Influent WW (25 mL)	
		$\%R_{app}$ (1 µg/L)	$\%R_{app}$ (5 µg/L)	$\%R_{app}$ (2 µg/L)	$\%R_{app}$ (10 µg/L)	$\%R_{app}$ (2 µg/L)	$\%R_{app}$ (10 µg/L)
BT	BT	52	43	53	53	64	71
	OHBT	48	55	61	59	62	71
	MeSBT	51	54	62	63	39	50
BTR	BTR	57	59	59	64	48	53
	5TTR	53	56	68	72	64	63
	4TTR	55	58	67	69	72	64
	ClBTR	51	62	63	62	57	64
	XTR	57	64	75	71	78	73
BSA	BSA	32	35	38	34	22	24
	TSA	55	61	65	62	55	64
	Me-p-TSA	64	65	77	75	70	74
	Et-p-TSA	68	64	80	72	85	78

*RSD (%) < 18%. (n = 4)

(except for BSA and TSA which were spiked at 20 and 100 µg/L) and the signal of a blank had been subtracted

. Spiking at lower concentrations was not evaluated because some of the compounds occurred naturally in the blank samples. Table 2 shows the $\%R_{app}$ obtained, which in most cases were above 50% (51% to 85%), except for BSA (whose low recoveries have been pointed out before), OHBT in river samples (48%), and MeSBT (39%) and BTR (48%) in influent wastewater samples. These results are comparable with those reported in other studies [9,27] in which similar compounds were determined in water with a commercial sorbent (Oasis HLB) to provide $\%R_{app}$ between 66% and 84% for river samples [27]. Another example was the use of a silica-based homemade sorbent to extract BTR, 5TTR, BT, OHBT and MeSBT, which gave recoveries between 70% and 114% in river samples [9].

Attending to the matrix effect (%ME), as can be observed in Figure 5, in all cases (except for MeSBT) it was below ±30%. For influent wastewater samples, the signal of all the analytes was enhanced, and for river and effluent samples they were both suppression and enhancement of signal. All in all, excluding MeSBT, the %ME ranged from -19% to 30% for all analyzed samples. In particular, it should be pointed out that for BTRs the %ME was lower than ±20% even in influent wastewater samples. These results were remarkable since a clean-up step was not included to improve the selectivity of the method. The results were comparable to those obtained by other methods [11] which did contain a clean-up step, where the %ME ranged from -32% to +24% for the determination of similar compounds with commercial sorbents (Oasis MCX and MAX). In comparison with other studies without a selective clean-up step, the %ME was lower. For example, for the determination of BSA and TSA in effluent

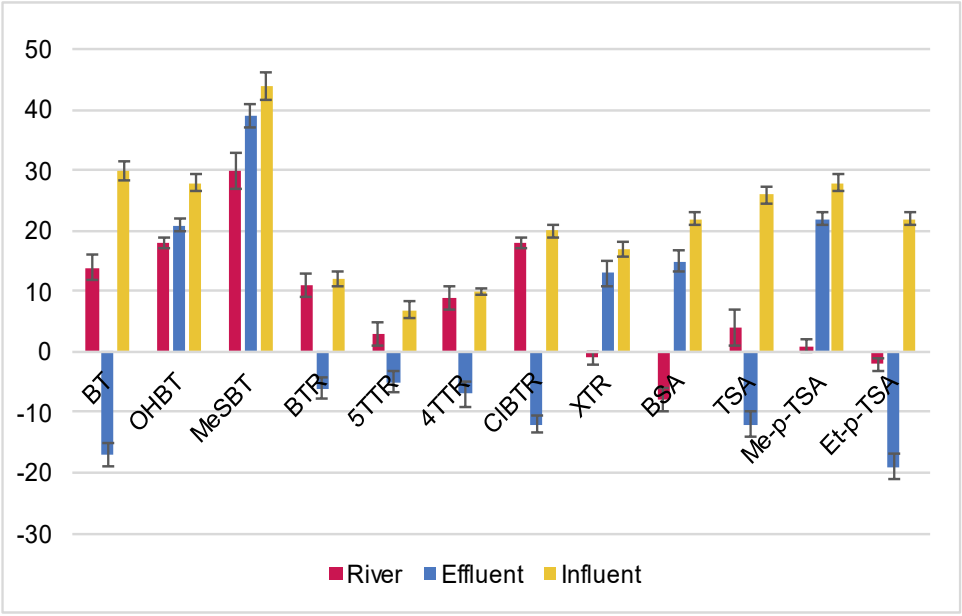

Figure 5. Matrix effect obtained for each analyte in river, effluent and influent wastewater samples.

and influent wastewater samples with a commercial sorbent (Polyclean polymeric), %ME ranged from -40% to -65% [6]. In both cases, a polymer sorbent based on reversed phase interactions was used, while our sorbent was silica-based. Silica-based sorbents present lower non-specific interactions than polymer-based sorbents. In addition, since the interactions exploited in this article are the π-π ones thanks to the graphene, the selectivity of the extraction is enhanced

MQLs and MDLs were also estimated from the instrumental limits and applying the recovery of each compound in each matrix. The results can be observed in Table S3, where MQL were lower than 18 ng/L in all cases except for BSA and TSA which, because of their high IQLs, were two orders of magnitude higher (300 - 900 ng/L). MDLs were lower than 10 ng/L in all cases except for BSA and TSA which were also considerably higher (154 – 455 ng/L). Several studies have reported similar MQLs and MDLs [6,9,11], for instance, Salas *et al.* [11] obtained MDLs in the range 10 – 42 ng/L and MQLs in the range 30 – 126 ng/L. Moreover, the high MDLs of BSA and TSA had been reported previously [7].

Both repeatability (% RSD, n = 4) and reproducibility between days (% RSD, n = 4) were lower than 18% for all analytes in all the matrixes.

3.6 Analysis of real samples

After the method had been validated, several river (Ebro), effluent wastewater and influent wastewater (Tarragona and Reus, Spain) samples were analyzed. Quantification was done by applying external calibration cuveres and using the apparent recoveries obtained

previously. To confirm the occurrence of the compounds, the mass error of the molecular ion had to be lower than 5 ppm and the signal of at least one fragment ion had to be higher than 10^3 AU.

Table 3 shows the results obtained. In river samples, all BTs and BTRs were found in most samples. Only MeSBT, ClBTR and XTR were below the MQL in some samples since the signal of fragment ions did not reach 10^3 AU. BSAs were not detected except for few samples where Et-p-TSA could have been quantified. BT and OHBT were the compounds detected at the highest concentrations (175 and 104 ng/L, respectively). The highest levels of BT (150 – 500 ng/L) and XTR (370 – 1200 ng/L) were observed by Xu *et al.* [39] in waters from German rivers. Herrero *et al.* [27] and Hidalgo *et al.* [7] analyzed similar river samples and the concentration of BTRs was comparable. More specifically, Herrero *et al.* quantified 33 – 153 ng/L (BTR) and 26 – 69 ng/L (4TTR) [27] and Hidalgo *et al.* quantified 34 – 78 ng/L (BTR) and 17 – 44 ng/L (4TTR) [7].

In wastewater samples, the compounds detected at highest concentrations were the BTRs, especially BTR, 4TTR and 5TTR in the influent wastewater samples which had concentrations around 500 ng/L. BSA and TSA were not detected due to their high detection limits, but Me-p-TSA and Et-p-TSA were detected in all samples and quantified in most of them. In general, the concentrations in influent wastewater samples were higher than in effluent wastewater, which suggests that these compounds had been partially removed in wastewater treatment plants. Salas *et al.* [11] determined BTs and BTRs in effluent and influent wastewater samples: the levels of BTs were similar (243 to 358 ng/L in effluent wastewater samples and 286 to 767 ng/L in influent wastewater samples) but BTRs were significantly higher (4TTR ranged from 666 to 1697 ng/L in effluent samples and from 743 to 1236 ng/L in influent wastewater samples). Dominguez *et al.* [40] found similar concentrations in effluent and influent wastewater samples. Concentrations of BT were close to 250 ng/L, which were similar to

Table 3. Range of concentrations (ng/L) obtained after the analysis of river, effluent wastewater and influent wastewater samples through SPE/LC-HRMS using the SiO_2-G-SAX/SCX sorbent.

		River (n=5)	Effluent WW (n=5)	Influent WW (n=5)
BT	BT	40 - 175	133 - 223	112 - 256
	OHBT	25 - 104	68 - 137	46 - 238
	MeSBT	<MQL - 18	<MQL	96 - 197
BTR	BTR	11 - 52	244 - 306	192 - 561
	5TTR	12 – 22	135 - 499	309 - 632
	4TTR	11 - 23	117 - 401	409 - 512
	ClBTR	<MQL - 12	MQL - 41	24 - 62
	XTR	<MQL - 11	MQL - 12	35 - 83
BSA	BSA	<MDL	<MDL	<MDL
	TSA	<MDL	<MDL	<MDL
	Me-p-TSA	<MDL	<MQL - 14	<MQL - 14
	Et-p-TSA	<MQL - 16	22 - 35	15 - 45

the concentrations found in the present study (112 – 256 ng/L).

BSA and TSA were not detected due to their high detection limits. Moreover, the other BSAs were found in low concentrations (<45 ng/L) in the samples.

4. Conclusions

A novel mixed-mode zwitterionic sorbent based on a silica network modified with graphene microparticles was synthesized. The sol-gel synthesis provided a facile pathway to molecular level integration of organic/inorganic polymer, organically modified sol-gel precursors as well as high surface area carbonaceous particles. The sol-gel synthesis maximizes the available surface area of the carbonaceous particles. In the current study, graphene with its high surface area and inherent capability to exert π-π interactions towards the analytes complements to the overall selectivity of the resulting SiO_2-G-SAX/SCX sorbent.

The sorbent was successfully applied for the extraction of a group of BTs, BTRs and BSAs. During the optimization of the sorbent, it was observed that the analytes were mainly retained in the sorbent through π-π interactions and various SPE parameters were optimized to exploit these interactions.

A method was successfully developed to determine the group of BTs, BTRs and BSAs in river, effluent wastewater, and influent wastewater samples using SPE followed by LC-HRMS. The results in terms of matrix effect were extremely low, considering that there was no clean-up step to improve the selectivity. These results suggest that this sorbent can be evaluated with other compounds with which π-π interactions can be performed.

Acknowledgements

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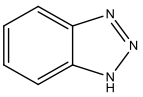
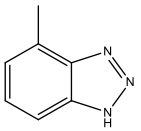
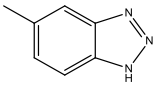
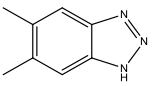
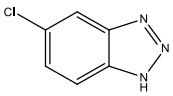
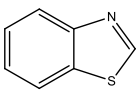
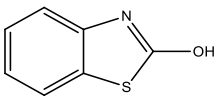
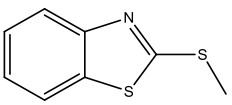
Suplementary data

Table S1. Structure, quantification ion and fragment ion of the compounds.

	Compound	Structure	Molecular ion (m/z)	Fragment ions (m/z)
BTR	1-H-benzotriazole (BTR)		$[M+H]^+$ 120.05572	$C_5H_5^+$ (65.03947) $C_6H_6N^+$ (92.05046)
	4-methyl-1-H-benzotriazole (4TTR)		$[M+H]^+$ 134.07138	$C_6H_7^+$ (79.05501) $C_6H_5N_2^+$ (105.04518)
	5-methyl-1-H-benzotriazole (5TTR)		$[M+H]^+$ 134.07138	$C_6H_7^+$ (79.05501) $C_6H_5N_2^+$ (105.04518)
	5,6-dimethyl-1-H-benzotriazole (XTR)		$[M+H]^+$ 148.08664	$C_7H_7^+$ (91.05423) $C_6H_5N_2^+$ (105.04517)
	5-chloro-1-H-benzotriazole (ClBTR)		$[M+H]^+$ 154.01628	$C_6H_4N^+$ (90.03444) $C_5H_4Cl^+$ (99.00011)
BT	Benzothiazole (BT)		$[M+H]^+$ 136.02129	$C_5H_5S^+$ (109.01125) $C_5H_5^+$ (65.03944)
	2-hydroxybenzothiazole (OHBT)		$[M+H]^+$ 152.01620	$C_6H_5S^+$ (109.01264) $C_5H_5^+$ (65.03946)
	2-(methylthio)-benzothiazole (MeSBT)		$[M+H]^+$ 182.00943	$C_7H_5NS_2^+$ (166.98601) $C_6H_5S^+$ (109.01104)

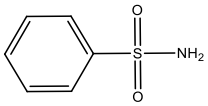
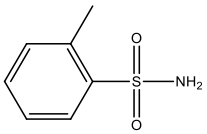
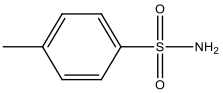
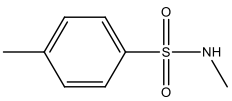
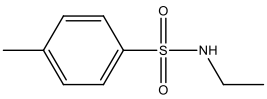
BSA	Benzenesulfonamide (BSA)		$[M-H]^-$ 156.01154	$C_6H_6N^-$ (92.05057) SO_2NH^- (78.97335)
	o-toluenesulfonamide (o-TSA)		$[M-H]^-$ 170.02703	SO_2NH^- (78.97335) $C_7H_8N^-$ (106.06522)
	p-toluenesulfonamide (p-TSA)		$[M-H]^-$ 170.02703	SO_2NH^- (78.97225) $C_7H_8N^-$ (106.06513)
	N-methyl-*p*-toluenesulfonamide (Me-p-TSA)		$[M+H]^+$ 186.05797	$C_7H_7^+$ (91.05480) $C_5H_5^+$ (65.03946)
	N-ethyl-*p*-toluenesulfonamide (Et-p-TSA)		$[M+H]^+$ 200.07376	$C_7H_7^+$ (91.05480) $C_5H_5^+$ (65.03946)

Table S2. Recoveries obtained when 100 mL of ultrapure water was spiked at 1 and 5 µg/L.

		%R_{SPE} (1 µg/L)	**%R_{SPE} (5 µg/L)**
BT	BT	86	84
	OHBT	85	92
	MeSBT	87	91
BTR	BTR	83	88
	5TTR	86	84
	4TTR	84	82
	ClBTR	90	87
	XTR	88	86
BSA	BSA	36	33
	TSA	71	75
	Me-p-TSA	79	77
	Et-p-TSA	85	81

Table S3. Method quantification limits (MQL) and method detection limits (MDL) expressed in ng/L for each analyte in river, effluent and influent wastewater samples.

		River		Effluent		Influent	
		MQL	MDL	MQL	MDL	MQL	MDL
BT	BT	10	2	9	2	16	8
	OHBT	21	10	16	8	13	3
	MeSBT	10	2	8	2	10	2
BTR	BTR	9	2	8	2	8	2
	5TTR	9	2	7	1	7	1
	4TTR	9	2	7	1	18	9
	ClBTR	20	10	16	8	6	1
	XTR	9	2	7	1	10	2
BSA	BSA	600	300	500	250	900	400
	TSA	300	180	300	150	350	180
	Me-p-TSA	18	8	18	6	25	9
	Et-p-TSA	20	7	20	6	25	10

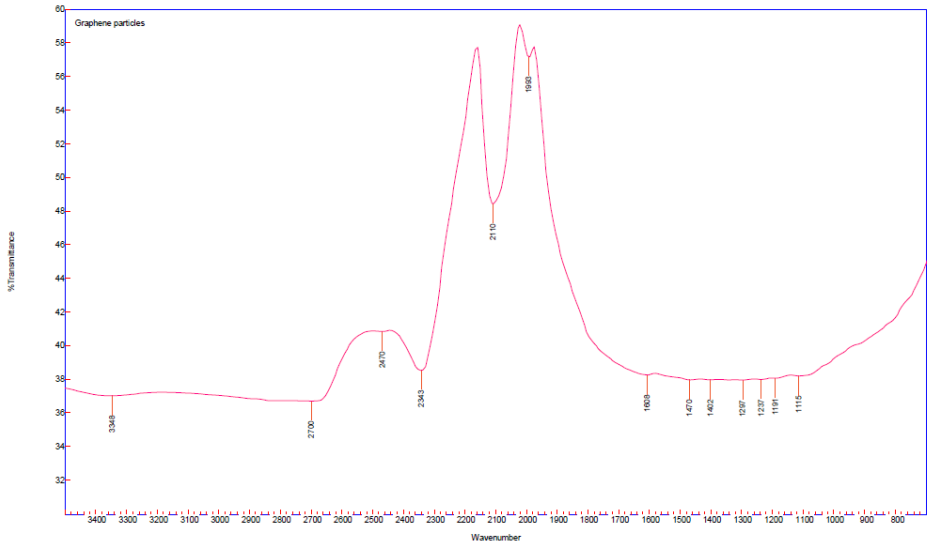


Figure S1. FT-IR spectra of graphene nanoparticle dispersion.

3.1.3. Selective determination of 2-aminobenzothiazole in environmental water and organic extracts from fish and dust samples

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

Selective determination of 2-aminobenzothiazole in environmental water and organic extracts from fish and dust samples

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Abstract

In the present study, a homemade mixed-mode ion-exchange sorbent based on silica with embedded graphene microparticles is applied for the selective extraction of 2-aminobenzothiazole (NH_2BT) followed by determination through liquid chromatography coupled to high resolution mass spectrometry.

The sorbent was evaluated for the solid-phase extraction of NH_2BT from environmental water samples (river, effluent wastewater and influent wastewater) and NH_2BT was strongly retained through the selective cation-exchange interactions. Therefore, the inclusion of a clean-up step of 7 mL of methanol provided good selectivity for the extraction of NH_2BT. The apparent recoveries obtained for environmental water samples ranged from 62 to 69% and the matrix effect from -1 to -14%.

The sorbent was also evaluated in the clean-up step of the organic extract for the extraction of NH_2BT from organic extracts of indoor dust samples (10 mL of ethyl acetate from pressurized liquid extraction) and fish (10 mL of acetonitrile from QuEChERS extraction). The organic extracts were acidified (adding a 0.1% of formic acid) to promote the cation-exchange interactions between the sorbent and the analyte. The apparent recoveries for fish samples ranged from 22 to 36% depending on the species. In the case of indoor dust samples, the recovery was 41%. It should be highlighted the low matrix effect encountered in such complex samples, with values ranging from -7 to 5% for fish and dust samples.

Finally, various samples were analyzed. The concentration in river samples ranged from 31 to 136 ng/L, in effluent wastewater samples, from 55 to 191 ng/L; in influent wastewater samples, from 131 to 549 ng/L; in fish samples, from 14 to 57 ng/g dried weight; and in indoor dust samples, from <MQL to 114 ng/g.

Keywords: homemade mixed-mode ion-exchange sorbent; clean-up; selective solid-phase extraction; fish; dust; complex water samples.

1. Introduction

The development of sorptive techniques is one of the leading research areas in the field of sample preparation and solid-phase extraction (SPE) is one of the most used sorptive techniques because of its reproducibility, low cost and simplicity [1,2]. However, one of the main drawbacks of this technique is that most commercial sorbents are unable to selectively extract target analytes [3] so there is increasing interest in obtaining selective materials for extraction. Of these sorbents, particular mention should be made of molecular imprinted polymers (MIPs) [4,5], tailor-made materials prepared using a template molecule during the polymerization, making the material very selective to the template molecule or similar ones. However, their applications are limited by the template. Another group of selective materials are mixed-mode ion-exchange sorbents, which combine reversed-phase and ionic interactions [6]. The selectivity of mixed-mode ion-exchange sorbents is achieved thanks to a clean-up step that uses an organic solvent (e.g. methanol) to disrupt the reversed-phase interactions. Thus, the compounds retained through ionic interactions can be eluted selectively with an acidic or basic eluent, depending on the nature of the retention.

This selectivity can be exploited to clean up the organic extracts obtained from complex solid matrices. SPE is applied after a previous extraction step such as solid-liquid extraction (SLE) or pressurized liquid extraction (PLE) [7–9] to purify the extract and increase the selectivity of the procedure. In most cases, the analytes are transferred to an organic extract (methanol, hexane, ethyl acetate, etc.) together with various interfering compounds, so a clean-up step is needed. The use of an organic loading solution has its drawbacks, since in SPE the breakthrough is lower than when aqueous solutions are used, which hampers the retention of the analytes. Therefore, to increase the retention capacity of sorbents, organic extracts are usually diluted in water [8,10,11] or partially evaporated to reduce the loading volume [8,12] or the extract is completely reconstituted in an aqueous solution [8,13,14]. Only in a few studies, the organic extract is directly transferred to the clean-up step. For instance, Tsuruoka et al. [15] developed a method for the determination of amantadine, rimantadine and memantine in chicken products where the QuEChERS organic extract (15 mL of acetronitrile with 0.1% of acetic acid) was directly cleaned up with a commercial mixed-mode ion-exchange sorbent (Oasis MCX).

The determination of contaminants in complex samples is a challenge that has yet to be solved. Benzothiazoles are one family of contaminants [16,17] which occur in samples such as water [18–20], sludge [21], fish [22,23], dust [24–26] and urine [27], among others. The biological activity of benzothiazoles has been applied to develop a wide range of pharmaceuticals (antimicrobial, analgesic, antitumor…) [16,28,29]. Focusing in 2-aminobenzothiazole (NH_2BT), its derivatives have been synthesized due to their biological activity [30,31]. However, these compounds with biological activity can cause also negative effects, and some studies have already evaluated their

effects of exposure on animals [32–34] and humans [35–37].

In the present study, a mixed-mode ion-exchange silica-based sorbent modified with graphene has been evaluated using SPE for the selective determination of NH_2BT in complex samples such as aqueous environmental water samples or solid samples such as dust and fish. In the case of solid samples, the sorbent was used to clean up organic extracts from complex matrices like fish or dust samples by introducing a SPE clean-up step after QuEChERS or PLE, respectively. All the SPE extracts were then analyzed by liquid chromatography coupled to high resolute mass spectrometry (LC-HRMS).

2. Experimental

2.1. Reagents and standards

Ultrapure water was provided by a water purification system (Millipore, Burlington, MA, USA), and "HPLC grade" methanol (MeOH), ethyl acetate (EtOAc) and acetonitrile (ACN) were acquired from Carlo Erba (Val de Reuil, France). "MS grade" MeOH, ACN and water were also acquired from Carlo Erba. Hydrochloric acid, formic acid and ammonium hydroxide were acquired from Sigma-Aldrich (St. Louis, MO, USA).

A solid standard of 2-aminobenzothiazole (NH2BT), 97% purity, was acquired from Sigma Aldrich. A stock solution was prepared in MeOH at a concentration of 1000 mg/L and stored at −20 °C. Working solutions were prepared weekly in a mixture of ultrapure water and MeOH (85/15, v/v) and stored at 4°C in amber bottles in the dark.

2.2. Structure of the sol-gel mixed-mode sorbent

The sorbent used in the present study was synthesized using a sol-gel approach with tetramethyl orthosilicate, methyl trimethoxysilane, octadecyl trimethoxysilane, N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride, and 3-mercaptopropyl trimethoxysilane as building blocks. The

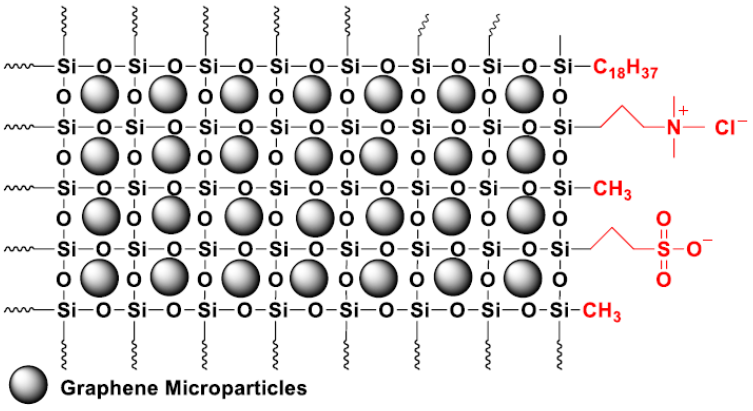

Figure 1. Scheme of the structure of SiO_2-G-SAX/SCX.

conditions of the sol-gel synthesis and its characterization have been described in detail in a previous study [38].

The resulting sorbent, SiO_2-G-SAX/SCX (Figure 1), is based on a silica modified with graphene microparticles and functionalized with C_{18} chains, quaternary amines and sulfonic groups, so that it can perform reversed-phase, strong anion- and strong cation-exchange interactions, respectively. Moreover, the addition of graphene microparticles makes π-π interactions possible. The advantage of strong ionic functionalization is that the groups will remain charged at any pH.

2.3. Extraction methods

2.3.1. Procedure for environmental water samples

All water samples were collected from the Tarragona region (Spain) and filtered through a 0.45 µm nylon membrane filter (Scharlab, Barcelona, Spain). River samples were collected from the Ebro River. Effluent and influent wastewater treatment plant samples were previously filtered with a 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK).

A total of 200 mg of the SiO_2-G-SAX/SCX sorbent was packed in an empty 6 mL SPE cartridge (Symta, Madrid, Spain) between two 10 µm polyethylene frits (Symta). The SPE procedure was performed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. With water samples, the first step was to condition the sorbent with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3. Samples of 100 mL of river water or 50 mL of influent and effluent wastewater were adjusted to pH 3 with HCOOH and loaded in the cartridge. The washing step was carried out with 7 mL of MeOH, and the elution step with 5 mL of 5% NH_4OH in MeOH.

The eluted volume was evaporated with a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to an approximate volume of 0.1 mL and reconstituted to 1 mL of 0.1% HCOOH in water. The reconstituted extracts were filtered with 0.45 µm PTFE (polytetrafluoroethylene) syringe filters (Scharlab) before analysis.

2.3.2. Procedure for indoor dust samples

Dust samples were collected using a vacuum cleaner from houses in the Tarragona region (Spain). The samples were pulverized and sieved (75 µm). Then, they were extracted by pressurized liquid extraction (PLE) with an ASE 350 accelerated solvent extractor system (Dionex, Sunnyvale, USA).

The extraction method was adapted from a previous study [25] in which a 10 mL stainless steel extraction cell was filled with 1 g of diatomaceous earth, 0.10 g, dried weight (d.w.), of sieved dust sample and 1 g of diatomaceous earth. The parameters for the extraction were 1 cycle of EtOAc at 70ºC and 1500 psi for 10 min. Flush volume was 80% of the cell volume and preheating and purge time were 5 min and 2 min, respectively.

Approximately 20 mL of EtOAc was obtained, which were evaporated to approximately 10 mL with a miVac Duo centrifuge evaporator and, before the

clean-up with SPE, 0.1% of HCOOH was added to the extract.

The SPE cartridge was conditioned with 5 mL of EtOAc and 5 mL of 0.1% HCOOH in EtOAc. The evaporated PLE extract was then loaded in the cartridge and eluted with 5 mL of 5% NH_4OH in MeOH. The eluted volume was evaporated and reconstituted as in section 2.3.1.

2.3.3. Procedure for fish samples

Fish samples were acquired from markets in Tarragona (Spain), cleaned, frozen and lyophilized using a miVac Duo sample concentrator with a SpeedTrap freeze-drying system evaporator (Genevac). The extraction method was adapted from a previous study [39]. The samples were ground and sieved (500 µm). A total of 0.10 g of sieved fish sample was placed in a 50 mL centrifuge tube (Serviquimia, Constantí, Spain) with 10 mL of ultrapure water and 10 mL of ACN. The tube was vortexed for 1 min. A QuEChERS extraction salt packet (Standard Method EN15662) (Scharlab) containing 4 g of magnesium sulfate, 1 g of sodium chloride, 0.5 g of sodium hydrogencitrate and 1 g of sodium citrate was then added to the tube, and it was vortexed for 3 min. The tube was then centrifuged at 4000 rpm for 5 min. The ACN phase was separated and 0.1% of HCOOH was added before the clean-up with SPE.

The SPE procedure was applied as reported in section 2.3.2 except that the SPE cartridge was conditioned with 5 mL of the ACN and 5 mL of 0.1% HCOOH in ACN, instead of EtOAc.

2.4. Liquid chromatography – high resolution mass spectrometry

Chromatographic analysis was performed with an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) with an automatic injector (Accela Autosampler) and a quaternary pump. The chromatographic column used was Acquity UPLC HSS T3 (100 mm x 2.1 mm, 1.8 µm particle size) acquired from Waters (Milford, MA, USA). The mobile phase was a mixture of 0.1% of HCOOH in H_2O (solvent A) and MeOH (solvent B). The gradient profile started with 0% of B, and was increased to 20% within 8 min and then to 100% in 1 min, where it was held for 5 min before returning to the initial conditions in 2 min where it was held for 2 min. The injection volume was 20 µL, the flow rate was 400 µL/min and the column oven temperature was 45 ºC.

The LC system was coupled to an Exactive Orbitrap mass spectrometer from Thermo Scientific with a heated electrospray ionisation (HESI) source and a high-energy collision dissociation (HCD) collision cell for the fragmentation and confirmation of the analytes.

In the HRMS, the signal was acquired in positive polarity. It was optimized in full scan at high resolution (50,000 FWHM) in a mass range of 50 – 500 m/z. The optimal parameters were a sheath gas flow rate of 40 AU (arbitrary units), an auxiliary gas flow rate of 20 AU, sweep gas 0 AU, spray voltage 4 kV, capillary voltage 37.5 V, tube lens voltage 85 V, skimmer voltage 20 V, capillary temperature 350 ºC and heater temperature 400 ºC.

Two scan events were used in a range of 50 – 250 m/z. The first event was a full scan at 50,000 FWHM with 250 ms of injection time. The second was a fragmentation scan at 10,000 FWHM with 50 ms of injection time, applying a collision voltage of 30 eV in the HCD. For quantification, the molecular ion selected was 151.03244 m/z ($[M+H]^+$) and it was measured with a mass extraction window of 5 ppm. Two fragments – 109.01145 ($[C_6H_5S]^+$) and 65.03942 ($[C_5H_5]^+$) m/z – were used to confirm, as well as the ratio of the molecular ion and its fragments. The confirmation was based on three criteria: the three ions must appear with a signal higher than 10^3, the ratio between the molecular ion and their fragments must be constant with a variation within ±30% range and the retention time must be constant in a ±0.1 min range.

The instrumental linear range was obtained by analyzing seven solutions, which gave a calibration curve from 0.5 to 500 µg/L (R^2>0.995). The instrumental detection limit (IDL) was 0.1 µg/L, which was the concentration that had a signal-to-noise ratio higher than 3 with one of the fragments providing a signal higher than 10^3. The instrumental quantification limit (IQL) was 0.5 µg/L, the lowest concentration of the calibration curve.

3. Results and discussion

3.1. SPE for environmental water samples

NH_2BT is a benzothiazole, a compound formed by a benzene ring fused with a 1,3-thiazole ring with an amine group, so it can interact with the sulfonic groups in the sorbent through cation-exchange interactions. Moreover, thanks to the electronic delocalization through the fused rings, the compound might interact with the graphene of the sorbent through π-π. As we are interested in exploiting the selectivity of the sorbent, the SPE protocol was studied to exploit the ion-exchange interactions. Based on previous experience [6,40] the initial protocol consisted of loading the sample at an acidic pH in ordet to protonate the NH_2BT, washing with 1 mL of MeOH and elution with 5% of NH_4OH in MeOH. Nevertheless, this protocol was optimized to further exploit these interactions.

To choose the loading pH, the load of 25 mL of ultrapure water adjusted to pH 3 and pH 6 with a washing step of 1 mL of MeOH were evaluated. Since the $\%R_{SPE}$ (SPE recovery, recovery when standard solutions are loaded) at pH 3 (93%) was slightly higher than at pH 6 (85%), pH 3 was selected as the loading pH. Other studies used an acidic pH for the loading solution to determine NH_2BT in water [18,19,40,41] and urine [42] samples. For instance, Salas *et al.* [40] adjusted the pH of their loading solution to 3 when analyzing environmental water samples with a commercial mixed-mode cationic-exchange sorbent (Oasis MCX).

The next parameter to optimize was the washing volume. The volume of MeOH used was increased, evaluating the use of 5 and 7 mL of MeOH. The recoveries of NH_2BT were not affected by the increasing of MeOH, being 92 and 91% for 5 mL and 7 mL of MeOH respectively. Since the highest volume of MeOH would provide the highest selectivity possible, 7 mL were selected

as the washing volume. The use of ACN as washing solvent was also evaluated, testing 3 and 5 mL. It was discarded since the recoveries of NH_2BT decreased slightly (83% with 3 mL and 79% with 5 mL).

The use of MeOH was also successfully evaluated for determining benzothiazoles and benzotriazoles with commercial mixed-mode sorbents (Oasis MCX and MAX) [40]. Other studies used water for the washing step with commercial reversed phase sorbents such as Oasis HLB [18,42,43] or Strata-X [19], although water would not have provided the selectivity obtained with MeOH.

The increase in loading volume was also evaluated, increasing from 25 to 100 mL. The recoveries in ultrapure water were similar with values between 87 and 91%. The use of 100 mL of river, effluent wastewater and influent wastewater was also evaluated. To evaluate the yield of the extraction when working with environmental samples, the apparent recovery ($\%R_{app}$) was used, which was calculated as the ratio between the experimental concentration of the spiked sample (considering the concentration of the blank sample) and the theoretical concentration. When 100 mL of river sample were percolated, the results were satisfactory, however, with effluent and influent wastewater samples, $\%R_{app}$ decreased to less than 36%. The loading volume was then reduced to 50 mL, which gave a $\%R_{app}$ of 69% for effluent wastewater samples and of 62% for influent wastewater samples.

The method for water samples was validated in terms of $\%R_{app}$ at two levels of concentration, matrix effect, detection and quantification limits, intra-day precision (%RSD, n=4) and inter-day precision (%RSD, n=4), and accuracy, expressed as relative recovery ($\%R_{rel}$), which was calculated as the ratio between the experimental concentration obtained from the analytical method (considering the concentration of the blank) and the concentration at which the sample was spiked.

Table 1 shows the results of $\%R_{app}$, spiked at different concentrations depending on the sample volume and complexity; for river samples was 0.1 µg/L, for effluent wastewater samples, 0.2 µg/L and for influent wastewater samples, 0.5 µg/L. As can be seen in Table 1, $\%R_{app}$ ranged from 63% to 64%, showing good extraction yields. The recoveries were also evaluated at a

Table 1. Validation parameters for environmental water samples.

Sample	$\%R_{app}$	%ME	Intra-day precision (%RSD, n=4)	Inter-day precision (%RSD, n=4)	$\%R_{rel}$	MDL (ng/L)	MQL (ng/L)
River	64	+1	5	7	105	3	8
Effluent wastewater	63	-9	3	5	94	9	21
Influent wastewater	64	-17	9	13	96	14	30

higher level of concentration, 1 µg/L for river samples, and 2 µg/L for effluent and influent wastewater samples, showing similar results, ranging the recoveries from 62 to 69%.

The matrix effect (%ME) was calculated using the formula: %ME = (C_{exp}/C_{the} x 100) – 100, where "C_{exp}" is the experimental concentration obtained by spiking a blank sample after SPE (subtracting the signal of the blank) and "C_{the}" is the theoretical concentration. A negative value indicates suppression of the signal while a positive value indicates enhancement. The extracts were spiked at 10 and 100 µg/L for river and effluent wastewater samples and 25 and 100 µg/L for influent wastewater samples. Table 1 shows the results for the low concentration, in the case of the high concentration the values were -1, -7 and -14% for river, effluent wastewater and influent wastewater samples respectively.

Comparing the results with those of other studies [18,19,40,41] that used polymeric sorbents, it can be observed that the $\%R_{app}$ are slightly higher than those obtained in the present study. However, the matrix effect was lower with the sorbent applied in the present study because silica sorbents tend to have less capacity than polymeric sorbents. On the other hand, silica sorbents have less non-specific interactions than polymeric sorbents, so the matrix effect is lower. For instance, the recoveries in the present study were lower than the ones reported by Hidalgo *et al.* [41], who obtained a recovery of 83% for NH_2BT when using a commercial polymeric reversed-phase sorbent (Oasis HLB) to analyze river samples, However, it should be highlighted that the matrix effectwas significantly higher (-25%), compared to the ±1% in the present study. Salas *et al.* [40]

reported similar results to the present study in terms of recovery for influent wastewater sampels ($\%R_{app}$ of 64%) , however, the matrix effect was higher (%ME of -25%) when they used a polymeric mixed-mode ion-exchange sorbent (Oasis MCX). It should be pointed out that in both the studies by Hidalgo and Salas the amount of sorbent was 500 mg, while in our study the amount of sorbent was 200 mg. The loading volume should also be considered: it was 1 L in Hidalgo's study [41] and 100 mL in Salas' [40].

The method detection limit (MDL) and method quantification limit (MQL) presented in Table 1 were estimated from the instrumental limits (which were based in the signal to noise approach) by applying the recoveries obtained for each matrix and the preconcentration factor. MDLs and MQLs were at low ng/L as can be observed in Table 1.

The precision was evaluated in terms of intra-day (%RSD, n=4) and inter-day precision (%RSD, n=4). The intra-day precision was lower than 9%. Meanwhile, the inter-day precision between days was lower than 13% as can be observed in Table 1. Accuracy also was evaluated as $\%R_{rel}$ at the same levels of concentration used for apparent recoveries, Table 1 shows the results for the low concentration levels, ranging from 94 to 105%.

Several samples were analyzed with the procedures developed. Table 2 shos the concentrations of NH_2BT obtained.

Table 2. Occurrence of NH_2BT obtained when analyzing different type of complex samples.

Sample	NH_2BT concentration
River water (n = 4)	31 – 136 ng/L
Effluent wastewater (n = 4)	55 – 191 ng/L
Influent wastewater (n = 4)	131 – 549 ng/L
Dust samples (n = 4)	<MQL – 114 ng/g
Cod (n = 3)	14 – 57 ng/g d.w.
Hake (n = 3)	21 – 42 ng/g d.w
Sole (n = 3)	24 – 38 ng/g d.w

The concentrations for river samples ranged from 31 to 136 ng/L, for effluent wastewater samples from 55 to 191 ng/L and for influent wastewater samples from 131 to 549 ng/L. Salas *et al.* [40] analyzed similar samples six years ago and found lower levels of NH_2BT in river (31 – 43 ng/L), effluent wastewater (30 – 59 ng/L) and influent wastewater (70 – 160 ng/L) samples. Hidalgo *et al.* [41] also analyzed river samples from the same area but only quantified NH_2BT in one (31 ng/L). They did not detect the compound in the other three samples (in that study, MDL = 12 ng/L). In samples from other areas, Ao *et al.* [20] found NH_2BT in a river in Taiwan at significantly higher concentrations (3.9 to 5.1 µg/L). Moreover, Asimakopoulos *et al.* [19] analyzed wastewater samples from Athens but did not detect NH_2BT (the MDL was at low ng/L).

3.2. SPE as clean-up for organic extracts from solid samples

The high retention of NH_2BT when a clean-up of MeOH or ACN was included made us evaluate the sorbent as a clean-up step for organic extracts. Initially, MeOH and ACN were evaluated as the loading solution. The procedure consisted of a conditioning step with an organic solvent (MeOH or ACN), a loading step with 10 mL of the solvent spiked at 1 µg/L and an elution step with 5 mL of 5% NH_4OH in MeOH. NH_2BT was retained but the recoveries –54% for MeOH and 25% for ACN – were not very high (Figure 2). Since the selectivity

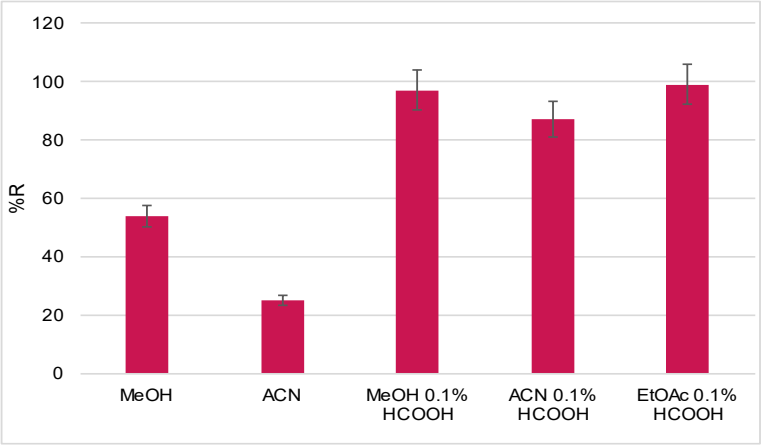

Figure 2. Recoveries obtained when different organic solvents were spiked with NH_2BT and loaded into the SiO_2-G-SAX/SCX cartridge.

was achieved thanks to the cationic exchange interactions between the sorbent and NH_2BT, 0.1% of HCOOH was added to the solvents to protonate the NH_2BT and enhance these interactions. As can be observed in Figure 2, the recoveries improved significantly when the loading solvents were acidified and were higher than 87% for all the solvents tested. Moreover, when a mixture water:MeOH (1:1, v/v) was also evaluated, the recovery was 73%. Because the addition of HCOOH is demonstrated to improve recovery, EtOAC with 0.1% of HCOOH was tested and the recovery was good (99%).

Since the tests with pure solvents provided good results, the sorbent was evaluated as a SPE clean-up for extracts from solid samples like dust or fish.

3.2.1 Analysis of indoor dust samples

For dust samples, 0.10 g of pulverized and sieved sample spiked at 1 µg/g was extracted through PLE using the method described in a previous study by Nuñez *et al.* [25]. The approximated 20 mL of EtOAc obtained after PLE was acified with 0.1% of HCOOH before the SPE clean-up.. The $\%R_{app}$ obtained after the PLE and the SPE clean-up was 30%. To improve the recovery, the PLE extract was evaporated to about 10 mL. Loading 10 mL of 0.1 %HCOOH in EtOAc extract increased the $\%R_{app}$ to 41%, as can be observed in Table 3 together with other validation parameters. So, the evaporation step was included in the procedure. The matrix effect was also evaluated and compared with the %ME obtained without the SPE. When the SPE clean-up was applied, the %ME was +5%, and when it was not, it was -62%. Therefore, SPE prevented a significant suppression of the signal. The intra-day and inter-day precision were evaluated as the %RSD (n=3) being lower than 12% in both cases. Accuracy was also evaluated as the $\%R_{rel}$, being 88%. Dust is a complex matrix, that when analyzed using LC-MS instruments tends to present high matrix effect values. For instance, Du *et al.* [44] determined aryl organophosphate esters in indoor dust and had to apply a clean-up step with Florisil to the extract of ultrasound assisted extraction. The application of the clean-up made it possible to reduce the matrix effect to levels ranging from -15 to -2%.

The use of a clean-up step with mixed-mode sorbents for PLE organic extracts has been studied very little, and mainly with commercial polymeric sorbents [14,45,46]. For instance, Romera-Torres *et al.* [45] used a commercial polymeric sorbent (Strata-X-C) for the clean-up after the PLE of tropane alkaloids from animal feed. They added the acidic moiety directly to the solvent for PLE and the SPE cartridge was eluted with a solution 3% NH_4OH in MeOH.

NH_2BT was determined in indoor dust samples collected from houses in the Tarragona region, the concentration found (Table 2) was between 32 and 114 ng/g in three samples, and in one sample, it was detected below the MQL (16 ng/g). Nuñez *et al.* [25] did not detect NH_2BT when they used GC-MS to analyze similar samples, probably due to their high MDL (1.5 µg/g). If it is possible to work with a low matrix effect,

benzothiazoles should be determined with LC-MS rather than GC-MS due to the higher sensitivity.

When Li *et al.* [26] analyzed indoor dust samples from China, they found NH_2BT in concentrations ranging from 16.5 to 142 ng/g. Wang *et al.* [24] analyzed indoor dust samples from USA, China, Korea and Japan. If detected, NH_2BT was found in concentrations between the MQL (0.5 ng/g) and 31.7 ng/g.

3.2.2 Analysis of fish samples

For fish samples, 0.10 g d.w. of sieved fish (cod for the initial trials) were spiked at 1 µg/g and the QuEChERS extraction described in section 2.3.2. was applied. Approximately 10 mL of ACN was obtained after QuEChERS. 0.1% of HCOOH was added before the SPE clean-up procedure. The $\%R_{app}$ obtained after the whole process was 31%, and the matrix effect was only -1%. This method was compared with another method developed by our research group for determining benzothiazoles in fish using LipiFiltr® for cleaning-up [39]. LipiFiltr® is a cartridge that traps lipids and fats to purify the extract and make it suitable for analysis by GC-MS or LC-MS. The $\%R_{app}$ for NH_2BT using the LipiFiltr® clean-up was 51% and the %ME was +19%. The $\%R_{app}$ obtained with the SiO_2-G-SAX/SCX sorbent was similar to the one obtained with LipiFiltr®, since the use of LipiFiltr® enhanced the signal by 19% while the use of the sorbent SiO_2-G-SAX/SCX removed practically the matrix effect (-1%). So, the proposed methodology based on the mixed-mode cation-exchange sorbent provided similar retention and lower %ME than the use of LipiFiltr®. The complexity of fish samples usually provides high matrix effects, for instance, Castro *et al.* [47] reported matrix effects reaching values between -60 to -80% when determining organophosphorus flame retardants in mussels when applying a clean-up step with Florisil. Lu *et al.* [48] applied a clean-up with graphene after QuEChERS extraction to reduce the matrix effect to less than 20% when they determined sulfonamides in fish samples.

As can be observed in Table 3, the $\%R_{app}$ was also evaluated with hake, sole and other cod samples, which have different fat content, ranging from 22% (sole) to 37% (cod). The matrix effect was also evaluated, ranging from -7% (hake) to +4% (cod). The intra-day and

inter-day precision were evaluated for all the species, and in both cases %RSD (n=3) was lower than 15%. In the case of the accuracy, the $\%R_{rel}$ was higher than 94%.

Table 3. Validation parameters for organic extracts from dust and fish samples**.**

Sample	$\%R_{app}$	%ME	Intra-day precision (%RSD, n=4)	Inter-day precision (%RSD, n=4)	$\%R_{rel}$	MDL (ng/g)	MQL (ng/g)
Fish	22 - 37	-7 - +4	8	15	94 - 96	5	12
Dust	41	+5	6	12	88	6	16

The use of mixed-mode ion-exchange sorbents for the clean-up of organic extracts from food samples has been studied very little [13,15,49]. For instance, one example is the study by Tsuruoka *et al.* [15], who extracted amantadine, rimantadine and memantine (basic pharmaceuticals) from processed chicken products and performed a clean-up step with a commercial polymeric mixed-mode sorbent (Oasis MCX) after a QuEChERS extraction. They also acidified the organic extract with 0.1% of acetic acid. The elution was also performed with a solution of NH_4OH in MeOH. The use of a mixed-mode sorbent reduced the matrix effect to remarkably low levels (±5%).

Three samples of each fish (cod, sole and hake) from Tarragona (Spain) were analyzed, and the concentration of NH_2BT was found to be between 14 and 57 ng/g d.w. (Table 2). Trabalón *et al*. [22] analyzed samples found similar concentration levels, ranging from 11 to 70 ng/g d.w., in similar fish (cod, sole and hake). Chen *et al.* [50] analyzed marketed fish (bass, billfish, tilapia and grouper) from Taiwan and did not detect NH_2BT. Moreover, when Jia *et al.* [50] analyzed molluscs from China, they found concentrations ranging from 53.3 to 93.9 ng/g d.w, slightly higher than the ones in the present study.

4. Conclusions

A mixed-mode ion-exchange sorbent based on silica modified with graphene has been successfully evaluated for the selective determination of NH_2BT. The sorbent was applied for the analysis of environmental water samples (river, effluent, and influent wastewater samples) with an optimized SPE protocol that includes 7 mL of MeOH as a washing step.

When the sorbent was tested as a clean-up of organic extracts from PLE (dust samples) and QuEChERS (fish samples). A total of 10 mL of acetonitrile and ethyl acetate extracts acidified with a 0.1% of formic acid could be loaded through the sorbent without significant losses of NH_2BT, resulting in a significant decrease of the matrix effect, showing high selectivity.

NH_2BT was determined in environmental water samples, fish and dust with low detection and quantification limits. These results are encouraging for the use of this sorbent for the selective extraction of basic compounds from matrices ranging from environmental water samples to complex matrices whose organic extracts need to be cleaned up.

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3.1.4. Application of a homemade silica-based mixed-mode ion-exchange sorbent for the determination of drugs in environmental water samples

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

Application of a homemade silica-based mixed-mode ion-exchange sorbent for the determination of drugs in environmental water samples

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Abstract

Two homemade silica-based mixed-mode ion-exchange sorbents with strong moieties were evaluated for the solid-phase extraction (SPE) of a group of pharmaceuticals, drugs of abuse and metabolites. The best performing sorbent was selected and its SPE protocol was optimized for the selective extraction of the compounds retained through cationic exchange interactions. The parameters optimized were loading pH, loading volume and washing step, and the optimal conditions were, respectively, pH 3, a variable volume depending on the matrix (river water, effluent wastewater or influent wastewater) and 2 mL of methanol.

The optimized method was validated for the analysis of water samples of environmental interest (river water, effluent wastewater and influent wastewater). Apparent recoveries ranged from 22 to 68%, matrix effect values were lower than ±20 for most compounds, and method detection limits ranged from 1 to 28 ng/L and method quantification limits from 2 to 56 ng/L. Precision was obtained as percentual relative standard deviation (RSD%, n=4) and the repeatability and reproducibility between days were always lower than 18%.

The best performing sorbent was compared with the commercial sorbents Oasis MCX, showing the new sorbent lower recoveries but lower matrix effect. The sorbent was used to determine the analytes in environmental water samples, where most compounds were found in the range from 2 to 180 ng/L in river samples, from 3 to 2547 ng/L in effluent wastewater samples and from 24 to 8086 ng/L in influent wastewater samples.

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Keywords: mixed-mode ion-exchange sorbent, homemade sorbent, drugs of abuse and pharmaceuticals, solid-phase extraction, environmental water samples.

1. Introduction

Sample preparation is one of the most important steps in the analytical procedure. This step can improve both the sensitivity of the determination, since in most cases the analytes are preconcentrated, and the selectivity by removing matrix interferences. Moreover, analytical instruments (e.g., columns, mass spectrometers) are protected due to the removal of harmful species that can damage them [1,2].

For the analysis of liquid samples, the use of sorptive techniques has replaced classic liquid-liquid extraction (LLE) and one the most representative of these techniques is solid phase extraction (SPE) [3]. Modifications to SPE have led to new techniques such as dispersive solid phase extraction (dSPE) [4] or pipette tip solid phase extraction (PT-SPE) [5]. Other relevant sorptive techniques are solid-phase microextraction (SPME) [6] and stir bar sorptive extraction (SBSE) [7] and its modifications such as stir bar sorptive-dispersive microextraction (SBSDME) [8] or stir-cake sorptive extraction (SCSE) [9]. In recent years, in compliance with the principles of Green Chemistry [10], sustainable supports such as fabric [11], wood [12] or paper [13] have been successfully explored.

The most used sorbents in SPE are polymer-based due to their good performance and the wide range of different characteristics that are commercially available. The most common ones are the Strata X (commercialized by Phenomenex) and the Oasis HLB (commercialized by Waters) [14–16]. These sorbents are characterized for their capacity to retain a wide range of compounds due to their hydrophilic-lipophilic balance. However, they can lead to low selectivity in the extraction.

The search for more selective extraction methods has provided new materials such as molecular imprinted polymers (MIPs) [17], mixed-mode ion-exchange sorbents [18] or aptamers [19]. The mixed-mode ion-exchange sorbents can perform both reversed phase and ion-exchange interactions. The compounds can engage in specific interactions (ion-exchange interactions) or non-specific interactions (hydrogen bonding, dipole-dipole, or Van der Waals forces). When a clean-up step is included, the non-specific interactions can be disrupted, rinsing the compounds retained with these interactions. Then, the compounds retained through ion-exchange interactions can be selectively eluted [18].

Mixed-mode ion-exchange sorbents can be acquired commercially. For example, Water offers the polymer-based sorbents “Oasis MCX”, “Oasis MAX”, “Oasis WCX” and Oasis “WAX”. One of the drawbacks of commercial mixed-mode ion-exchange sorbents is that they only perform one kind of interaction, either cationic (Oasis MCX and WCX) or anionic (Oasis MAX and WAX). The difference between Oasis MCX and WCX is that the former is based on strong ion-exchange interactions while the latter is based on weak ion-exchange interactions. The same occurs with Oasis MAX and WAX. There are also commercial mixed-mode ion-exchange sorbents silica-based: for example, Isolute HCX (cation-exchange) or Isolute HAX (anion-exchange), commercialized by Biotage.

A part from the commercial ones, in recent years, homemade mixed-mode ion-exchange sorbents have been developed [20]. Among these new sorbents, there are the zwitterionic mixed-mode sorbents, sorbents able to perform both cationic and anionic interactions. Few of these sorbents have been developed, either polymer-based [21–23] or silica-based ones [24–27]. Polymer-based sorbents are more stable to extreme pH, and have a greater capacity so recoveries are higher but matrix effects are too. Silica-based sorbents are easy to functionalize thanks to silanol groups in the silica network but their capacity is lower and they tend to present fewer non-specific interactions than the corresponding polymer-based ones.

Mixed-mode ion-exchange sorbents are designed to increase the selectivity of the extraction of acidic/basic compounds. Pharmaceuticals and drugs of abuse are two groups in which most compounds are acidic or basic and there is increasing concern about their presence in environment [28,29]. These compounds reach aquatic systems and have an impact on human health and aquatic life [30,31]. They are released as parent compounds or metabolites through human waste or improper disposal [29,31] and in recent years their occurrence and effects have been evaluated [28,30–33].

In the present study, two homemade silica-based sorbents were evaluated for the SPE of a group of pharmaceuticals, drugs of abuse and metabolites followed by LC-MS/MS. The protocol of the best-performing sorbent was optimized, validated and compared with Oasis MCX. Finally, the method developed was applied to determine the analytes in environmental water samples.

2. Experimental

2.1. Reagents and standards

Solid standards of nicotine (NIC), cotinine (COT), morphine (MOR), ranitidine (RAN), codeine (COD), 4-acetamidoantipyrine (4-APY), caffeine (CAF), ketamine (KET), benzoylecgonine (BE), tramadol (TRA), cocaine (COC), venlafaxine (VEN), oxazepam (OXA), methadone (MET) and diazepam (DIA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The structures and pK_a of these compounds are presented in Table S1. Standard solutions of 1000 mg/L were prepared in methanol (MeOH) and stored in the dark at -20ºC. Working solutions were prepared weekly in water/MeOH (80/20).

HPLC grade MeOH, MS grade water and MS grade acetonitrile (ACN) were acquired from Carlo Erba (Val de Reuil, France). Formic acid, hydrochloric acid, acetic acid and ammonium hydroxide were purchased from Sigma-Aldrich. Ultrapure water was obtained through a Millipore water purification system (Burlington, MA, USA).

2.2 Synthesis of the homemade mixed-mode ion-exchange sorbents

The synthesis and characterization of the homemade mixed-mode ion-exchange sorbents were deeply described in previous studies [26,27]. Briefly, SiO_2-SCX/SAX and SiO_2-G-SCX/SAX sorbents were synthesized using a sol-gel approach using tetramethyl orthosilicate, methyl trimethoxysilane, octadecyl trimethoxysilane, N-trimethoxysilyl propyl

N,N,N-trimethyl ammonium chloride, and 3-mercaptopropyl trimethoxysilane as building blocks. In the case of SiO_2-G-SCX/SAX, graphene microparticles were added to the reaction mixture. A scheme of the structures of the sorbents are presented in Figure S1. Both sorbents are based on silica modified with sulfonic groups and quaternary ammines to perform strong ion-exchange interactions and with C_{18} chains to perform reversed-phase interactions.

2.3. SPE procedure

For SPE with homemade sorbents, 200 mg of each sorbent was placed in an empty 6 mL SPE cartridge (Symta, Madrid, Spain) with two 10 µm polyethylene frits (Symta) above and below the sorbent. The sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3.

The SPE protocol optimized for river samples was a loading volume of 100 mL adjusted to pH 3 with HCl, a clean-up step with 2 mL of MeOH and an elution step with 5 mL of 5% NH_4OH in MeOH. The eluate was evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1 mL of water/ACN, (95/5, v/v). The reconstituted extracts were filtered with 0.45 µm PTFE (polytetrafluoroethylene) syringe filters (Scharlab, Barcelona, Spain). The same protocol was applied for wastewater samples, except for the loading volume, which was 50 mL for effluent wastewater and 25 mL for influent wastewater.

For Oasis MCX cartridge containing 500 mg of sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3. Then 250 mL of river water (or 50 mL of influent wastewater) adjusted to pH 3 with HCl was loaded into the cartridge. The clean-up step was 5 mL of MeOH and the elution step 5 mL of 5% NH_4OH in MeOH. The eluate was treated with the same protocol described above.

2.4. Chromatographic conditions

A liquid chromatograph Agilent 1260 Infinity II coupled to a triple quadrupole mass spectrometer detector Agilent 6460 was used. The chromatographic column was Luna Omega 5 µm Polar C18 (150 mm x 3.0 mm, 5 µm particle size) with a precolumn (3 mm x 3 mm), both acquired from Phenomenex (Torrance, CA, USA). The mobile phase was water with 0.1% of HCOOH as solvent A and ACN with 0.1% of HCOOH as solvent B. The column was thermostatized at 30º C, the flow rate was 0.4 mL/min and the injection volume was 5 µL.

The gradient profile started with 5% of B for 3 min, and then the % of B was increased to 75% in 27 min, and to 100% in 4 min. Then, it was held at 100% for 3 min before being returned to the initial conditions in 2 min, where it was held for 4 min to stabilize the column.

The electrospray ionization source conditions were optimized. The N_2 flow rate was tested between 6 and 14 mL/min, gas temperature between 200 and 400ºC, nebulizer pressure between 20 and 60 psi and capillary voltage between 2500 and 5000 V. The optimum values were N_2 flow rate of 12 mL/min, gas temperature of 340 ºC, nebulizer pressure of 40 psi and capillary voltage of 2500 V in positive mode.

For each compound, the fragmentor potential was optimized between 50 and

175 V, being 100 V the optimal value. The collision energy (CE) was also evaluated for each product ion of each compound between 0 and 30 eV, and the best conditions for each fragment were selected (Table S2). The acquisition was performed in dynamic multiple reaction monitoring (dMRM) mode, using two transitions for each compound except for tramadol.

The instrumental linear range was obtained after analyzing the solutions with concentrations from 0.01 to 250 µg/L in triplicate. In all cases, two calibration curves were used, one for low concentrations and the other for high concentrations. In both cases, the determination coefficient (r^2) was always higher than 0.99. The instrumental limits of detection ranged from 0.01 µg/L (NIC, MET or TRA) to 0.25 µg/L (CAF or VAL), and the instrumental limits of quantification ranged from 0.05 to 0.5 µg/L.

2.5. Validation parameters

The method was validated in terms of apparent recovery at two levels of concentration, matrix effect, method limits of detection and quantification, and precision (repeatability and reproducibility between days).

The $\%R_{SPE}$ was obtained as the ratio between the experimental concentration after the SPE and the theoretical concentration of the standard solutions prepared in ultrapure water. When environmental samples were used, the apparent recoveries ($\%R_{app}$) were calculated as the ratio between the experimental concentration after SPE and the theoretical concentration in the samples.

The matrix effect (%ME) was calculated with the following formula $\%ME = (C_{exp}/C_{the} \times 100) - 100$, where "$C_{exp}$" is the concentration obtained by spiking a non-spiked sample after SPE and "C_{the}" is the theoretical concentration. The signal is enhanced when %ME is positive, whereas when it is negative, it is suppressed.

The method limits of detection (MDL) and quantification (MQL) were estimated by applying the apparent recovery and the preconcentration factor to the instrumental limits due to the occurrence of some compounds in the samples.

Repeatability was obtained as the intra-day %RSD (relative standard deviation) (n=4) by analyzing samples spiked at the same concentration on the same day. The reproducibility between days was obtained as the inter-day %RSD (n=4) by analyzing samples spiked at the same concentration on different days.

3. Results and discussion

3.1. Optimization of the SPE procedure

Both homemade sorbents have been tested in previous studies. SiO_2-SCX/SAX was evaluated for the selective determination of six basic pharmaceuticals in environmental water samples [26], while SiO_2-G-SCX/SAX was applied for the determination of a group of benzothiazoles, benzotriazoles and benzenesulfonamides in environmental water samples [27] and for the selective determination of 2-aminobenzothiazole in environmental water, fish, and indoor dust samples [34].

Considering the structures and pK_a of the compounds (Table S1) and the

previous experience with the sorbents, the use of different loading pHs was evaluated. A total of 25 mL of ultrapure water standard solutions with a pH adjusted to 3, 5, 7 and 9 was percolated in both sorbents. A clean-up step of 1 mL of MeOH was included to rinse the compounds retained through non-specific interactions and increase the selectivity. The elution was performed with, consecutively, 5 mL of 5% NH_4OH in MeOH and 5 mL of 5% HAc in MeOH.

As can be seen in Table 1, the retention of compounds decreased as the pH increased, and the retention of the analytes in both sorbents was acceptable when the loading pH was 3, with results being higher than 50% in most cases. It should be pointed out that some compounds showed a significant decrease in recoveries between pH 3 and pH 5. For instance, COT had a recovery of 55% at pH 3 and 11% at pH 5 when SiO_2-G-SCX/SAX was used. And RAN had a recovery of 83% at pH 3 and 38% at pH 5 when SiO_2-SCX/SAX sorbent was used. In general, pH 3 showed the highest recoveries, so it was selected for the subsequent experiments. However, three compounds presented low SPE recoveries at all pHs: OXA, 4-APY and CAF. The clean-up portion (MeOH) was analyzed and it was observed that these three compounds were retained in the sorbent but eluted with MeOH, meaning that they were retained mainly through reversed-phase interactions instead of through ion-exchange ones. Regarding the structures and pK_a of OXA, 4-APY and CAF, these three analytes behaved as neutral compounds in the working pHs. Moreover, as CAF can present an aromatic character [35]. The π electronic density present in this compound is higher than in the other compounds, allowing it to interact better with SiO_2-G-SCX/SAX than with SiO_2-SCX/SAX, due to the π-π interactions. For this reason, the retention of CAF in SiO_2-G-SCX/SAX was better than in SiO_2-SCX/SAX, but unfortunately, π-π interactions are disrupted with MeOH. Since OXA, 4-APY and CAF did not presented ion-exchange interactions, the subsequent experiments were performed without these compounds.

Table 1. SPE recoveries of the compounds when 25 mL of ultrapure water were percolated in each sorbent at different pH.

Compound	SiO_2-G-SCX/SAX				SiO_2-SCX/SAX			
	pH 3	pH 5	pH 7	pH 9	pH 3	pH 5	pH 7	pH 9
NIC	40	44	30	31	80	65	67	62
COT	55	11	14	7	65	15	3	5
MOR	58	53	64	47	95	78	75	42
RAN	30	6	20	0	83	38	21	6
COD	63	44	57	44	94	87	76	45
4-APY	10	7	12	4	8	2	14	3
CAF	15	16	21	13	10	12	12	6
KET	30	38	27	16	77	72	52	24
BE	52	15	17	9	81	30	24	10
TRA	43	13	40	32	96	81	67	63
COC	54	8	35	21	88	55	48	33
VEN	72	65	58	47	92	84	78	62
OXA	17	12	18	10	18	10	15	9
MET	8	0	3	2	55	45	35	21
DIA	45	18	24	14	81	16	21	15

In terms of performance, SiO_2-SCX/SAX showed better SPE recoveries for the compounds. For instance, NIC and MOR showed recoveries of 80 and 95% for SiO_2-SCX/SAX respectively, and 40% and 58% for SiO_2-G-SCX/SAX. The addition of graphene allows the sorbent to perform π-π interactions. However, the presence of graphene in the sorbent inherently reduces the number of ion-exchange sites. Therefore, since the group of compounds selected does not present high π density, SiO_2-SCX/SAX should give better results than SiO_2-G-SCX/SAX and was selected for the subsequent experiments.

For the elution step, the acidic elution (5 mL of 5% AcOH in MeOH) was discarded since the analytes were retained through SCX interactions and they were rinsed with the basic elution (5 mL of 5% NH_4OH in MeOH). This protocol was consistent with that used in studies which determined basic compounds. The use of commercial mixed-mode cation-exchange sorbents has been widely reported [29,33,36–40], Oasis MCX being the most commonly used. The studies on these sorbents used an acidic pH for the loading solution, most frequently pH 2. We also decided to use an acidic pH (pH = 3), but not pH 2 since the sorbent was silica-based and there was a risk that it would be damaged by extreme pH conditions.

The clean-up step is important when working with mixed-mode ion-exchange sorbents because it increases the selectivity of the extraction. This step should remove the compounds retained through non-specific interactions (reversed-phase interactions) without removing the compounds retained through specific interactions (ionic interactions). A higher clean-up volume was evaluated (2 mL and 5 mL of MeOH). Figure 1 shows the results obtained for each clean-up protocol. The increase in

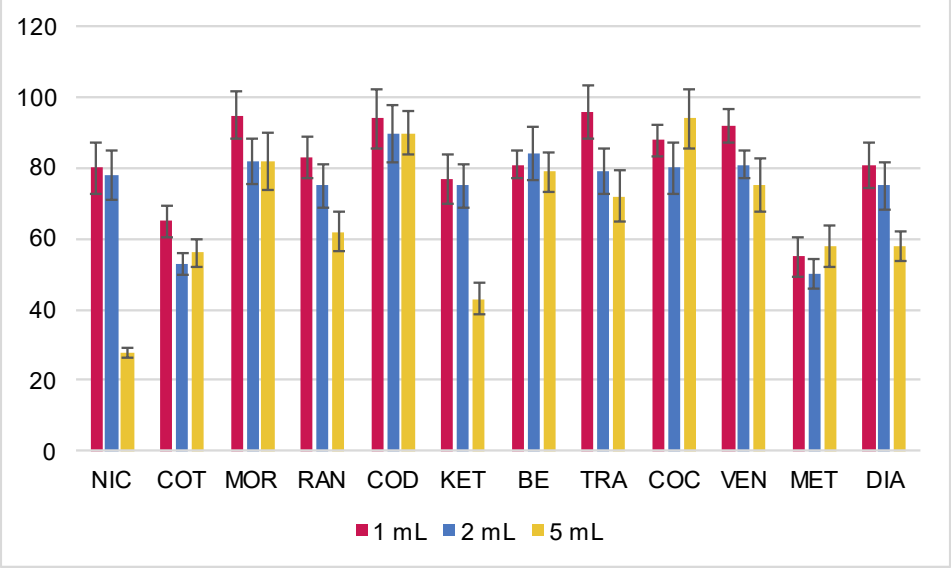

Figure 1.- SPE recoveries of the compounds when 25 mL of ultrapure water was percolated evaluating different clean-up volumes**.**

the volume of MeOH reduced the SPE recoveries for most of the compounds. When the volume increased from 1 mL to 2 mL, this reduction was slight: for instance, $\%R_{SPE}$ (TRA) decreased from 96% to 79% and $\%R_{SPE}$ (MOR) from 95% to 82%. When 5 mL of MeOH was used in the clean-up step, most of the compounds showed a slight decrease in the SPE recoveries (e.g., TRA, VEN or VEN). But compounds such as NIC, KET and RAN showed a considerable decrease (80 to 28%, 77 to 43% and 83 to 62%). Thus, 2 mL of MeOH was selected for the clean-up step. The clean-up step reported in several papers was based on water [33,37–40], and the elution step on a sequence of MeOH and basic MeOH (NH_4OH in MeOH). This led to a less selective extraction. The present study, however, takes a more selective approach in the extraction and the search for reducing matrix effects may involve a slight decrease in the recoveries.

The loading volume was evaluated with ultrapure water and was increased from 25 to 100 mL. The recoveries did not decrease more than 6% (KET) when 100 mL of ultrapure water was percolated and were higher than 60% for all compounds except MET and COT. Therefore, 100 mL was selected as the loading volume.

After optimization, the loading pH was 3, the clean-up was 2 mL of MeOH and the elution step was 5 mL of 5% NH_4OH in MeOH. The loading volume with ultrapure water was 100 mL. However, when the complexity of the samples increases, the loading volume should be reduced taking into account the breakthrough volume. In this sense, the loading of different volumes of river water, effluent wastewater and influent wastewater samples was tested. The volumes selected were 25 mL for influent wastewater samples, 50 mL for effluent

Table 2.- Apparent recoveries of the compounds at two levels of concentration and matrix effect in different matrices.

	River			Effluent			Influent		
	$\%R_{app}$		%ME	$\%R_{app}$		%ME	$\%R_{app}$		%ME
	0.1 µg/L	0.5 µg/L	0.5 µg/L	0.1 µg/L	0.5 µg/L	0.5 µg/L	0.1 µg/L	0.5 µg/L	0.5 µg/L
NIC	34	40	4	29	34	-1	-*	36	-17
COT	46	44	-5	42	39	9	32	30	1
MOR	31	25	-10	32	28	-3	24	23	-5
RAN	30	34	-10	41	49	-20	48	41	-17
COD	53	64	-10	53	60	-10	51	57	-17
4-APY	40	43	-10	34	37	-7	31	36	-8
CAF	20	14	-1	14	17	-4	22	18	-8
KET	58	65	-8	52	44	-10-	-*	38	-14
BE	41	45	-7	48	43	-8	37	34	-13
TRA	52	57	-11	65	64	-18	46	42	-12
COC	59	68	1	63	58	-5	57	53	-15
VEN	25	22	-5	32	29	-9	22	26	-28
OXA	34	40	4	29	34	-1	-*	36	-17
MET	46	44	-5	42	39	9	32	30	1
DIA	31	25	-10	32	28	-3	24	23	-5

%RSD (n = 3) < 14%.
*Natural occurrence higher than spiked concentration

wastewater samples and 100 mL for river samples.

3.2.Method validation

The method was validated for river water, effluent wastewater and influent wastewater samples in terms of apparent recovery at two levels of concentration, matrix effect, method limits of detection and quantification, and precision (repeatability and reproducibility between days). Details were presented in section 2.5.

The apparent recoveries were obtained at two levels of concentration: for river samples, 0.1 and 0.5 µg/L; for effluent wastewater samples, 0.2 and 1 µg/L; and for influent wastewater samples, 0.4 and 2 µg/L. Table 2 presents the recoveries obtained for each matrix. Most of analytes presented recoveries higher than 30%, discarding BE and DIA, the apparent recoveries ranged from 25 to 68% in river samples, from 28 to 65% in effluent wastewater samples and from 23 to 57% in influent wastewater samples. In the case of BE and DIA, the good SPE recoveries (84 and 81%) obtained with ultrapure water decreased significantly when the matrix was more complex, and the apparent recoveries ranged from 14 to 26%. The apparent recovery of NIC and TRA when the samples were spiked at 0.4 µg/L could not be obtained in influent wastewater samples because these compounds were present at levels higher than the spiked values.

As can be observed in Table 2, the %ME ranged from -11 (VEN) to +4 (NIC) in river samples, from -20 (RAN) to +9 (COT) in effluent wastewater samples, and from -17 (NIC, RAN, COD) to +1 (COT) in influent river samples, except for DIA (-28%). The sorbent proved to be selective since the matrix effect for all compounds was lower than ±20% except for DIA in influent wastewater (-28%). Yuan *et al.* [36] obtained values between -33 and +14% for KET, MOR, 4-APY, COD, COC and BE (among others) in influent wastewater samples when they used Oasis MCX.

The MDLs and MQLs are presented in Table S3. For river samples, the MDLs were lower than 7 ng/L and the MQLs lower than 15 ng/L. For effluent wastewater samples, the MDLs were lower than 17 ng/L and the MQLs lower than 34 ng/L. And for influent wastewater samples, the MDLs were lower than 28 ng/L and the MQLs lower than 56 ng/L. In all cases, the compound with the highest limit was NIC, whereas RAN, COC and MET presented limits lower than 10 ng/L in the three matrices.

In the case of precision, repeatability (% RSD, n = 4) was lower than 13% and reproducibility between days (% RSD, n = 4) was lower than 18% for all compounds in all the matrices.

3.3. Comparison with Oasis MCX

Since SiO_2-SCX/SAX behaved like a mixed-mode strong cation-exchange sorbent (SCX), it was interesting to compare it with Oasis MCX, a commercial mixed-mode SCX sorbent. The sorbent was compared for river and influent wastewater samples (being the least and the most complex samples) applying the procedures described in section 2.3. The procedure for Oasis MCX was adapted from a previous study performed in the group [41] where seven drugs of abuse and their metabolites

were determined in river, effluent wastewater and influent wastewater samples. In that study the loading volume was 250 mL for river samples and 100 mL for influent wastewater samples, 5 mL of MeOH as washing step and 15 mL of 5% NH_4OH in MeOH as elution step. In the present study, the loading volume with influent wastewater samples was reduced to 50 mL and the elution volume was reduced from to 5 mL.

Table 3 shows the apparent recoveries and matrix effect obtained when 250 mL of river water spiked at 0.5 µg/L and 50 mL of influent wastewater spiked at 2 µg/L were percolated in an Oasis MCX cartridge. Most apparent recoveries are above 40% and the recoveries in both matrices are largely similar, except for NIC (52% in river water and 13% in influent wastewater) and COT (87% and 51%). As expected, the matrix effect is higher in influent wastewater samples because of their complexity, except for NIC, RAN, KET and COC.

The results obtained for Oasis MCX can be compared with the ones obtained for SiO_2-SCX/SAX (Table 2) even though the amount of sorbent and the loading volume were not the same. For Oasis MCX and SiO_2-SCX/SAX, the amount of sorbent was 500 mg and 200 mg, respectively. Likewise, for Oasis MCX the loading volume was 250 mL and 50 mL for river water and influent wastewater, respectively, and for SiO_2-SCX/SAX it was 100 and 25 mL. As far as the results of river samples are concerned, the recoveries with Oasis MCX were higher than the ones with SiO_2-SCX/SAX. These differences were more pronounced with compounds like MOR (91% with Oasis MCX and 25% with SiO_2-SCX/SAX) or BE (81% and 20%). Moreover, in terms of the matrix effect, SiO_2-SCX/SAX showed better results than Oasis MCX, with the %ME being lower in almost all compounds. The

Table 3.- Apparent recoveries and matrix effect of Oasis MCX for river (0.5 µg/L) and influent wastewater (2 µg/L) samples

Compound	River (250 mL)		Influent wastewater (50 mL)	
	$\%R_{app}$	%ME	$\%R_{app}$	%ME
NIC	52	-23	13	-20
COT	87	-12	51	-46
MOR	91	-6	87	-32
RAN	79	+13	88	-3
COD	100	-5	87	-35
4-APY	81	+16	82	-34
CAF	45	+30	68	-12
KET	84	+14	78	-25
BE	79	-34	72	-33
TRA	22	+10	27	-26
COC	76	+16	54	-18
VEN	72	+8	62	-24
OXA	52	-23	13	-20
MET	87	-12	51	-46
DIA	91	-6	87	-32

%RSD (n = 3) < 14%.

greatest differences were NIC (-23% with Oasis MCX and +4% with SiO_2-SCX/SAX), BE (+30% and -1%) and COC (-34% and -7%).

In influent wastewater samples, the recoveries with Oasis MCX are again higher than the recoveries with SiO_2-SCX/SAX (except for NIC). The biggest differences were MOR (87% with Oasis MCX and 23% with SiO_2-SCX/SAX), BE (68% and 18%), KET (82% and 36%) and DIA (62% and 26%). The matrix effect was also lower with SiO_2-SCX/SAX in most cases. COT (-46% with Oasis MCX and +1% with SiO_2-SCX/SAX), MOR (-32% and -5%) and KET (-34% and -8%) were the highest differences.

It should be highlighted that SiO_2-SCX/SAX is silica-based and Oasis MCX is polymer-based. As mentioned in the introduction, polymer-based sorbents tend to have a greater capacity, showing higher recoveries while the silica-based sorbents tend to perform fewer non-specific interactions, showing lower matrix effect. The results confirmed this. Oasis MCX presented higher recoveries while SiO_2-SCX/SAX presented a lower matrix effect, so SiO_2-SCX/SAX proved to be more selective than Oasis MCX.

There are other studies where Oasis MCX was used for the determination of similar compounds. For instance, in the study previously mentioned [41], performed by our group, the apparent recoveries for MOR, COC, COD and MET ranged from 82 to 154% for river samples and from 55 to 128% in influent wastewater samples. However, the matrix effect observed was significantly higher, ranging from +8 to +41% in river samples and from -20 to -57% in influent samples. In fact, the influent samples were diluted 1:5 to reduce the matrix effect.

There are more studies where these analytes were determined using Oasis MCX. For example, Wang *et al.* [40] obtained recoveries ranging from 85 to 111% for COC, MOR and COD in river samples, analyzing both MeOH and basic MeOH extracts. Krizman *et al.* [38] also analyzed MeOH and basic MeOH to determine MOR, COD, TRA, MET and 4-APY (among others), and obtained recoveries ranging from 73 to 109% in river water, from 73 to 116% in effluent

Table 4.- Range of concentrations (ng/L) obtained after the analysis of river, effluent wastewater and influent wastewater samples (n=4).

Compound	River (100 mL)	Effluent wastewater (50 mL)	Influent wastewater (25 mL)
NIC	54 – 180	160 – 310	956 - 8086
COT	10 – 13	20 – 36	160 - 1453
MOR	<MQL - 15	12 - 51	323 - 383
RAN	<MDL	8 - 10	25 - 103
COD	9 – 13	20 -72	66 - 153
KET	<MDL	28 – 58	24 - 127
TRA	40 - 80	1311 - 2547	1058 - 2166
COC	2 - 14	3 - 25	408 - 1222
VEN	15 - 30	234 - 697	373 – 1245
MET	2 - 14	99 - 210	84 – 189
DIA	<MDL - 7	38 – 72	54 -428

wastewater and from 79 to 97 in influent wastewater samples. It should be pointed out that both studies used deuterated compounds as internal standards, then the relative recoveries are reported.

3.4. Analysis of environmental samples

Four samples from river water (Ebro River, Spain), effluent wastewater (Tarragona, Spain) and influent wastewater (Tarragona, Spain) were analyzed with the optimized method. The analytes were quantified by applying the external calibration curves described above and taking into account the $\%R_{app}$ and the dilution factor. It was decided not to use the matrix-matched calibration curves since the matrix effects calculated (Table 2) were low and the natural occurrence of the compounds in the samples.

Table 4 shows the concentrations obtained. In the river samples, all compounds were detected except for RAN and KET in all samples and DIA in two. NIC had the highest occurrence, with concentrations from 54 to 180 ng/L. The concentration of TRA was also high, ranging from 40 to 80 ng/L. Wang *et al.* [40] quantified COC, MOR and COD in river samples from China. MOR was not detected, COC was found between 0.05 and 13 ng/L, similar to the concentration observed in the present study (2 – 14 ng/L), while COD was found in lower concentrations (0.8 – 1.8 ng/L versus 9 – 13 ng/L). Brieudes *et al.* [42] quantified TRA, MET, COD, COC and VEN in samples from the Seine River (France). VEN (3 – 27 ng/L), COC (MQL – 8 ng/L), COD (3 – 14 ng/L) and TRA (12 – 90 ng/L) were quantified at similar levels, while the concentration of MET was slightly lower (0.5 – 1.8 ng/L). RAN and VEN were quantified in similar samples from the Ebro River in a previous study [26], where RAN occurrence ranged from 24 to 32 ng/L and VEN occurrence ranged from 32 to 72 ng/L.

In the effluent wastewater samples, all compounds were quantified. The compound found at the highest concentration was TRA (1311 – 2547 ng/L) followed by VEN (234 – 697 ng/L) and NIC (160 – 310 ng/L). The concentration of the other compounds was lower than 100 ng/L (except MET). Gómez-Canela *et al.* [43] determined RAN, TRA, VEN and DIA (among others) in effluent wastewater samples from Barcelona (Spain). TRA was also the most occurring compound (141 – 1200 ng/L), VEN and DIA were observed at similar concentrations (104 – 820 ng/L and 52 – 100 ng/L) and RAN was detected at higher levels (26 – 97 ng/L). Masemola *et al*. [33] determined MOR and COC (among others) in effluent wastewater samples from Florida (USA) at significantly higher concentrations of 65 – 142 ng/L and 265 – 328 ng/L, respectively. It should be highlighted that the differences between concentrations from different origins may be due to the influent water treatment carried out at wastewater treatment plants.

In influent wastewater samples, all compounds were quantified in all samples. Moreover, some compounds were quantified in concentrations higher than 1 µg/L. This was the case of NIC and TRA in all samples and COT, COC and VEN in some. Krizman et al. [38] determined MOR, COD, TRA and MET (among others in influent wastewater samples from Split, Croatia). The concentration of TRA was significantly lower (586 – 717 ng/L) while the

concentration of COD was higher (237 – 625 ng/L), and the concentration of MOR and MET was similar (142 – 445 and 71 – 94 ng/L). Gilart *et al.* [44] determined MOR, RAN, COD, MET and COC in samples from the same waste water treatment plant (Tarragona, Spain). The concentration of RAN was significantly higher (1028 – 3293 ng/L), and the concentration of COC was slightly lower (163 – 500 ng/L). The concentrations of MOR, COD and MET were similar. It can be unexpected that in some cases, such as TRA or MET, the concentrations in effluent are similar or higher than the concentrations found in influent samples. It can be explained since the samples analyzed were collected in different periods of time, so the effluent and the influent were not related.

4. Conclusions

When two novel silica-based homemade materials were compared for the solid-phase extraction of a mixture of basic pharmaceuticals, drugs of abuse and metabolites, SiO_2-SCX/SAX performed better than SiO_2-G-SCX/SAX. A method based on SiO_2-SCX/SAX was succesfully developed for the selective extraction of these analytes and it was optimized by exploiting the strong cation-exchange (SCX) interactions.

Good validation parameters were obtained for different types of sample (river water, effluent wastewater and influent wastewater). The homemade sorbent, SiO_2-SCX/SAX, showed higher selectivity (lower matrix effect) but lower capacity (lower recoveries) than Oasis MCX.

The methodology developed for SiO_2-SCX/SAX was successfully applied to determine the analytes in river water, effluent wastewater and influent wastewater samples.

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Suplementary data

Table S1. Structures and pK_a of the analytes.

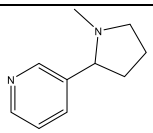
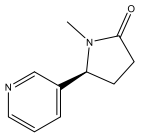
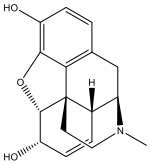
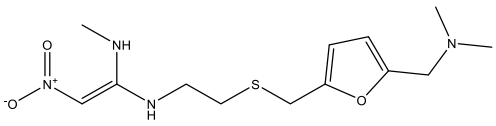
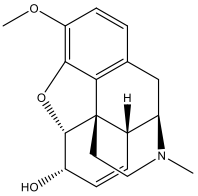
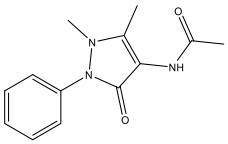
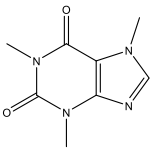
Compound	pK_a	Structure
Nicotine	8.1	
Cotinine	4.8	
Morphine	8.2	
Ranitidine	7.8	
Codeine	8.2	
4-acetamido-antipyrine	12.8	
Caffeine	14	

Table S1. Structures and pK_a of the analytes (cont.).

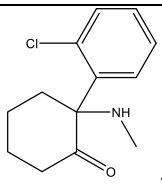
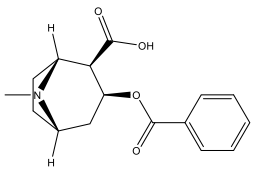
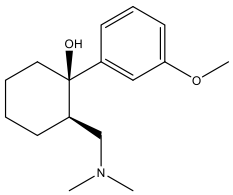
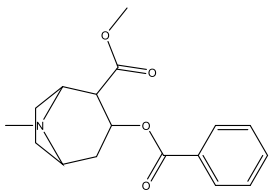
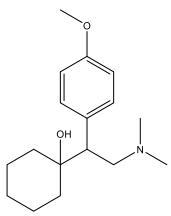
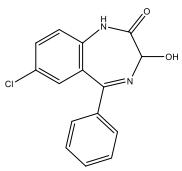
Ketamine	7.5	 .1
Benzoylecognine	8.6	
Tramadol	9.2. 13.1	
Cocaine	8.7	
Venlafaxine	9.5	
Oxazepam	1.5, 10.9	

Table S1. Structures and pK_a of the analytes (cont.).

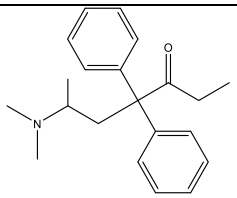
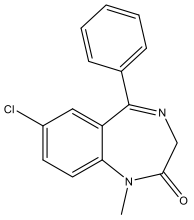
Methadone	9.2	
Diazepam	3.4	

Table S2. Precursor and product ions and collision energies for each compound.

Compound	Precursor ion (m/z)	Product ion 1 (m/z)	Collision energy 1 (eV)	Product ion 2 (m/z)	Collision energy 2 (eV)
NIC	163.1	130	25	117	30
COT	177.1	98.1	20	146.1	20
MOR	286.1	165	40	153.1	40
RAN	315.1	176	15	270.1	10
COD	300.1	215.1	30	199	30
4-APY	246.1	104	20	159	20
CAF	195.1	138	20	110	25
KET	238.1	207	10	179	10
BE	290.1	168.1	15	105	20
TRA	264.2	58.2	20	-	-
COC	304.1	182.1	15	150.1	20
VEN	278.2	147	20	215.1	20
OXA	287	241	15	269	10
MET	310.2	265.1	10	105	30
DIA	285.1	193.1	30	222.1	25

Table S3. Method quantification limits (MQL) and method detection limits (MDL) for each analyte in river, effluent and influent wastewater samples in ng/L.

Compound	River (100 mL)		Effluent wastewater (50 mL)		Influent wastewater (25 mL)	
	MDL	MQL	MDL	MQL	MDL	MQL
NIC	7	15	17	34	28	56
COT	2	5	5	12	13	31
MOR	2	3	3	6	8	17
RAN	2	3	2	5	4	8
COD	5	9	9	19	20	39
KET	1	3	3	6	6	13
TRA	1	2	2	4	5	11
COC	1	2	1	2	1	5
VEN	5	10	8	15	22	43
MET	1	2	2	3	4	7
DIA	2	4	3	6	9	18

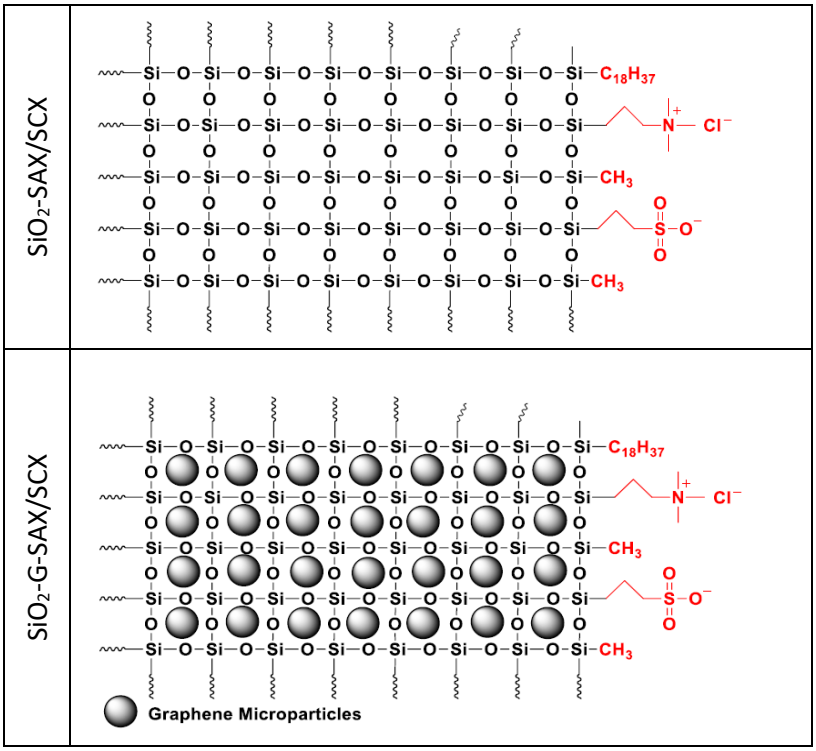


Figure S1. Structure of the homemade silica-based sorbents.

3.1.5. Introduction of zwitterionic functional groups in silica sorbent for the simultaneous determination of acidic and basic pharmaceuticals

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

Application of a homemade silica-based mixed-mode ion-exchange sorbent for the determination of drugs in environmental water samples

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Abstract

Three homemade mixed-mode zwitterionic ion-exchange silica-based materials were synthesized through a sol-gel approach. Three different zwitterionic additives were used to introduce sulfonic groups and quaternary amines, to perform strong cation- and strong anion-exchange interactions.

The sorbents were evaluated for the solid-phase extraction (SPE) of basic and acidic pharmaceuticals, and the sorbent functionalized with 2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide was the best performing one. The optimal conditions for SPE were pH 5, a variable volume depending on the matrix (100 mL for river samples, 50 for effluent wastewater samples and 25 mL for influent wastewater samples) and elution with 5 mL of 1% NH_4OH in MeOH.

The method validation was carried out attending to apparent and relative recoveries, matrix effect, intra-day and inter-day precision and method detection and quantification limits. It can be highlighted that apparent recoveries were higher than 30% for most compounds and method detection limits were in the range of low ng/L. The method was applied to determine the pharmaceuticals in several river, effluent wastewater and influent wastewater samples.

Keywords: zwitterionic functional group, silica-based sorbent, mixed-mode ion-exchange interactions, pharmaceuticals, environmental water samples.

1. Introduction

Traditionally there have been two main types of extraction sorbents for sorptive extraction: organic polymer-based sorbents and inorganic silica-based sorbents. Organic polymer-based sorbents have a wider pH tolerance and may suffer swelling effects when exposed to organic solvents [1]. On the other hand, silica-based sorbents have high organic solvent resistance and mechanical stability but may be damaged at extreme pH [2]. One of the main advantages of silica-based sorbents is the ease to modify to introduce a wide variety of functional groups such as quaternary amines [3,4], humic acids [5], or aliphatic chains (C_{12}, C_{18}) [6,7].

Sorbents applied in extraction techniques are classified depending on the interactions they perform with the analytes, like reversed-phase, hydrophilic-lipophilic balance, or ion-exchange interactions. If they can perform more than one kind of interaction, they are considered mixed-mode sorbents, being the most common ones, the sorbents that combine ion-exchange and reversed-phase interactions [8].

Mixed-mode ion-exchange sorbents are classified depending on the type of ion-exchange interaction they perform, being anion exchangers if they retain anionic compounds or cation exchangers if they retain cationic compounds. The strong anion-exchange (SAX) sorbents are functionalized with quaternary amines and the weak anion-exchange (WAX) sorbents are functionalized with primary or secondary amines. In the same way, strong cation-exchange (SCX) sorbents and weak cation-exchange (WCX) sorbents are mainly functionalized with sulfonic and carboxylic acid groups, respectively to retain anionic compounds [8].

Mixed-mode ion-exchange commercial sorbents have been developed, being “Strata” and “Oasis” brands the most commonly used. Although these two are polymeric based, there are also mixed-mode ion-exchange sorbents based on silica like the “Isolute” brand. Aside from the commercial ones, homemade sorbents have been developed in the recent years such as polymer-based materials with cation-exchange [9] and anion-exchange moieties [10,11]. In the same way, silica-based materials have been also developed with cation-exchange [12] and anion-exchange [3,13,14] moieties. All of them have been widely applied with good performance; however, they are only functionalized with one type of ion-exchanger moiety, so they are only capable of extracting one type of compounds, either cationic or anionic ones.

Moreover, some homemade sorbents have been synthesized with both anion- and cation-exchange moieties, so they are zwitterionic exchangers. There are examples of polymer-based sorbents [15–17] and silica-based sorbents [18–21]. For instance, the study performed by Wang *et al.* [20] functionalized silica with primary amines and carboxylic acid groups to extract acidic and basic analytes simultaneously from soft drinks thanks to the dual functionalization. In that study, the performance was compared with Oasis WAX and WCX, showing similar extraction yields but using only one sorbent.

The mixed-mode ion-exchange sorbents are designed to retain ionizable compounds, compounds with acidic or basic behavior. An example of such compounds includes pharmaceuticals, whose determination in environmental samples has attracted growing interest. Pharmaceutical pollution is a problem with increasing concern [22] because the presence of pharmaceuticals in surface waters pose substantial risks for public health and aquatic ecosystems [23,24]. Therefore, the quantification of pharmaceuticals in surface and wastewater water samples is relevant to evaluate the presence of these pollutants [25].

In the present study, three homemade mixed-mode zwitterionic ion-exchange sorbents have been synthesized, characterized and evaluated for the solid-phase extraction (SPE) of basic and acidic pharmaceuticals. The optimized method was applied for the determination of acidic and basic pharmaceuticals in river, effluent wastewater, and influent wastewater samples through liquid chromatography with high resolution mass spectrometry detection (LC-HRMS).

2. Experimental

2.1. Reagents and standards

Zwitterionic functional groups 2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide, 3-(N,N-Dimethylmyristylammonio) propane sulfonate, and 3-Benzyldimethylammoniopropanesulfonate; and reagents used for synthesis: dichlormethane, acetone, trifluoroacetic (TFA), dichloromethane, methyltrimethoxysilane (MTMS) and tetramethylortosilicate (TMOS) were acquired from Sigma–Aldrich (St. Louis, MO, USA). NaOH and HCl were acquired from Thermo Fisher Scientific (Milwaukee, WI, USA). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Fisher Scientific (Steinheim, UK). A Milli-Q purification system (Millipore, Bedford, MA, USA) was employed to obtain ultrapure water. MS grade water and ACN were purchased from Carlo Erba (Val de Reuil, France). Ammonium hydroxide, acetic acid and formic acid were acquired from Sigma-Aldrich.

Solid standards of five acidic pharmaceuticals: valsartan (VAL), diclofenac (DIC), clofibric acid (CLO), fenoprofen (FEN) and bezafibrate (BEZ), and five basic pharmaceuticals: propranolol (PRO), venlafaxine (VEN), metoprolol (MTO), trimethoprim (TRI) and atenolol (ATE) were purchased as pure standard (purity >96%) from Sigma-Aldrich. Standard solutions of 1000 µg/L were prepared in MeOH and stored in the freezer (-20 ºC) in dark bottles. Working solutions were prepared weekly in water:MeOH (90/10, v/v) and stored in the refrigerator at 4 ºC in the dark.

2.2 Preparation of sol-gel Carbowax 20M/zwitterionic composite sorbents

Sol-gel Carbowax 20M/ZW composite sorbents were prepared using the following pre-synthesis, synthesis and post-synthesis processing steps: (a) hydrolysis of sol-gel precursors; (b) preparation of aqueous zwitterionic additives (presented in Table 1) solution; (c) dissolving Carbowax 20M (CW20M) polymer in hydrolyzed sol solution, addition of zwitterionic additive solution

and initiating condensation process by adding methanolic ammonium hydroxide to the sol solution; (d) aging and conditioning of the bulk sol-gel composite monolith; (e) crushing, grinding and drying of sol-gel CW20M/ZW composite sorbent; (f) disintegrating dry sol-gel CW 20M/ZW composite sorbent into fine microparticles using mortar and pestle; (g) cleaning sol-gel CW 20M/ZW composite sorbent in methanol: dichloromethane (50:50 v/v) in a sonicator for 30 min; and (h) drying sol-gel CW 20M/ZW composite sorbent at 100 °C for 8 h.

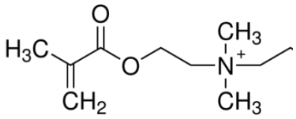
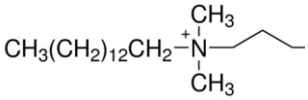
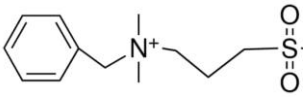
2.3. Instruments and equipment

For synthesis, an Eppendorf Centrifuge Model 5415R (Eppendorf North America Inc., Hauppauge, NY, USA) was used to obtain particle free sol solutions. A Fisher Scientific Digital Vortex Mixer (Fisher Scientific, Pittsburg, PA, USA) was employed for the thorough mixing of different solutions. A 2510 BRANSON Ultrasonic Cleaner (Branson Ultrasonics, Danbury, CT, USA) was used to prepare a bubble-free sol solution.

For characterization, PerkinElmer Spectrum 100 Fourier transform infrared (FT-IR) Spectrometer equipped with Universal ATR Sampling Accessory (PerkinElmer Inc., Akron, OH, USA) was used to perform FT-IR characterization of sol-gel composite sorbents and a Philips XL 30 Scanning Electron Microscope (SEM) equipped with an EDAX detector was employed to obtain SEM images of the sol-gel sorbents.

For chromatographic analysis, a liquid chromatograph (LC) Agilent 1100 series equipped with a diode array detector (DAD) (Agilent, Waldbronn, Germany) was used for the optimization of the extraction method. When working with samples, an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) was used. The chromatograph was equipped with an Accela Autosampler automatic injector and a quaternary pump. The LC system was coupled to an Exactive Orbitrap mass spectrometer from Thermo Scientific mass spectrometer. The mass spectrometer was equipped with a heated electrospray ionization (HESI) source and a high-energy collision dissociation (HCD) cell.

Table 1. Name, zwitterionic additives and their structures

Name	Zwitterionic additives	Structure of the functional group
CW 20M/ZW-1	2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide	
CW 20M/ZW-2	3-(N,N-Dimethylmyristylammonio) propane sulfonate	
CW 20M/ZW-3	3-Benzyldimethyl-ammoniopropane sulfonate	

2.4. SPE procedure

To prepare the SPE cartridges, 150 mg of the corresponding sorbent were packed on a SPE cartridge (Symta, Madrid, Spain) between two 10 µm polyethylene frits (Symta). Before loading the sample, 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 5 were used to condition it. After conditioning the sorbent, a volume of sample (100 mL for river samples and 50 mL for effluent and 25 mL for influent wastewater samples) adjusted to pH 5 was loaded into the cartridge at an approximated flow rate of 10 mL/min. The elution step consisted of passing 5 mL of 1% NH_4OH in MeOH, that was evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1 mL of water/ACN (95/5, v/v). Before SPE, all samples were filtered with a 0.45 µm nylon membrane filter (Scharlab). Moreover, wastewater samples were previosuly filtered with a 1.2 µm glass-fibre membrane filter (Fisher brand, Loughborough, UK).

2.5. Chromatographic conditions

The chromatographic column selected was a Luna Omega Polar C_{18} 100 (150 mm x 3.0 mm, 5 µm particle size) equipped with a precolumn (4 x 3 mm, 5 µm particle size) with the same filling supplied by Phenomenex (Torrance, CA, United States). The injection volume was 20 µL, the flow rate 0.4 mL/min and the column oven was set at 30 ºC. The mobile phase was formed by ultrapure water adjusted to pH 3 with HCl (solvent A) and ACN (solvent B). The gradient began with 5% of B, increasing the % of B to 60% within 6 min, then it was increased to 100% of B within 6 min, where it was held for 3 min before returning to the initial conditions in 1 min. The initial conditions were maintained for 3 min to stabilize the column. Two different wavelengths were used: ATE, MTO, DIC and BEZ were measured at 230 nm; TRI, VEN, PRO, VAL and CLO were measured at 210 nm.

In the case of LC-HRMS conditions, “solvent A” was water with 0.1% of HCOOH and the rest of chromatographic conditions were the same as LC-DAD. Basic analytes were determined in positive ionization mode with the following conditions and acidic analytes were determined in negative ionization mode. Both conditions are presented in Table S1.

Two windows were used for data acquisition, the first one for basic compounds in positive ionization mode and the second one for acidic compounds in negative ionization mode. Each window had two alternative scan events with a range of 50 – 450 m/z. The first of them was a full scan at 50,000 FWHM (Full Width Half Maximum) and 250 ms for injection time. The second event consisted of a fragmentation scan at 10,000 FWHM with a collision voltage of 20 eV and 50 ms for the injection time in both windows. Table S2 shows the mass of the selected ions and their fragments and the ratios.

3. Results and discussion

3.1. Sol-gel sorbent synthesis

Sol solution was prepared in a 100 mL polypropylene reaction vessel by sequentially adding MTMS, TMOS, and MeOH into the nickel vessel. The mixture was vortexed for 3 minutes. Subsequently, 0.1 M HCl was added to begin the hydrolysis. Hydrolysis of the sol

solution continued for 8 h at ambient laboratory conditions.

Zwitterionic additives, 2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide for ZW-1, 3-(N,N-Dimethyl myristylammonio)propane sulfonate for ZW-2 or 3-Benzyldimethylammoniopropane sulfonate for ZW-3, were accurately weighed in a 50 mL centrifuge tube. Deionized water was added to the zwitterionic additive, then the solution was vortexed until complete dissolution.

Carbowax 20M (CW 20M) polymer and zwitterionic additive solution were then added to the hydrolyzed sol solution, to facilitate the integration, the mixture was vortexed (3 min) and sonicated (30 min). Subsequently, 1 M NH_4OH was added to the sol solution and briefly sonicated to remove any trapped air bubbles. The molar ratio between TMOS:MTMS:CW 20M:Zwitterionic additive:MeOH:HCl (0.1 M) and NH_4OH (1 M) in the sol-gel sorbent were maintained at 1:1:0.15:0.2:30:8:10.

The solution formed a transparent monolithic bed in an hour. The monolithic sol-gel bed was conditioned and aged at 50 °C for 24 h. The aged and conditioned monolithic bed was crushed and transferred into a borosilicate glass drying vessel and dried at 100 °C for 12 h. Subsequently, the dried sol-gel CW20M/ZW composite sorbent was disintegrated into fine microparticles in a mortar and pestle. The fine particles were then rinsed with MeOH/dichloromethane (50:50 v/v) under sonication for 30 minutes. The sol-gel CW 20M/ZW particles were then filtered and dried at 100 °C for 8 h.

3.2. Characterization of sorbents

The three sorbents were characterized through Fourier transform infrared spectroscopy (FT-IR spectroscopy); scanning electron microscopy (SEM); and elemental analysis using scanning electron microscopy-energy dispersibe spectroscopy (SEM-EDS). The characterization of CW 20M/ZW-1 sorbent is discussed in the main text while the characterization of the two other sorbents (CW 20M/ZW-2 and CW 20M/ZW-3) are included in Supplementary Material.

3.2.1. Fourier transform infrared spectroscopy

FT-IR spectra of the major functional building blocks of sol-gel is presented in Figure 1 (a-d). The FT-IR spectra of CW 20M is presented in Figure 1 (a). The most representative bands correspond to the alkyl chain of the polymer (2883 cm^{-1}), the C-H bending vibration (1342 cm^{-1}), the C-H twisting vibration (1240 cm^{-1}) and the C-O stretching vibration (1091 cm^{-1}) [26,27].

The FT-IR spectrum of MTMS is presented in Figure 1 (b). The presence of Si-CH3 from MTMS can be confirmed by the bands at at 1276 cm^{-1}, 833 cm^{-1} and 791$cm^{-1,}$ meanwhile the bands at 1188 cm^{-1} and 1074 cm^{-1} are from Si-OCH_3 in MTMS [26,28]

The FT-IR spectrum of the zwitterionic additive, 2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide is presented in Figure 1 (c). The most representative bands from the additive are C=O bond in the ester group (1716

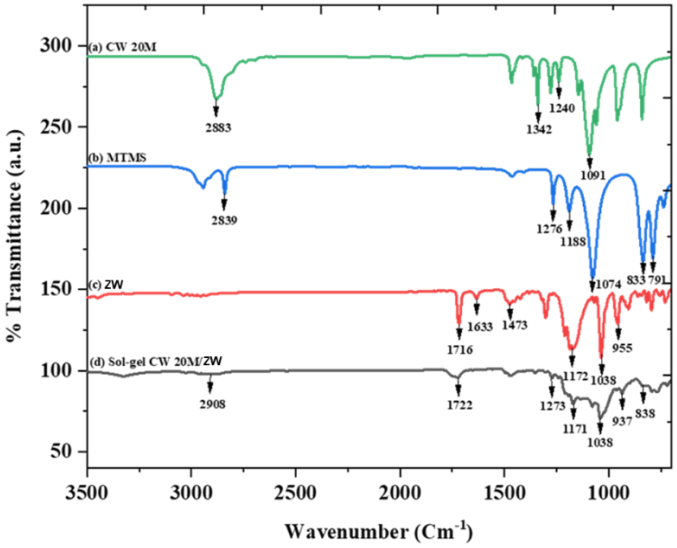


Figure 1. FT-IR spectra of (a) pristine Carbowax 20M polymer; (b) methyl trimethoxysilane (MTMS); (c) 2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide; (d) sol-gel Carbowax 20M/2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide composite sorbent.

cm^{-1}), the stretching vibration of $C\text{-}N^{+}$ (1633 cm^{-1}) and the symetrical (1038 cm^{-1}) and asymmetrical (1165 cm^{-1}) stretching vibration of S=O in the sulfonate group [29,30].

The FT-IR spectrum of sol-gel CW 20M/ZW-1 is presented in Figure 1 (d). Many peaks in the FT-IR spectrum of sol-gel CW 20M/ZW-1 also appeared spectra presented in Figure 1 (a – c). The peak at 2908 cm^{-1} may correspond to the mentioned peak from CW 20M to the alkyl chain of the polymer (2883 cm^{-1}), the peaks at 1722 cm^{-1} may correspond to the C=O bond in the ester group from the zwitterionic additive (1716 cm^{-1}) and the peak at 1171 cm^{-1} may correspond to the $Si\text{-}OCH_3$ band from MTMS spectrum (1188 cm^{-1}). Since several peaks from the building blocks appear in the FT-IR spectrum from the final product, the successful integration of the building block components in the sol-gel composite sorbent can be considered achieved.

3.2.2. Scanning electron microscopy

SEM images of sol-gel Carbowax 20M/ZW-1 sorbent can be observed Figure 2 (a-b). It can be seen in the SEM image at 500x magnifications (Figure 2a) the polydispersed particle sizes, that facilitates the packing in the SPE cartridge. Figure 2b presents the SEM image at 10,000x magnifications, that reveals the sponge-like microstructure of sol-gel sorbent.

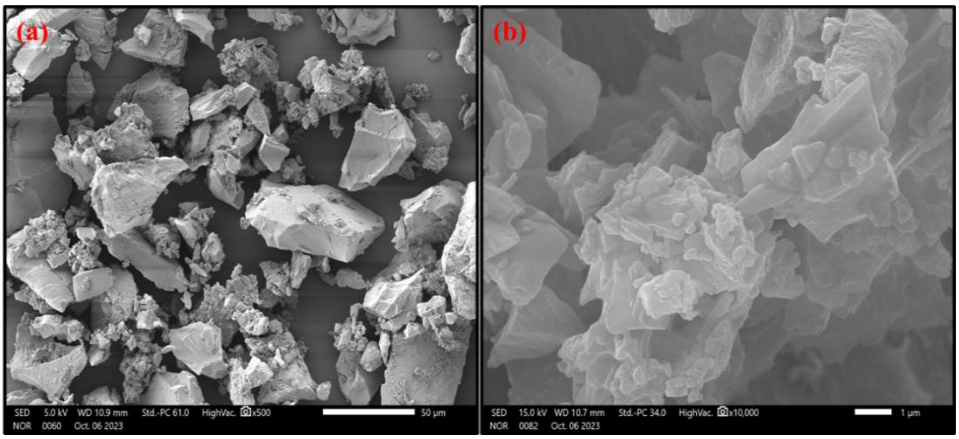


Figure 2. Scanning electron microscopy images of sol-gel Carbowax 20M/2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide composite sorbent at (a) 500x magnifications; (b) 10,000x magnifications.

3.2.3. Scanning electron microscopy-energy dispersive spectroscopy

SEM-EDS was applied to estimate the elemental composition of the sol-gel Carbowax 20M/ZW-1 composite sorbent. The SEM image and EDS spectra are presented in Figure 3 (a-b). As the data revealed (inseeted table), the primary components include C (38%), O (33%), Si (22%), S (5%) and N (2%). The presence of S and N reveals that the zwitterionic additive has been successfully integrated in the silica structure.

3.3. Evaluation of the three sorbents and evaluation of the SPE protocol

The three synthesized sorbents (CW 20M/ZW-1, CW 20M/ZW-2, and CW 20M/ZW-3) were evaluated for the SPE of the selected 5 acidic (CLO, BEZ, VAL, FEN and DIC) and 5 basic (ATE, TRI,

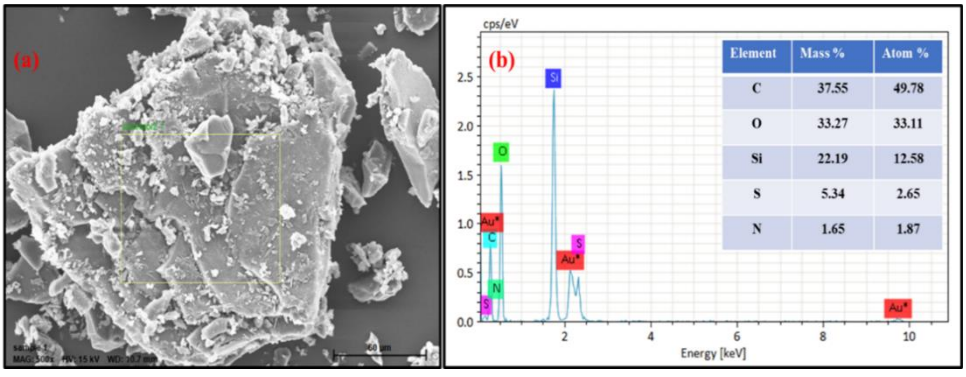


Figure 3. Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) images of sol-gel Carbowax 20M/2-(methacryloxy)ethyldimethyl-3-(sulfopropyl)ammonium hydroxide (a) SEM image; (b) EDS graph with elemental composition in the inset.

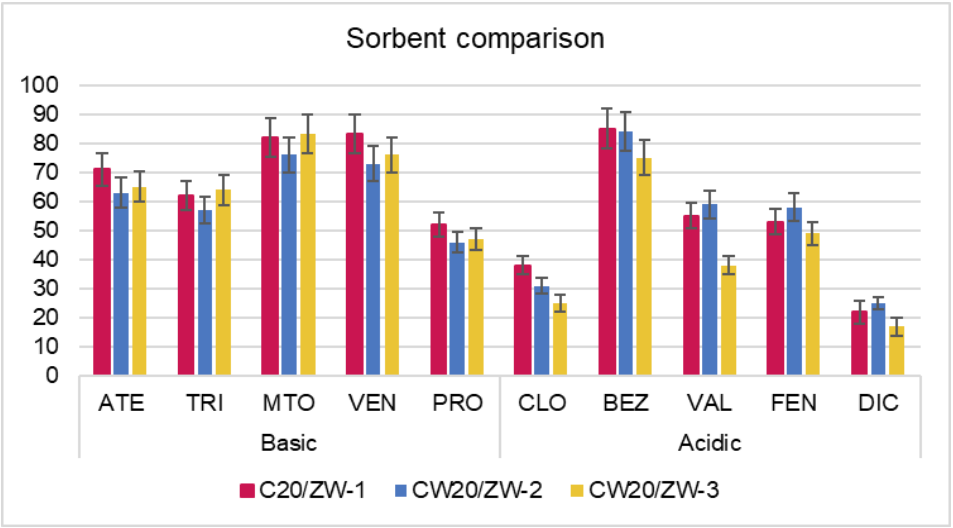


Figure 4. Recoveries obtained when 25 mL of ultrapure water at pH 5 was loaded in the three different sorbents.

MTO, VEN and PRO) pharmaceuticals. Based on previous experience with zwitterionic sorbents [18] and the pK_a of the analytes (Table S2), the initial conditions selected were a 25 mL of ultrapure water as loading step and 5 mL of 5% NH_4OH in MeOH followed by 5 mL of 5% CH_3COOH in MeOH as elution step. The three sorbents were evaluated with solutions adjusted at pH 3, 5, 7 and 9 to choose both the best performing sorbent and the optimal pH at the same time. $\%R_{SPE}$ was used to determine the best conditions and was calculated diving the concentration obtained after the SPE by the theoretical concentration of the solutions prepared in ultrapure water.

Figure 4 shows the $\%R_{SPE}$ obtained for the three sorbents at pH 5 and Figures S5, S6 and S7 show the $\%R_{SPE}$ at pH 3, 7 and 9, respectively. It can be observed that the three sorbents provided acceptable values (>50%) for most of the compounds, except for CLO and DIC. Because CW 20M/ZW-1 presented the highest values for all the basic compounds and for some of the acidic ones (CLO and BEZ) at any pH. It was selected as the best performing sorbent.

For comparative purposes, Figure 5 shows the $\%R_{SPE}$ at pH 3, 5, 7 and 9 for CW 20M/ZW-1. As it can be observed in this figure, the optimal pH for the acidic compounds was pH 3, meanwhile the best pH for the basic ones was pH 5. However, for the basic analytes, the retention of ATE, MTO and PRO at pH 3 were significantly lower than at pH 5, meanwhile, for the acidic ones, the differences between pH 3 and pH 5 were only remarkable for VAL ($\%R_{SPE}$ of 75% at pH 3 and 55% at pH 5). Therefore, the working pH selected was 5. Wang et al. [20] also chose an acidic pH (pH 6) when determining acidic and basic compounds in soft drinks with a mixed-mode zwitterionic exchange silica-based sorbent.

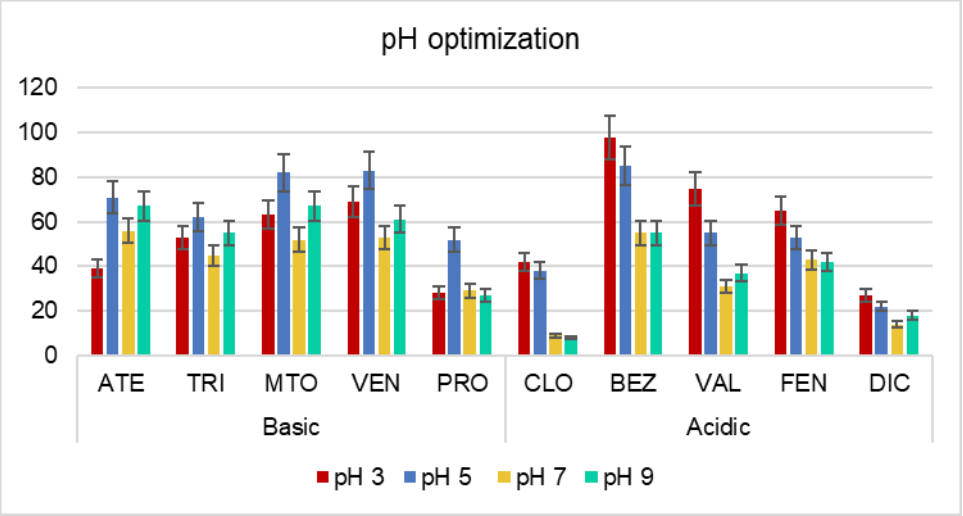


Figure 5. Recoveries obtained when 25 mL of ultrapure water at different pH was loaded in sorbent CW 20/ZW-1.

During the selection of the best performing sorbent and pH, it was observed that the elution with acidified MeOH (5 mL of 5% CH_3COOH in MeOH) could be removed since all the analytes were eluted with the basic MeOH (5 mL of 5% NH_4OH in MeOH). Furthermore, it was observed that when successive extractions were done with the same cartridge, the performance of the sorbent decreased. And it was considered that the basic elution could damage the sorbent, so it was decided to evaluate the use of a solution 1% NH_4OH in MeOH. After these experiments, it was observed that the decrease in the percentage of NH_4OH prevented the material to be damaged without affecting the elution of the compounds.

To increase the selectivity of the extraction, the addition of a washing step with MeOH was evaluated. The objective of this washing step was to remove the compounds retained through reversed-phase interactions and then, rinse the compounds retained through ion-exchange interactions in the elution step. Considering the previous experience with other silica-based sorbents functionalized with strong ion-exchange moieties [18,31], 1 mL of MeOH was initially tested. Similarly to a previous study [18], the basic analytes remained retained in the sorbent but the acidic ones were rinsed with MeOH. This could be explained by the electrostatic repulsions that can occur due to the high concentration of charges. The charges of sorbent and analytes may involve that the retention through ion-exchange interactions of the acidic compounds is not strong enough to prevent their rinsing with MeOH. In our previous study [18], it was decided to maintain the washing step and only determine the basic analytes with high selectivity. However, in the present study, we removed the washing step and determined both acidic and basic analytes, despite partially sacrificing the selectivity.

The loading volume was increased from 25 mL to 100 mL to improve the preconcentration factor without observing significant losses in the recoveries. It should be noted that 100 mL was maintained when loading river samples but it was lowered to 50 mL for effluent wastewater samples and to 25 mL influent wastewater samples due to the complexity of these samples.

To sum up, the optimized protocol consisted of loading 100 mL of river water, 50 mL of effluent wastewater or 25 mL of influent wastewater adjusted to pH 5, and an elution step with 5 mL of 1% NH_4OH in MeOH.

3.4. Validation of the method

Once optimized, the method was validated with LC-HRMS quantification in terms of apparent recovery ($\%R_{app}$) at two levels of concentration, matrix effect (%ME), method detection and quantification limits, accuracy (relative recovery) and precision (repeatability and reproducibility between days) with river, effluent wastewater and influent wastewater samples.

The $\%R_{app}$ were calculated dividing the measured concentration after subtracting the blank concentration by the theoretical concentration. It was obtained spiking at two levels of concentration, 1 and 2 µg/L for river samples, 2 and 5 µg/L for effluent and 4 and 10 µg/L for influent wastewater samples. As can be observed in Table 2, good recoveries were obtained in the three types of samples for most of the compounds. ATE and CLO were the analytes with lower recoveries; in fact, CLO presented low retention yields when loading ultrapure water samples (as can be observed in Fig. 5) and this behavior was enhanced by the complexity of the samples. In the case of ATE, a significant decrease in the recoveries when working with more complex samples was already observed in a previous study [18], when the recoveries decreased from 94% (ultrapure water) to 40% (river samples). For the rest of the compounds, recoveries higher than 30% were observed for all of them except for DIC in influent wastewater samples, which can be explained by the high matrix effect (-84%). In the case of VAL in influent wastewater samples, the natural occurrence in the samples was higher than the concentration spiked, so the $\%R_{app}$ could not be calculated. Similar compounds were determined by Nadal *et al.* [15] with a polymeric mixed-mode zwitterionic exchange sorbent, obtaining recoveries ranging from 57 to 81% in river samples and from 25 to 105% in effluent wastewater samples. Jaukovic *et al.* [32] determined cardiovascular drugs in river and wastewater samples from Serbia using Oasis HLB for the extraction and the recoveries obtained ranged from 26 to 121% in river samples and from 45 to 107% in wastewater (no differences between effluent and influent where done in the mentioned study).

The matrix effect was determined using the following expression: $\%ME = (C_{exp}/C_{the} \times 100) - 100$, where "$C_{exp}$" represents the concentration obtained by spiking (1 µg/L for river samples, 2 µg/L for wastewater samples) a non-spiked sample after SPE, subtracting the blank signal, and "C_{the}" represents the theoretical concentration. A positive value indicates signal enhancement

Table2. Apparent recoveries ($\%R_{app}$) at two levels of concentration and matrix effects (%ME) in river, effluent wastewater, and influent wastewater samples.

		River			Effluent wastewater			Influent wastewater		
		$\%R_{app}$ high conc	$\%R_{app}$ low conc	%ME	$\%R_{app}$ high conc	$\%R_{app}$ low conc	%ME	$\%R_{app}$ high conc	$\%R_{app}$ low conc	%ME
Basic	ATE	11	10	-31	18	20	-41	14	14	-53
	TRI	50	42	-46	61	60	-45	33	31	-58
	MTO	68	62	-25	81	81	-41	48	44	-54
	VEN	64	63	-21	64	59	-49	38	38	-62
	PRO	48	43	+14	59	53	-12	52	43	-31
Acidic	CLO	9	8	+4	9	11	-3	14	14	-16
	BEZ	75	65	-13	68	55	-30	64	67	-31
	VAL	69	78	13	65	88	-7	-*	-*	-*
	FEN	69	76	+14	83	72	-26	45	56	-51
	DIC	33	38	-4	33	32	-24	16	15	-84

%RSD (n = 3) < 14%.
*Natural occurrence higher than spiked concentration.

while a negative value indicates signal suppression. As can be observed in Table 2, low %ME were obtained for river samples, being lower than ±25% except for ATE (-31%) and TRI (-46%). Effluent wastewater samples suffered higher signal suppression, mainly for basic compounds, ranging from -41 to -49% for ATE, TRI, MTO and PRO. For acidic compounds, the matrix effect was lower, being BEZ the most suppressed one (-30%). In the case of influent wastewater samples, the complexity of the sample caused higher suppression. Only PRO, CLO and BEZ presented a %ME lower than ±35%, meanwhile VEN or DIC reached values of -62 and -84%, respectively. The lack of a washing step led to high matrix effect values, especially when the complexity of matrices increased. Salas *et al.* [33] combined two commercial mixed-mode strong anion- and cation-exchange sorbents in one cartridge to extract similar compounds from river, effluent wastewater and influent wastewater samples. In their study, 15 mL of MeOH were used as washing step, obtaining %ME ranging from +5 to -36% for river samples, from -10 to -49% for effluent wastewater samples and from +10 to -42% for influent wastewater samples. Thus, in spite of using such a high volume of washing solvent, matrix effect was similar to the values obtained in this study except for influent wastewater samples.

The accuracy of the method was evaluated as the relative recovery, that was obtained as the ratio between the calculated concentration after the correction with the apparent recovery and the theoretical concentration. It was obtained at two levels of concentration (1 and 2 µg/L for river samples and 2 and 5

µg/L for effluent and influent wastewater samples). The relative recoveries ranged from 88 to 111% in river samples, from 85 to 113% in effluent wastewater samples and from 92 to 115% in influent wastewater samples. Relative recoveries were not obtained for ATE and CLO due to their low apparent recoveries.

The method detection (MDL) and quantification limits (MQL) were estimated applying the preconcentration factor and the apparent recoveries to the instrumental limits. The instrumental detection limit was determined as the concentration exhibiting a signal-to-noise ratio greater than 3, with one of the fragments yielding a signal exceeding 10^3 arbitrary units (AU). Additionally, the instrumental quantification limit (MQL) was established as the lowest point on the calibration curves, ensuring a signal-to-noise ratio higher than 10. Table S3 presents the MDLs and MQLs. All MDLs were lower than 5 ng/L and all MQLs were lower than 12 ng/L except for FEN, whose limits were significantly higher. The low ionization yield of FEN produced high instrumental limits that led to high method limits.

The precision evaluated as the repeatability (% RSD, n = 4) and the reproducibility between days (% RSD, n = 4) was obtained at 1 µg/L for river samples and 2 µg/L for wastewater samples. The repeatability was lower than 14% and the reproducibility between days was lower than 18% for all type of samples.

3.5. Analysis of real environmental samples

River, effluent wastewater and influent wastewater samples (n=3) collected in the Tarragona region (Spain) were analyzed to determine the acidic and basic analytes. Quantification was done by using external calibration curves and applying the apparent recoveries obtained previously and the preconcentration factor. To confirm the occurrence of the compounds, the difference in the retention time should be lower than 0.1 min, the mass error of the molecular ion had to be lower than 5 ppm, the signal of at least one fragment ion had to be higher than 10^3 AU and the relative intensities of at least one fragment ion and the molecular ion should be within ± 40 % relative deviation (following the indications of 2021/808/EC [34]).

Table 3 shows the natural occurrence found of the compounds in river, effluent wastewater and influent wastewater and in brackets, the ranges of uncertainty for each type of sample. Starting with river samples, they showed the lowest concentration and some compounds were not detected (FEN and TRI in all samples, and PRO in some samples). It was possible to confirm the presence of DIC and VEN in all the samples analyzed. For MTO, PRO and BEZ (in one of the samples), peaks were detected in the retention time of these compounds but the ratios between the fragment ions and the molecular ion were not in the range of acceptance. The highest concentration found was DIC with 401 ng/L. Nadal *et al.* [35] determined similar compounds in river samples from Ebro river. In that study, DIC concentrations were lower ranging from 23 to 30 ng/L, and ATE were higher ranging from 8 to 45 ng/L. Klancar *et al.* [36] also determined several pharmaceuticals in river samples from

Table2. Concentrations found (ng/L) in river, effluent wastewater, and influent wastewater samples (n=3).

		River	Effluent	Influent
Basic	TRI	<MDL	<MDL– 1,178 (184 – 202)	577 – 19,137 (57 – 1,154)
	MTO	<MDL	89 – 178 (15 – 29)	326 – 1,414 (53 – 231)
	VEN	22 – 27 (2 – 4)	206 – 1,091 (30 – 163)	324 – 1,123 (48 – 168)
	PRO	<MDL	<MDL – 249 (2 – 33)	<MDL – 1,546 (6 – 170)
Acidic	BEZ	<MDL – 81 (3 – 11)	48 – 422 (8 – 58)	204 – 1,537 (24 – 211)
	VAL	<MQL – 235 (4 – 10)	881 – 11,847 (184 – 2,483)	-
	FEN	<MDL	<MDL	<MQL
	DIC	16 – 401 (2 – 45)	692 – 4,666 (80 – 670)	1,734 – 6,456 (196 – 728)

Slovenia, and ATE, DIC, MTO or TRI were detected at concentrations below the quantification limits which were 80, 1.6, 0.4 and 0.4 ng/L, respectively. Garrido *et al.* [37] determined some emerging pollutants in river samples in the south of Spain, among which ATE, TRI, CLO and BEZ. ATE and CLO were not detected (MDL of 0.67 and 6.2 ng/L respectively), and similar concentrations than the present study of TRI and BEZ were found, ranging from <MDL (0.14 ng/L) to 25 ng/L and from <MDL to 139 ng/L, respectively.

Regarding the effluent wastewater samples, the occurrence of the analytes was higher than in river samples. All compounds could be detected in at least one sample except for FEN. Regarding the ratios between fragment and molecular ions, they were acceptable for all compounds except for PRO in one sample. The compounds with higher concentration were VAL and DIC ranging from 881 to 11,847 ng/L and from 428 to 2,938 ng/L, respectively. When Vergeynst *et al.* [38] determined pharmaceuticals (DIC, TRI and VEN, among others) in wastewater samples from Belgium, TRI was not detected (MDL = 28 ng/L), meanwhile the concentrations of DIC and VEN were similar to the one found in the present study, ranging from 542 to 1,391 ng/L and from 205 to 365 ng/L. When Zhang *et al.* [39] determined several pharmaceuticals in Chinese effluent wastewater samples, the average concentrations of TRI and VEN were lower than the present study, being 4.4 and 34.2 ng/L. In the case of MTO, the average concentration found was similar to the present study, 165 ng/L.

In the case of influent wastewater samples, as expected, the concentrations were the highest among the three types of samples. All compounds except for FEN and VAL could be quantified; FEN due to the low occurrence and the high quantification limits of the method and VAL because the recoveries could not be calculated. The ratios between the fragment ions and the molecular ion were acceptable in all cases except for PRO in one sample. All compounds were present in at least one sample with concentrations above 1,000 ng/L. Gilart *et al.* [40] determined similar compounds in wastewater samples from Tarragona region and MTO and TRI were

quantified at similar levels of concentration (116 – 1,857 and 97 – 155 ng/L, respectively). Vergili *et al.* [41] also determined pharmaceuticals in influent wastewater samples, and DIC concentration ranged from 810 to 4,300 ng/L, meanwhile TRI and VEN were found at lower concentrations than in the present study (140 to 290 ng/L and 130 to 300 ng/L, respectively). When Van Nuijs *et al.* [42] determined MTO and VEN in influent wastewater samples from Belgium, concentrations of MTO were similar, ranging from 252 to 1,190 ng/L. In the case of VEN, the concentrations found were lower (119 – 480 ng/L).

4. Conclusions

Three novel mixed-mode zwitterionic ion-exchange silica-based sorbents were synthesized using only one single additive. The additive was a molecule with both quaternary amine and sulfonic acid groups, making it possible to introduce the strong anion-exchange and the cation-exchange moieties. In addition, silica demonstrated to be a good substrate to insert the functional groups as it could be observed in the characterization.

A method based on SPE followed by LC-HRMS applying the best performing sorbent was successfully developed and validated for the simultaneous determination of five acidic and five basic pharmaceuticals in environmental water samples. Most of the compounds were detected when the developed method was applied to the determination of the pharmaceuticals in river, effluent wastewater, and influent wastewater samples.

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Suplementary data

Table S1. Mass spectrometer conditions.

	Positive ionization	Negative ionization
Sheath gas flow rate	40 AU	40 AU
Auxiliar gas flow rate	20 AU	20 AU
Sweep gas	0 AU	0 AU
Spray voltage	4 kV	4 kV
Capillary voltage	45 V	-25 V
Tube lens voltage	65 V	-60 V
Skimmer voltage	22 V	-22 V
Capillary temperature	330 ºC	330 ºC
Heater temperature	350 ºC	350 ºC

Table S2. pK_a, protonated (basic) or deprotonated (acidic) ion and fragment ion for the analytes.

	Compound	pK_a	Protonated ion/ Deprotonated ion	Fragment ion
Basic	ATE	9.6	267.17020	190.08649 145.06514
	TRI	7.1	291.14517	245.10388 261.09866
	MTO	9.7	268.19061	116.10733 191.10712
	VEN	10.1	278.21133	260.20111 58.06614
	PRO	9.4	260.16418	116.10773 183.08057
Acidic	CLO	3.2	213.03151	126.99430 85.02819
	BEZ	3.8	360.10059	274.06412 154.00548
	VAL	3.6	434.21912	350.16254 179.08580
	FEN	4.5	241.08667	93.03340 211.07599
	DIC	4.1	294.00919	250.01923 252.01630

Table S3. Method detection limits and method quantification limits in ng/L.

		River		Effluent wastewater		Influent wastewater	
		MDL	MQL	MDL	MQL	MDL	MQL
Basic	ATE	1	9	1	11	2	14
	TRI	1	3	2	6	3	8
	MTO	1	3	2	8	4	10
	VEN	1	3	3	8	5	12
	PRO	2	5	3	9	4	10
Acidic	BEZ	1	3	1	7	2	8
	VAL	2	4	3	8	-	-
	FEN	36	73	60	120	111	223
	DIC	1	7	1	8	6	31

Characterization of sorbents ZW-2 and ZW-3

The FT-IR spectra of sol-gel CW 20M/3-(N,N-Dimethylmyristylammonio) propane sulfonate composite sorbent (ZW-2) and its building blocks

The FT-IR spectra of sol-gel CW 20M/3-(N,N-Dimethylmyristylammonio) propane sulfonate composite sorbent and the individual building block components are provided in Figure S1.

The spectral information of pristine CW 20M and MTMS are presented in the main text.

The FT-IR spectra of the zwitterionic monomer, [3-(N,N-Dimethylmyristylammonio) propane sulfonate is presented in Figure S1 (c). The peak at 1637 cm^{-1} corresponds to the absorption from the stretching vibration of C-N^+ . The peaks at 1036 cm^{-1} and 1171 cm^{-1} correspond to the symmetrical and asymmetrical stretch of S=O bond in the sulfonate group.

The FT-IR spectra of sol-gel CW 20M/ZW-2 is presented in Figure S1 (d). Many peaks in the FT-IR spectra of sol-gel CW 20M/ZW-2 also appeared in either CW 20M, MTMS, or IL spectra. For example, the peak at 2931 cm^{-1} in Figure S1 (d) may correspond to the peak at 2883-1 in CW 20M, peak at 1743 cm^{-1} may correspond to peak at 1714 cm^{-1}in the zwitterionic monomer spectra, the peak at ~1171 cm^{-1} may correspond to 1188 cm-1 in the MTMS spectra. The appearance of the peaks in both the building block compounds as well as in sol-gel CW 20M/ZW-2 strongly suggests the successful integration of the building block components in the sol-gel composite sorbent.

The FT-IR spectra of sol-gel CW 20M/(Benzyldimethylammonio)propane sulfonate composite sorbent (ZW-3) and its building blocks

The spectral information of pristine CW 20M and MTMS are presented in the main text.

The FT-IR spectra of the zwitterionic monomer, (Benzyldimethylammonio)propane sulfonate is presented in Figure S2 (a). The peak at ~1543 cm^{-1} corresponds to aromatic double bond of the benzene ring. The peak at 1688 cm^{-1} corresponds to the absorption from the stretching vibration of C-N. The peaks at 1039 cm^{-1} and 1173 cm^{-1} correspond to the symmetrical and asymmetrical stretch of S=O bond in the sulfonate group

The FT-IR spectra of sol-gel CW 20M/ZW-3 is presented in Figure S2 (d). Many peaks in the FT-IR spectra of sol-gel CW 20M/ZW-3 also appeared in either CW 20M, MTMS, or IL spectra. For example, the peak at 2883 cm^{-1} in Figure S2 (d) may correspond to the peak at 2885-1 in CW 20M, peak at 1722 cm^{-1} may correspond to peak at 1668 cm^{-1}in the zwitterionic monomer spectra. The appearance of the peaks in both the building block

compounds as well as in sol-gel CW 20M/ZW-3 strongly suggests the successful integration of the building block components in the sol-gel composite sorbent.

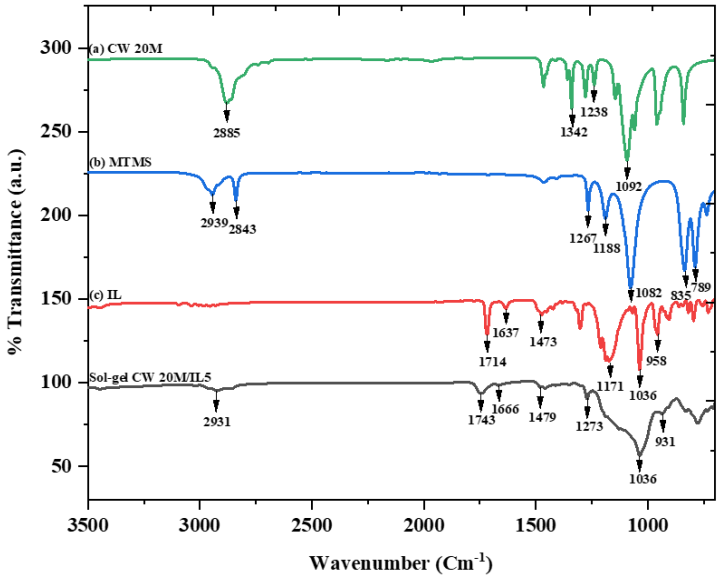

Figure S1. FT-IR spectra of (a) pristine Carbowax 20M polymer; (b) methyl trimethoxysilane (MTMS); (c) 3-(N,N-Dimethylmyristylammonio) propane sulfonate zwitterionic monomer (d) sol-gel Carbowax 20M/3-(N,N-Dimethylmyristylammonio) propane sulfonate composite sorbent.

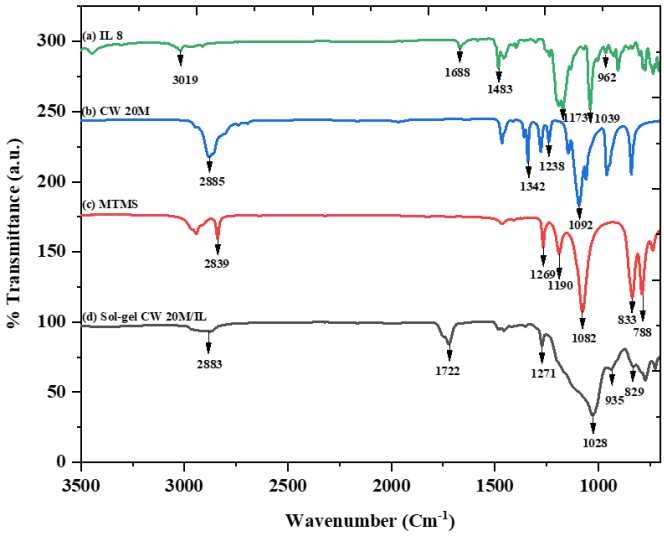

Figure S2. FT-IR spectra of (a) pristine Carbowax 20M polymer; (b) methyl trimethoxysilane (MTMS); (c) (Benzyldimethylammonio)propane sulfonate zwitterionic monomer (d) sol-gel Carbowax 20M/(Benzyldimethylammonio) propane sulfonate composite sorbent.

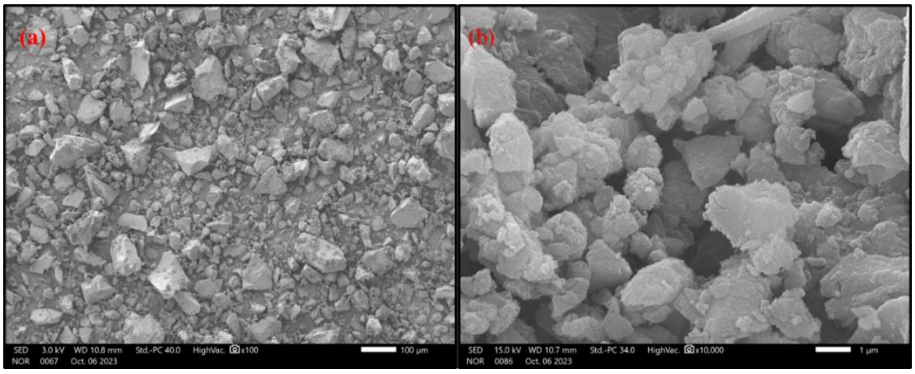

Figure S3. Scanning electron microscopy images of sol-gel Carbowax 20M/3-(N,N-Dimethylmyristylammonio) propane sulfonate composite sorbent at (a) 100x magnifications; (b) 10,000x magnifications.

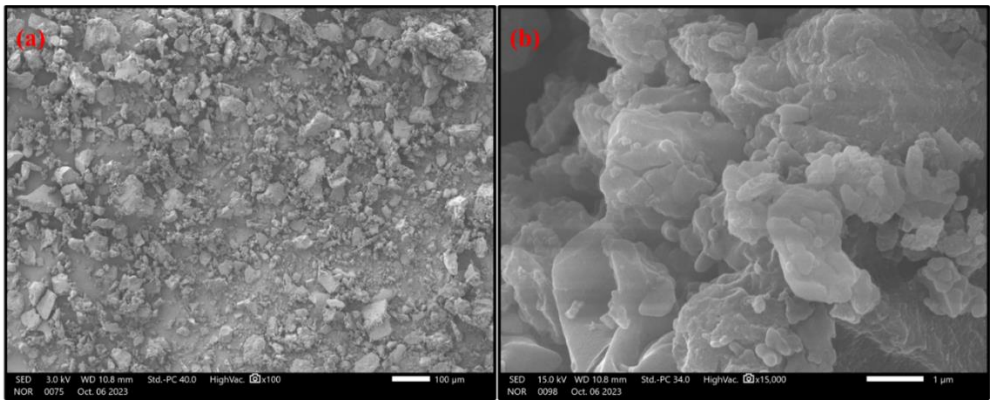

Figure S4. Scanning electron microscopy images of sol-gel Carbowax 20M/ N-(Dimethylbenzylammonio)propane sulfonate composite sorbent at (a) 100x magnifications; (b) 15,000x magnifications.

Recoveries at pH 3, 7 and 9 for all sorbents

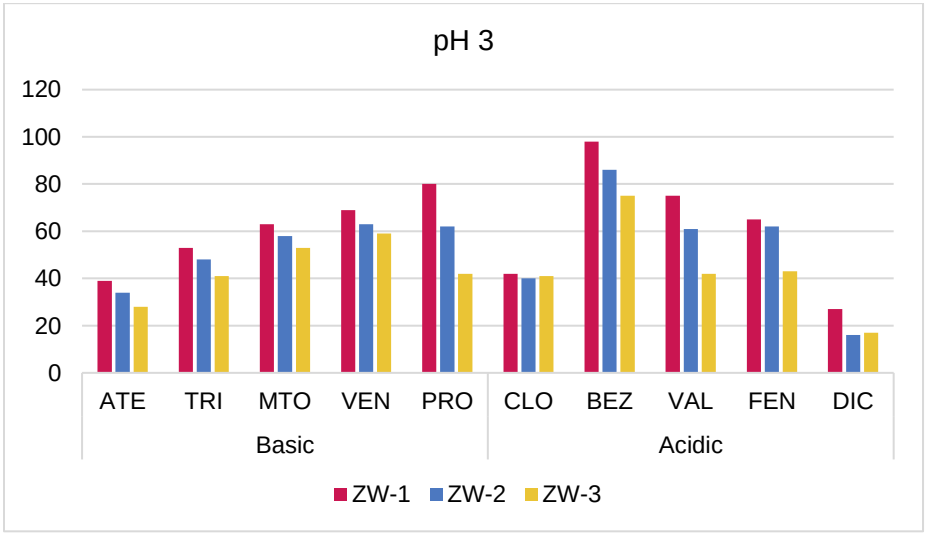

Figure S5. Comparison of the three sorbents when 25 mL of ultrapure water at pH 3 was loaded.

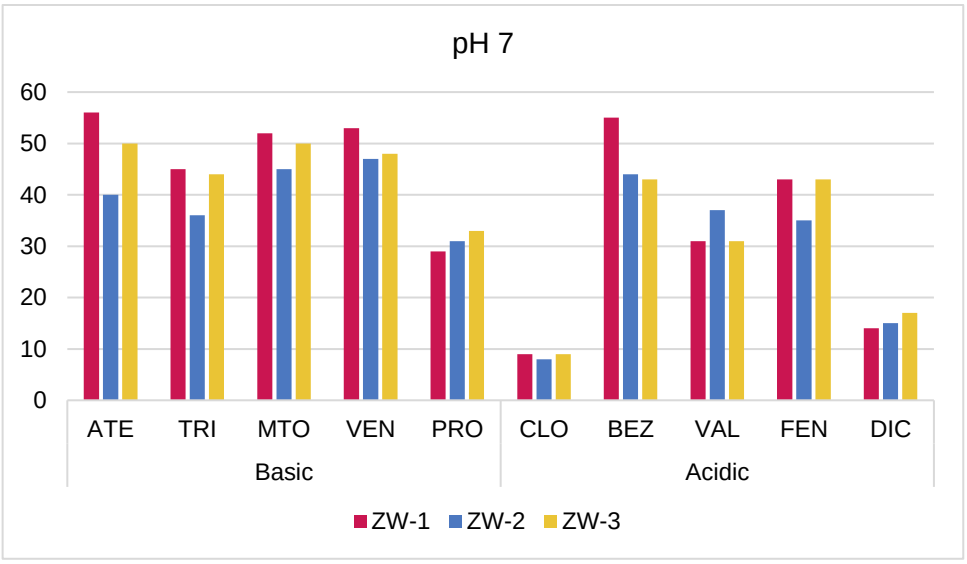

Figure S6. Comparison of the three sorbents when 25 mL of ultrapure water at pH 7 was loaded.

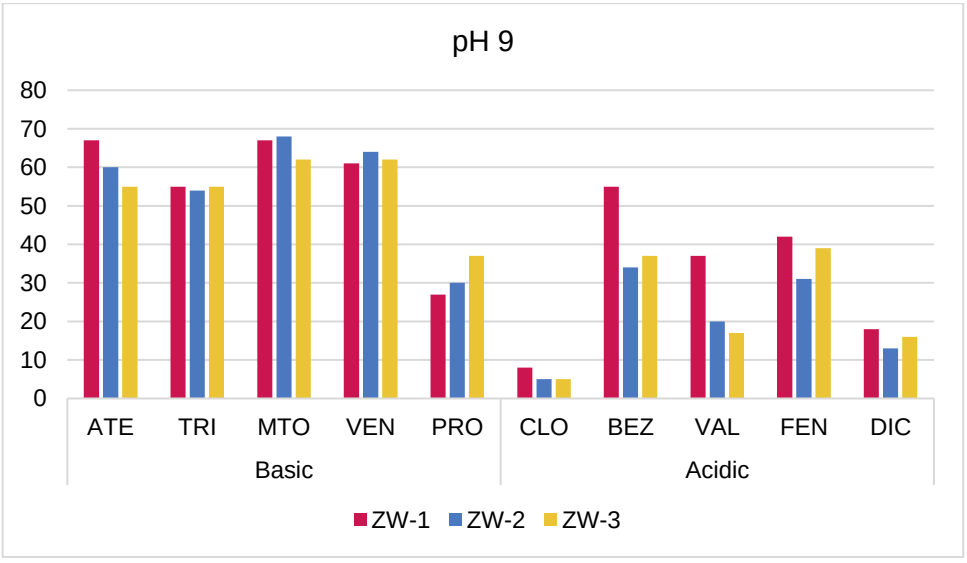

Figure S7. Comparison of the three sorbents when 25 mL of ultrapure water at pH 9 was loaded.

3.1.6. Discussion of results

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In this section, the silica-based sorbents that have been evaluated in the present chapter will be discussed in an overall point of view. Up to eight different sorbents have been evaluated in this chapter, that can be separated in two groups: the first group cluster the sorbents from the first four studies where the functional groups were included with different reagents and the second one, where the zwitterionic functional groups were included with only one additive. As it has been discussed in the studies, the best performing sorbents have been SiO_2-SAX/SCX, SiO_2-G-SAX/SCX and CW-20/ZW-1.

In all five studies, different types of analytes were extracted from water samples. Table 1 summarizes the analytes and the extraction conditions (loading pH, washing and elution) for each sorbent. It can be observed that the conditions changed depending on the application. For instance, when SiO_2-SAX/SCX was used to extract basic pharmaceuticals (section 3.1.1.), drugs of abuse and metabolites (section 3.1.4), the conditions were similar: pH 3, MeOH for the washing step and 5 mL of 5% NH_4OH in MeOH, changing only the volume of the MeOH. pH 3 was also selected when SiO_2-G-SAX/SCX was applied for the extraction of benzothiazoles (BTs), benzotriazoles (BTRs) and benzenesulfonamides (BSAs) (section 3.1.2.) or NH_2BT (3.1.3.). The differences were in the washing step and the elution, as it was highlighted, all the BTs, BTRs and BSAs, except for NH_2BT were mainly retained through π-π interactions and when percolating MeOH they were eluted. For this reason, in section 3.1.2. the analytes were eluted with 5 mL MeOH without washing, meanwhile in section 3.1.3. a washing step with 7 mL of MeOH could be included followed by elution with 5 mL of 5% NH_4OH in MeOH, meaning that ionic interactions played an important role on the retention of this analyte. Regarding CW20/ZW-1, with the purpose of extracting simultaneously acidic and basic pharmaceuticals, the loading pH was 5 and, to avoid the loss of acidic analytes, the washing step was removed. Moreover, the % of NH_4OH in the elution was reduced to prevent the damaging of the sorbent.

Table 1. Analytes, loading pH, washing step and elution from the studies in chapter 3.1.

	SiO_2-SAX/SCX (3.1.1)	**SiO_2-G-SAX/SCX (3.1.2)**	**SiO_2-G-SAX/SCX (3.1.3)**	**SiO_2-SAX/SCX (3.1.4)**	**CW 20/ZW-1 (3.1.5)**
Analytes	Basic pharmaceuticals	BT, BTR, BSA	NH_2BT	Basic pharmaceuticals, drugs of abuse and metabolites	Acidic and basic pharmaceuticals
Loading pH	pH 3	pH 3	pH 3	pH 3	pH 5
Washing	5 mL MeOH	-	7 mL MeOH	2 mL MeOH	-
Elution	5 mL 5% NH_4OH in MeOH	5 mL MeOH	5 mL 5% NH_4OH in MeOH	5 mL 5% NH_4OH in MeOH	5 mL 1% NH_4OH in MeOH

As it was pointed out in the studies of this chapter, the simultaneous extraction of both acidic and basic analytes could be achieved, but it was not possible to introduce a

washing step with MeOH without suffering high losses of the acidic analytes. Therefore, we had to choose between extracting selectively the basic analytes applying the washing step with MeOH and rinsing the acidic analytes like in section 3.1.1 or removing the washing step to extract both acidic and basic compounds like in section 3.1.5. but suffering a decrease in the selectivity of the extraction. In these studies, the analytes were mostly the same since all the pharmaceuticals present in section 3.1.5. were also present in section 3.1.1

After optimizing the SPE conditions, the methods were validated percolating 100 mL and 50 mL of river and effluent wastewater samples. In Table 2, the apparent recoveries and the matrix effects for the basic analytes that were in common in both studies are presented. Before comparing the results, it should be noted that LC-MS/MS was used in section 3.1.1 meanwhile LC-HRMS was used in section 3.1.5. The use of different detector and instruments with different ionization sources might affect the %ME results.

Table 2. Apparent recoveries and matrix effects for SiO_2-SAX/SCX and CW20/ZW-1 when analyzing river and effluent wastewater samples.

	River				**Effluent wastewater**			
	SiO_2-SAX/SCX (3.1.1)		**CW 20/ZW-1 (3.1.5)**		**SiO_2-SAX/SCX (3.1.1)**		**CW 20/ZW-1 (3.1.5)**	
	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME
ATE	48	-16	11	-31	71	-25	18	-41
TRI	60	-14	50	-46	66	-24	61	-45
MTO	66	-14	68	-25	58	-19	81	-41
VEN	85	-17	64	-21	64	-22	64	-49
PRO	63	-4	48	+14	40	-18	59	-12

As can be observed in Table 2, if we exclude ATE, which gave poor recoveries with CW20/ZW-1, the overall recoveries are similar, being in the range from 40 to 85% for both sorbents. However, focusing on the matrix effects reported, it can be observed that in all cases (except for PRO in effluent wastewater samples), the %ME with SiO_2-SAX/SCX were always lower than with CW20/ZW-1. These results are coherent, since the use of the washing step provided lower matrix effect values, but the determination of acidic compounds could not be performed in section 3.1.1., since these compounds were rinsed in the washing step.

Moreover, this type of sorbents can be applied with other purposes. Since the sorbents are functionalized with both SAX and SCX moieties, they can be applied to exploit only one of the interactions, like it was done in section 3.1.1 for basic analytes. An example of this application can be the study performed by Kechagia *et al.* [1] when a sorbent similar to the ones evaluated in section 3.1.5 was used to extract atorvastatin, pitavastatin, rosuvastatin, pravastatin and emamectin benzoate in urine samples. All of them have acidic behaviour, so, the method was optimized to exploit the SAX interactions. Another example can be the study performed by Ntorkou *et al.* [2] when a sorbent similar to CW20/ZW-3 was applied for the extraction of lanreotide (a basic pharmaceutical) in urine samples

In addition, sorbents that are able to perform different types of interactions can retain a wide range of compounds. For example, Ferracane *et al.* [3] applied a fabric phase to extract a wide variety of pesticides with different properties (carbamates, morpholines, nitrosamines, organochlorines, organophosphates, pyrethroids, and triazines), obtaining recoveries higher than 35% for most compounds. Therefore, the sorbents developed in this section could be applied in a similar way. In this sense, to compare the suitability of these sorbents to act as "multi-mode" sorbents, the recoveries of three basic (ATE, MTO, VEN) and three acidic pharmaceuticals (BEZ, VAL, FEN) when 25 mL of ultrapure water at pH 5 were analyzed, are shown in Figure 1. It can be observed that in most cases, SiO_2-SAX/SCX provided the higher recoveries, being higher than 80% for all compounds. The functionalization with C_{18} chains, sulfonic acid groups and quaternary amines allow this sorbent to perform a wide variety of interactions. SiO_2-C-SAX/SCX which is similar to SiO_2-SAX/SCX with activated carbon microparticles embedded also gave good results, but as it was pointed out in section 3.1.1, it seems that activated carbon has low contribution to enhance the retention of compounds. Focusing on the sorbents from section 3.1.5 (CW20/ZW-1, CW20/ZW-2 and CW20/ZW-3), they presented overall good performance but lower than SiO_2-SAX/SCX and SiO_2-C-SAX/SCX.

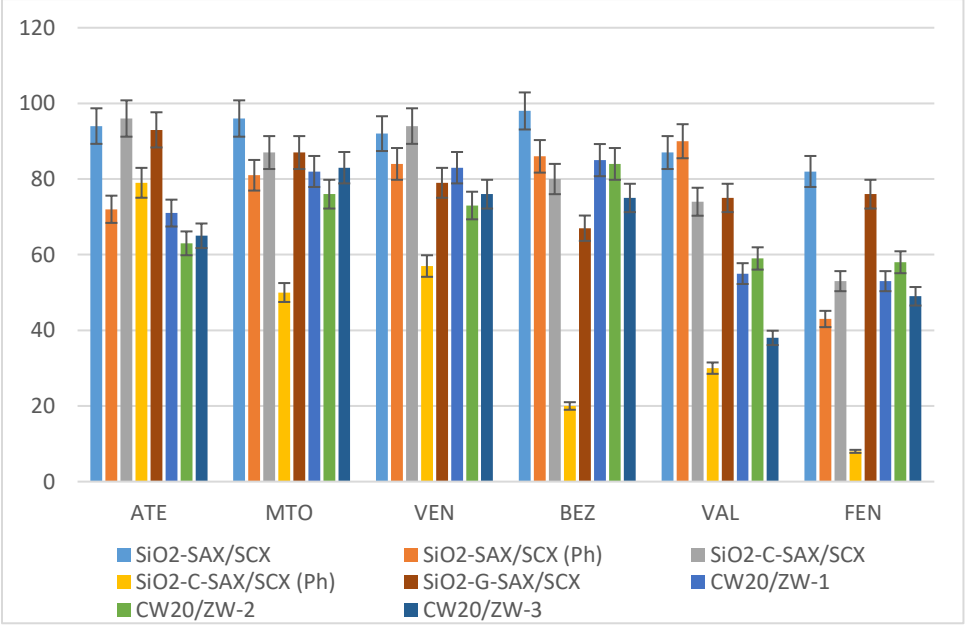

Figure 1. Recoveries for ATE, MTO, VEN, BEZ, VAL and FEN with different silica-based sorbents with ultrapure water.

The sorbent evaluated in sections 3.1.2 and 3.1.3 (SiO_2-G-SAX/SCX) should be the most appropriate sorbent to be applied as "multi-mode" material since apart from the reversed-phase, SCX and SAX interactions due to the C_{18} chains, sulfonic acid groups and quaternary amines respectively, it can perform π-stacking interactions thanks to graphene.

However, as can be seen in Figure 1, despite the recoveries of SiO_2-G-SAX/SCX are good, SiO_2-SAX/SCX shows better performance. As it was highlighted in section 3.1.4, the presence of graphene enhanced the recoveries of the compounds with high delocalized π density. Thus, to extract compounds without this high π-density, SiO_2-SAX/SCX looks like a better option.

Focusing on the sorbent SiO_2-G-SAX/SCX, which was applied to the extraction of BTs, BTRs and BSAs, there have been other studies where sorbents that perform π-π interactions have been applied such as the studies where a silica sorbent functionalized with humic acids [4] or multi-walled carbon nanotubes [5] were successfully applied for the extraction of these analytes from environmental water samples. In another study, Flores-López *et al.* [6] successfully applied a graphene aerogel for the extraction of benzotriazoles. It should be noted that the benzotriazoles were different to the ones determined in section 3.1.2 but they also presented high π density.

The study from section 3.1.3. is slightly different from the others, specially the part focused on the application of SiO_2-G-SAX/SCX as a clean-up for organic extracts from dust and fish samples. As it was pointed out in the study, the ability to retain compounds from organic extracts is remarkable. However, the study was only focused on one analyte, which is not commonly determined isolated, being more determined with other benzothiazoles [7,8]. This study could be extended evaluating the sorbent to extract similar analytes with high π-density and basic behaviour. Some examples of these analytes can be 5-aminobenzotriazole, of 2-aminobenzimidazole and thiabendazole. All of them have fused rings to delocalize the π-density and basic groups like NH_2BT. 5-aminobenzotriazole belongs to the BTR family and has been determined in previous studies where BTs and BTRs were quantified [9]. In the case of 2-aminobenzimidazole and thiabendazole, they are fungicides from the family of benzimidazoles [10].

One of the objectives of the studies from this chapter was to extract simultaneously acidic and basic pharmaceuticals including a washing step with an organic solvent to increase the selectivity, similarly to previous studies on the group where WAX/WCX [11] or SAX/WCX and WAX/SCX [12]. Considering these results and what will be presented in the following chapter, where another WAX/WCX was successfully applied with a washing step based on methanol, we can confirm that the problems with the SAX/SCX sorbents might be the combination of strong moieties. As it was highlighted in section 3.1.5., the use of strong exchange moieties combined with the charged analytes generates a considerable concentration of charges in the sorbent. This concentration of charges can make it possible for methanol to elute the analytes, since the retention through ion-exchange interactions might not be strong. In this sense, the introduction of at least one weak exchanger is desirable if acidic and basic analytes want to be selectively extracted with the same sorbent. However, the sorbents developed were successfully applied for the determination of different types of contaminants in environmental samples, mainly river, effluent wastewater and influent wastewater ones, but also in dust and fish.

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3.1. Evaluation of mixed-mode ion-exchange polymer-based sorbents

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This chapter focus on the synthesis, characterization and evaluation of two polymer-based sorbents. Unlike previous chapter, where the sorbents were synthesized by Kabir and Furton in FIU, the sorbents evaluated in this chapter were synthesized by myself during my stay in Glasgow (UK). The stay was done in P.A.G. Cormack group, in the Department of Pure and Applied Chemistry at University of Strathclyde. Our group and Cormack's group have been collaborating over the last two decades combining their knowledge in the field of polymer chemistry with our expertise in exploiting materials for sorptive extraction techniques. During this collaboration, most of the developed materials were applied to solid-phase extraction (SPE), as it will be described, although some studies were focused on the development of coatings for stir bar sorptive extraction (SBSE) [1,2].

The collaboration started in early 2000s with the development of molecular imprinted polymers (MIPs) [3], and several studies were performed using different templates such as 4-nitrophenol [3], naphthalene [4] or ciprofloxacin [5], being applied for the extraction of these analytes in water [3,4] and urine samples [5,6]. The next step was the development of hypercrosslinked polymers, as it was described in the introduction, hypercrosslinked polymers have enhanced specific surface area, increasing the capacity and the retention sites of the sorbent. Materials with 908 m^2/g [7] and 1320 m^2/g [8] were developed and applied for the on-line SPE of pesticides and phenolic compounds. The polymers were prepared using divinylbenzene (DVB) and vinylbenzene chloride (VBC) as monomers. The DVB and VBC were polymerized through a radical precipitation polymerization using 2,2'-azobisisobutyronitrile (AIBN) as radical initiator. Then, with the addition of $FeCl_3$ a Friedel-Crafts reaction between the chlorine from the VBC and the aromatic rings from DVB and VBC was performed, occurring the hypercrosslinking.

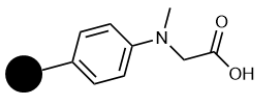
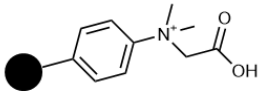
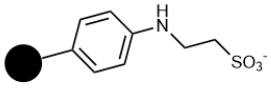
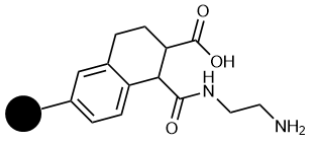
After hypercrosslinking, the "unreacted" chlorine atoms from VBC in the polymer can be exploited to modify the sorbent. The first study in this sense was the reaction with ethylenediamine to introduce primary, secondary and tertiary amine groups in the sorbent, obtaining a mixed-mode ion-exchange material able to perform weak anion-exchange (WAX) interactions [9]. In subsequent studies, materials with the other moieties were developed: strong anion-exchange (SAX) [10], weak cation-exchange (WCX) [11] and strong cation-exchange (SCX) [12]. Different routes were followed, for the SAX one, dimethylbutylamine was used to introduce the quaternary amines in a polyDVB-co-VBC material, similarly to the WAX study [10]. In the case of WCX, the carboxylic acid groups were not added in a functionalization step, but including methacrylic acid as monomer [11]. Regarding SCX, different approaches were evaluated, for instance, a DVB and 2-hydroxyethyl methacrylate were used as monomers, adding the sulfonic acid groups with concentrated sulfuric acid [12]. Moreover, in another study, a polyDVB-co-VBC material was sulfonated using lauroyl sulfate [13].

In the first study of this chapter (section 3.2.1.) a novel SCX polymer was developed. Taking into account the successful results from mixed-mode ion-exchange materials developed previously, a novel format was evaluated in this section, synthesizing core-shells. This type of core-shells were both core-and shell are polymer-based has not been commonly applied in SPE so far. In this sense, hypercrosslinked sulfonated core-shell particles were

synthesized and evaluated evaluated for the extraction of basic pharmaceuticals from environmental water samples and its determination through LC-HRMS.

As it was pointed out in the introduction of the previous chapter, in the recent years, the development of sorbents that combine both anion- and cation-exchange moieties has attracted the interest of our research group. To date, two studies developing amphoteric materials have been carried out in collaboration with Cormack's group. The first one was based on a combination of both weak-exchange moieties, the WAX/WCX material was developed functionalizing a hypercrosslinked polyDVB-co-VBC sorbent which was modified with sarcosine ethyl ester and subsequently hydrolyzation of the ester group [14]. The use of an amino acid such as sarcosine is interesting since they combine amine and carboxylic acid groups. Amino acids were also exploited in the following study where SAX/WCX and WAX/SCX materials were developed [15]. Once again, a hypercrosslinked polyDVB-co-VBC sorbent was functionalized, in the case of SAX/WCX, the same path mentioned for the obtention of WAX/WCX material was followed, including a methylation step to quaternize the amine group from sarcosine. On the other hand, the WAX/SCX was obtained functionalizing the sorbent with taurine, which already had the amine and the sulfonic groups. In both studies a mixture of acidic and basic compounds was successfully extracted from environmental water samples. In Table 1, schemes of the structures of the mentioned materials are presented.

Table 1. Structures of the different amphoteric groups introduced in polymer-based sorbents.

Study	Type of interactions	Structure
Nadal *et al.* (2020) [14].	WAX/WCX	
Nadal *et al.* (2022) [15]	SAX/WCX	
	WAX/SCX	
Section 3.2.2.	WAX/WCX	

In the present Doctoral Thesis, a different approach to obtain the amphoteric functionalization was evaluated. In this case, a polyDVB material was functionalized with maleic anhydride through a Diels-Alder cycloaddition. Maleic anhydride is formed by a ring

which can be opened through a nucleophilic addition. Previously our research group evaluated another maleic anhydride derivative synthesized by Ronka group from Wroclaw University [16]. In that study, the ring was opened using water, obtaining a WCX sorbent, but in the second study of the present chapter (section 3.2.2), the reagent used was ethylenediamine, obtaining a WAX/WCX material with the structure shown in Table 1. The difference between this material and the amino acid (sarcosine and taurine) derivatives evaluated previously [14,15] was the distance between the functional groups, having seven atoms despite the two atoms present in amino acids. The developed material was then applied to the simultaneous extraction of acidic and basic pharmaceuticals followed by the determination through LC-HRMS.

In contrast to the to the previous chapter, the studies of this chapter will not be presented in article format because they have not been yet submitted and they are not completely prepared for its publication. In this sense, a preliminary report of the results will be presented, pointing out the most relevant aspects of the studies. In the end of this chapter, there will be also included a section where the results from the presented studies will be discussed.

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3.2.1. Core-shell polymer microspheres with strong cation-exchange character for the extraction of basic pharmaceuticals from aqueous samples

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

Core-shell polymer microspheres with strong cation-exchange character for the extraction of basic pharmaceuticals from aqueous samples

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Abstract

The application of core-shell systems is very common in analytical chemistry as packing materials for liquid chromatography columns, however, their capacity to obtain efficient separations due to their short diffusion path lengths is barely exploited in solid-phase extraction (SPE). In this sense, in the present study, core-shell polymeric microspheres were developed to be applied to SPE. The core-shell microspheres were subjected to hypercrosslinking and sulfonation reactions in order to increase the specific surface area and include sulfonic acid groups to perform strong cation-exchange (SCX) interactions.

The synthesised sorbent was evaluated for the extraction of basic pharmaceuticals from environmental water samples. After optimizing the loading pH, washing with an organic solvent and loading volume, the method was validated in terms of apparent and relative recoveries, matrix effect, limits of detection and quantification and precision, obtaining remarkable results in terms of $\%R_{app}$ (>39%) and %ME (<±29%).The method was successfully applied for the determination of the basic pharmaceuticals in environmental water samples (river, effluent wastewater and influent wastewater).

Keywords: core-shell microparticles, polymer-based sorbent, strong cation-exchange interactions, pharmaceuticals, environmental water samples.

1. Introduction

The main application for core-shell polymer microspheres within the field of analytical chemistry is as stationary phase materials in liquid chromatography (LC) [1]. Typically for core-shell constructs in LC applications, a non-porous, non-functional core is encapsulated by a functional shell; the latter, in conjunction with the mobile phase, dictates the partitioning of analytes between the stationary and mobiles phase. Optionally, the shell can be porous or non-porous in the dry state. The interest in core-shell polymer microspheres as novel LC packing materials arises primarily from the fact that thin shells on uniform microspheres promotes high chromatographic efficiency and separation power, since the diffusion path lengths for analytes are relatively short compared to conventional stationary phase materials. Whilst ultra-performance liquid chromatography (UPLC) exploits columns which have particularly high separation power, arising from the low particle diameters of the stationary phase materials used, UPLC instruments are expensive on account of the very high back pressures which need to be accommodated/constrained. In contrast, core-shell polymer microspheres with mean particle diameters higher than the stationary phase particles used in UPLC are compatible with lower pressure HPLC methods, whilst still offering performance benefits over traditional HPLC packing materials, such as improved separation power.

Recent innovations in the field of core-shell materials include the introduction of molecularly imprinted components as well as the encapsulation of silica particles, magnetic nanoparticles and quantum dots within the interiors of particles [2,3]. Relevant to the present work are synthetic approaches to core-shell materials where the cores and shells are polymeric and prepared *via* sequential synthetic steps [4,5]. For example, non-porous cores can be prepared in a first step and porous, and functional shells prepared in a second step, yielding materials that are very attractive for use as sorbents in sorptive extraction techniques such as solid-phase extraction (SPE).

The application of core-shell polymer microspheres to sorptive extraction techniques is particularly appealing for several reasons, not least of all because the thicknesses of the shells, their porous morphologies and the chemical functionality embedded within the shells can all be brought under synthetic control. In this way, not only can the selectivity of the material be controlled through rational selection of the functional groups, but high sorption capacities and efficient separations can be expected. With respect to porous morphology, hypercrosslinking chemistry has been used by us to prepare ultra-high specific surface area polymer microspheres, where specific surface areas in excess of 1,000 m^2/g arise from the presence of a large number of small pores; whilst these materials performed well in SPE, they do not have a core-shell format [6]. Since there is a correlation between specific surface area and sorption capacity, core-shell polymer microspheres where the shells are hypercrosslinked are appealing. With respect to the installation of functional groups, this can be achieved during either the construction of the shells, through copolymerization strategies, or through post-polymerisation chemical modification strategies. For example, sulfonic acid groups can be installed into some polymers using sulfonation reactions [7], yielding products with strong cation-exchange (SCX) character [8] that can be applied to the

extraction of basic compounds through ion-exchange interactions, in a fashion similar to SPE reports exploiting commercial [9,10] and non-commercial SCX sorbents [11–13].

In the present study, core-shell polymer microspheres with SCX character in the shells have been prepared using a versatile synthetic strategy which exploits free radical polymerization chemistry for the production of the cores and the shells. More specifically the precipitation polymerisation of divinylbenzene-55 (DVB-55) under dilute monomer conditions yields high quality, non-porous cores that can be shelled in a second precipitation polymerization, where divinylbenzene-80 (DVB-80) and 4-vinylbenzyl chloride (VBC) are used as comonomers. The presence of VBC residues in the shells enables the crosslinked shells to be hypercrosslinked to increase their surface area, and this is followed by the installation of SCX character using sulfonation chemistry. In this paper we describe the preparation of the core-shell polymer microspheres and their exploitation as porous, functional sorbents in the SPE of basic pharmaceuticals from river water, effluent wastewater and influent wastewater samples.

2. Experimental

2.1. Reagents and standards

Core synthesis: Divinylbenzene-55 (DVB-55) (55 %, technical grade) was purchased from Sigma-Aldrich (Saint Louis, MO, USA) and purified by percolation through a column of neutral aluminium oxide. 2,2'-Azo*bis*isobutyronitrile (AIBN) (98 %) and acetonitrile (ACN) (99.8 %, HPLC grade) were used as received from Sigma-Aldrich and VWR Chemicals (Portland, OR, USA), respectively.

Shell synthesis: The DVB-55 cores were prepared in-house. Divinylbenzene-80 (DVB-80) (80 %, technical grade) and 4-vinylbenzyl chloride (VBC) (≥ 90 %) were procured from Sigma-Aldrich and purified by passing them through a plug of neutral aluminium oxide. 2,2'-Azo*bis*isobutyronitrile (AIBN) (98 %) and acetonitrile (ACN) (99.8 %, HPLC grade) were used as received from Sigma-Aldrich and VWR Chemicals, respectively.

Hypercrosslinking reactions: Iron (III) chloride ($FeCl_3$) (reagent grade, 97 %) and 1,2-dichloroethane (DCE) (anhydrous, 99.8 %) were obtained from Sigma-Aldrich and used as received.

Sulfonation reactions: Lauric acid (99.5 %) and chlorosulfonic acid (99 %) were purchased from Sigma-Aldrich and used as received. Cyclohexane (analytical grade, ≥ 99.8 %) was sourced from Fisher Chemicals (Pittsburgh, PA, United States) and used as received.

The solvents used for washing the polymer microspheres (acetone, methanol (MeOH), petroleum ether 60-80 and petroleum ether 30-40) were of standard laboratory grade and purchased from either Sigma-Aldrich and Fisher Chemicals.

The pharmaceuticals venlafaxine (VEN), propranolol (PRO), metoprolol (MTO), atenolol (ATE) and trimethoprim (TRI) were acquired as pure standards (purity >96%) from Sigma-Aldrich. 1000 µg/L solutions of the standards were prepared in methanol (MeOH) and stored

at -20 ºC in brown bottles. Working solutions of mixtures of analytes were prepared weekly in ultrapure water and MeOH (90/10, v/v) and stored at 4 ºC in brown bottles.

HPLC grade ACN, HPLC grade MeOH, MS grade water and MS grade ACN were purchased from Carlo Erba (Val de Reuil, France). Formic acid, hydrochloric acid, acetic acid and ammonium hydroxide were purchased from Sigma-Aldrich. Ultrapure water was obtained from a Millipore water purification system (Burlington, MA, USA).

2.2. Synthesis of core-shell polymer microspheres

Figure 1 presents a scheme of the synthetic procedure to obtain the core-shell polymer microspheres.

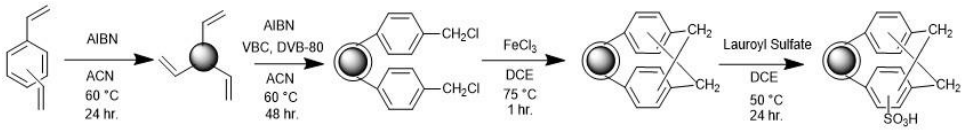

Figure 1. Synthethic procedure followed to prepare the core-shell polymer microspheres

(i) Synthesis of polyDVB-55 cores: DVB-55 (20 mL, 18.24 g, 140.10 mmol) and AIBN (0.365 g, 2.22 mmol) were dissolved in ACN (400 mL) in a 1 L Nalgene® bottle. The solution was degassed by ultrasonication for 10 minutes followed by sparging of the solution with N_2 gas for 15 minutes. The bottle was then sealed under N_2, placed onto a Stovall low-profile roller (which was housed inside a Stuart Scientific S160D incubator), and rolled about its long axis at a rate of 5-10 rpm. The temperature of the incubator was then ramped from ambient temperature to 60 °C over the course of approximately 2 hours and held at 60 °C for a further 22 hours. Once the polymerisation was complete, the bottle contents were in the form of a milky, white suspension of polymer particles. After cooling, a drop of the crude reaction mixture was spotted onto a microscope slide and the particles visualised using optical microscopy. The particles were then isolated by vacuum filtration on a 0.45 µm nylon membrane filter and washed on the filter with successive volumes (100 mL) of ACN and acetone. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing white powder (4.93 g, 27%).

(ii) Synthesis of poly(DVB-80-*co*-VBC) shells on polyDVB-55 cores: PolyDVB-55 cores (2 g), DVB-80 (3.60 g, 27.65 mmol), VBC (2.40 g, 15.73 mmol), AIBN (0.216 g, 1.32 mmol, 2 mol % relative to total number of polymerisable double bonds) and ACN (100 mL) were charged to a 500 mL Nalgene® bottle. The mixture was degassed for 10 minutes using an ultrasonic bath and the bottle contents then sparged with N_2 for a further 15 minutes. The bottle was then sealed under N_2, placed onto a Stovall low-profile roller (which was housed inside a Stuart Scientific S160D incubator) and rolled about its long axis at a rate of 5-10 rpm. The temperature of the incubator was then ramped from ambient temperature to 60 °C over the course of approximately 2 hours, and held at 60 °C for a further 46 hours. Once the polymerisation was complete, the bottle contents were in the form of a milky, white suspension of polymer particles. After cooling, a drop of crude reaction mixture was spotted

onto a microscope slide and the particles visualised using optical microscopy. The particles were then isolated by vacuum filtration on a 0.45 µm nylon membrane filter and washed on the filter with successive volumes (100 mL) of ACN and acetone. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing white powder (3.60 g, 27%).

(iii) Hypercrosslinking of poly(DVB-80-*co*-VBC) shells. Core-shell polymer microspheres (1.5 g) were added to a three-necked, round-bottomed flask together with anhydrous DCE (45 mL). An overhead stirrer and reflux condenser were fitted to the flask, the suspension of microspheres in DCE was sparged with N_2 gas and the mixture stirred for 1-2 hours at a rate of 50 rpm. Once the microspheres were fully solvated, $FeCl_3$ (0.57 g, 3.51 mmol, 1:1 molar ratio relative to polymer-bound chloromethyl groups) was added to the reaction vessel. The temperature and stirring rate were increased to 75 °C (from ambient temperature) and 80 rpm, respectively, for a further 1-2 hours. After this time, the reaction mixture was dark purple in colour. After cooling, the hypercrosslinked microspheres were isolated by vacuum filtration on a 0.45 µm nylon membrane filter and washed on the filter with successive volumes (50 mL) of each of the following solvents: ACN, methanol, 0.01 M $NaHCO_3$, distilled water and acetone. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing, yellow powder (1.22 g, 81 %).

(iv) Sulfonation of shells: For the sulfonation reactions, lauroyl sulfate was prepared freshly immediately prior to the sulfonation reactions. In a typical sulfonation reaction, lauric acid (0.335 g, 1.67 mmol) was dissolved in cyclohexane (12 mL). Chlorosulfonic acid (0.11 mL, 0.193 g, 1.652 mmol) was added and the mixture stirred for one hour at ambient temperature under an inert atmosphere. Whilst the lauroyl sulfate was being prepared, hypercrosslinked core-shell polymer microspheres (1.25 g) and DCE (30 mL) were charged to a three-necked, round-bottomed flask fitted with an overhead stirrer and reflux condenser. The mixture was then stirred at ambient temperature for one hour under N_2 to wet the microspheres. The lauroyl sulfate solution was then injected into the three-necked flask *via* syringe. The temperature was raised to 50 °C and the mixture stirred for 24 hours at 80 rpm, after which time the reaction mixture was red/brown in colour. After cooling, the particles were isolated by vacuum filtration on a 0.45 µm nylon membrane filter and washed on the filter with successive volumes (50 mL) of petroleum ether 60-80 and petroleum ether 30-40. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing, orange powder (1.27 g).

To calculate the ion-exchange capacity of the SCX sorbent (reported as mmol of sulfonic acid groups per gram of dry polymer), 50 mg of sorbent was stirred overnight in 15 mL of 0.1 M aqueous NaOH. The polymer was then isolated by filtration, the polymer washed with deionised water, and the combined liquids titrated using 0.1 M aqueous HCl.

2.3. SPE procedures

A 10 µm polyethylene frit (Symta, Madrid, Spain) was inserted into an empty 6 mL SPE cartridge (Symta), followed by a 2 µm stainless steel frit (Sigma-Aldrich) to prevent sorbent loss, followed by 150 mg of sorbent, followed by a second 10 µm polyethylene frit. The sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 3.

Sample volumes were 100 mL for river water samples, 50 mL for effluent wastewater and 25 mL for influent wastewater. Samples were adjusted to pH 3 and loaded onto the cartridges at an approximate flow rate of 10 mL/min. 5 mL of MeOH was used in the washing step and the elution step consisted of 5 mL of 5% NH_4OH in MeOH. The eluates were evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1 mL of water/ACN (95/5, v/v). Prior to SPE, river water samples were filtered with a 0.45 µm nylon membrane filter (Scharlab). Effluent and influent wastewater samples were filtered using a 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK) and a 0.45 µm nylon membrane filter.

2.4. Chromatographic conditions

An Agilent 1200 series liquid chromatograph equipped with a binary pump, an autosampler, an automatic injector and a diode array detector (DAD) (Agilent, Waldbronn, Germany) was used to analyse the SPE eluates. A Luna Omega Polar C_{18} 100 column (150 x 3.0 mm, 5 µm particle size) and a 3 mm precolumn containing the same stationary phase were both supplied by Phenomenex (Torrance, CA, United States). The flow rate was set at 0.4 mL/min, the injection volume was 20 µL, and the column temperature was 30 ºC. The mobile phases were ultrapure water adjusted to pH 3 with HCl (solvent A) and ACN (solvent B). The gradient began with 5% of B, increasing to 40% of B within 8 min., increasing to 100% of B within 14 min., holding at 100% B for 3 min., before returning to the initial conditions over 1 min. The initial conditions were then maintained for 3 min. to equilibrate the column. Two different wavelengths were used to detect the pharmaceuticals. ATE and MTO were quantified at 230 nm, whereas TRI, VEN and PRO were quantified at 210 nm.

The analysis of water samples was performed by LC-HRMS using an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) equipped with an automatic injector (Accela Autosampler) and a quaternary pump. The LC system was coupled to an Exactive Orbitrap (Thermo Scientific) with a heated electrospray ionisation (HESI) source and a high-energy collision dissociation cell (HCD) to fragment the analytes for confirmation purposes. Solvent A was changed to water with 0.1% of HCOOH, however the rest of chromatographic conditions were the same as used for LC-DAD.

The optimised conditions for ionisation were as follows: sheath gas flow rate, 40 arbitrary units (AU); auxiliary gas flow rate, 20 AU; sweep gas, 0 AU; spray voltage, 4 kV; capillary voltage, 45 V; tube lens voltage, 65 V; skimmer voltage, 22 V, capillary temperature, 330 ºC; heater temperature, 350 ºC.

For data acquisition, the mass range selected was 50 – 450 m/z, and two alternative scan events were employed; the first consisted of a full scan at 50,000 FWHM (Full Width Half Maximum) with an injection time of 250 ms, whereas the second consisted of a fragmentation scan at 10,000 FWHM with an injection time of 50 ms and a collision voltage of 20 eV. The protonated ion was measured with a mass accuracy window of 5 ppm. Table S1 includes information on the pK_a values, protonated ions, and the fragment ions and the ratios between them, for the analytes acquired for quantification and confirmation purposes. Instrumental calibration curves ($R^2 > 0.995$) were obtained through weighted calibration

regression for the compounds: the lower limit was 0.05 µg/L for TRI, MTO and PRO, and 0.1 µg/L for ATE and VEN. The upper limit was 500 µg/L in all cases.

3. Results and discussion

3.1 Synthesis of the sorbent

Core-shell polymer microspheres with hypercrosslinked shells and SCX character were synthesised using a four-step methodology, as follows: (i) Precipitation polymerisation of DVB-55 to give non-porous polyDVB-55 cores; (ii) Precipitation polymerisation of DVB-80 and VBC in the presence of polyDVB-55 cores to give core-shell polymer microspheres; (iii) Hypercrosslinking of the shells in a solvent-expanded state, using $FeCl_3$ as a Lewis acid; (iv) Sulfonation of the shells using lauroyl sulfate.

3.2. Characterisation of the sorbent

The physical format, porous morphology and chemical constitution of the sorbent was characterised using scanning electronic microscopy (SEM), Fourier Transform infrared (FTIR) spectroscopy and nitrogen sorption analysis. The ion-exchange capacity was determined by titration, and batch-to-batch reproducibility evaluated. In respect of batch-to-batch reproducibility, three different batches of polymer were synthesised and the precision between batches evaluated through SPE of the basic analytes from ultrapure water samples. The RSD% (n=3) was lower than 18% for all analytes.

Figure 2 shows SEM images of the core microspheres (Figure 2a) and the core-shell polymer microspheres (Figure 2b). The diameters of the core microspheres range from 2.5 to 2.7 µm and the diameters of the core-shell polymer microspheres range from 3.0 to 3.2 µm. This gives a mean shell thickness of 0.25 µm. Furthermore, it can be seen from the SEM images that both sets of microspheres are relatively uniform. Whilst not monodisperse, ImageJ analysis of the core-shell polymer microspheres reveals that the particles are quasi-monodisperse (size dispersity = 12%).

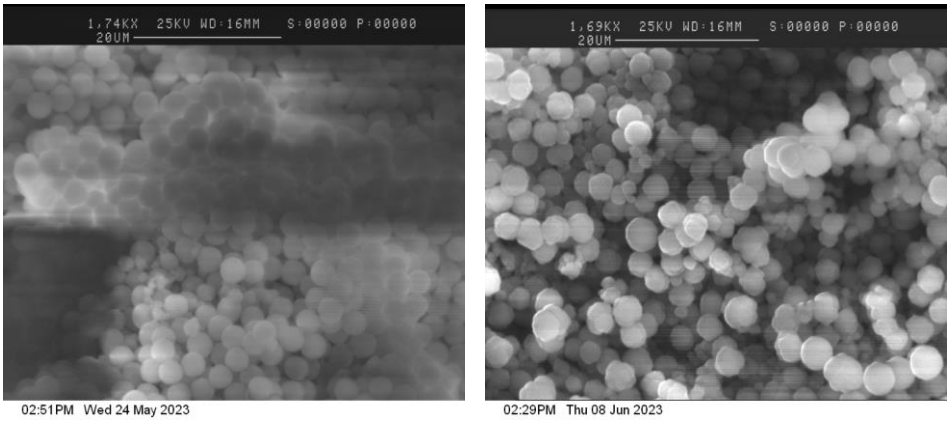

Figure 2. SEM images of core polymer microspheres (left) and core-shell polymer microspheres (right).

Figure S1 presents the FTIR spectra acquired for the core polymer microspheres (Figure S1a), the core-shell polymer microspheres before hypercrosslinking (Figure S1b), the hypercrosslinked core-shell polymer microspheres (Figure S1c) and the hypercrosslinked core-shell polymer microspheres with SCX character (Figure S1d). All four spectra are consistent with the proposed structures. In these spectra, the C-Cl band at 1265 cm^{-1}, which is assignable to VBC residues, is particularly diagnostic. A signal at around 1265 cm^{-1} appears in the FTIR spectrum of the poly(DVB-80-*co*-VBC) shells on polyDVB-55 cores (Figure S1b), and diminishes in relative intensity for the hypercrosslinked derivative (Figure S1c) because the hypercrosslinking reaction consumes chloromethyl groups. Thus, the signal at 1265 cm^{-1} verifies the incorporation of VBC into the microspheres through copolymerization and is a very convenient way to track the progress and success of the hypercrosslinking reaction.

Nitrogen sorption analysis was used to measure the specific surface areas of the core-shell materials. Compared to the core microspheres, which are non-porous in the dry state specific surface area < 5 m^2/g), the hypercrosslinked core-shell polymer microspheres with SCX character have a very well-developed porous morphology (specific surface area = 550 m^2/g). When one takes into account the fact that the cores are non-porous, one can estimate that the specific surface of the shells is 1380 m^2/g, undoubtedly because the shell contain a large number of small pores. This characteristic, combined with the facts that we are dealing with a core-shell format and that the ion-exchange capacity (IEC) is high (3.7 mmol/g of sulfonic groups, as measured by titration), suggests that the final material is an attractive SPE sorbent candidate. The calculations made to obtain the surface area and the IEC are presented in supplementary material.

3.3. Optimisation of the SPE protocol

Guided by the previous experiences of our group when working with other polymeric SCX sorbents [11,14], the initial conditions for the SPE were as follows: loading with 10 mL of ultrapure water adjusted to pH 3 or pH 5, and elution with 5 mL of 5% NH_4OH in MeOH. To evaluate the yields of the extractions, the $\%R_{SPE}$ values were calculated as the ratio of the measured concentration after SPE to the theoretical concentration when working with ultrapure water.

As can be observed in Figure 3, the recoveries were significantly lower at pH 5 for all compounds, except for ATE. The recoveries at pH 3 were higher than 87% in all cases, meanwhile at pH 5 the recoveries were lower than 60% for all compounds except for ATE. Since pH 3 provided the best results, it was selected as optimal pH. This result can be rationalized on the basis that the extraction of basic compounds with pK_a values ranging from 7.1 to 10.1 on an SCX sorbent is improved when the pH of the loading solution is lower. This finding is in agreement with other studies on SCX sorbents where basic compounds were also loaded at pH 3 [11,14,15].

The use of a washing step involving an organic solvent can be included in an SPE protocol to improve the selectivity of the extraction and reduce the matrix effects. In the

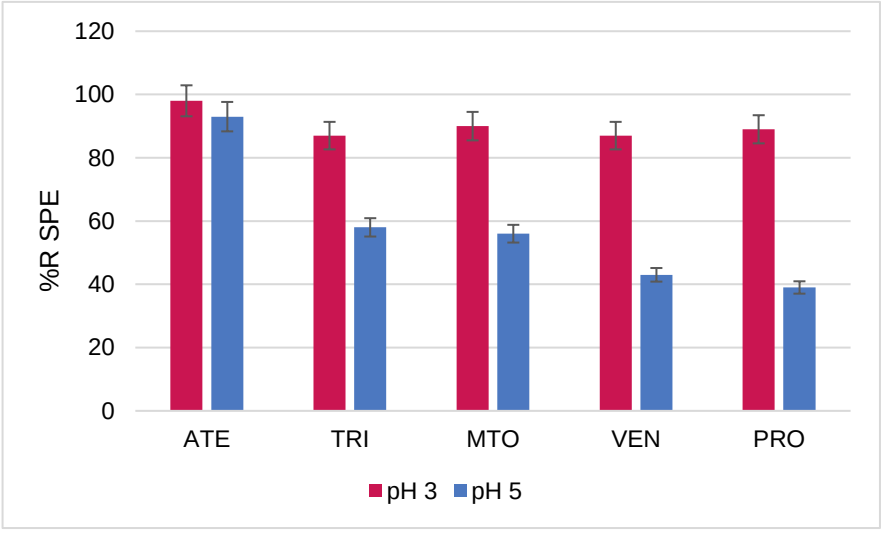

Figure 3. SPE recoveries when 10 mL samples were loaded at pH 3 and pH 5 without the inclusion of a washing step.

present study, the use of 1 and 5 mL of MeOH as washing solvent were evaluated; the $\%R_{SPE}$ values are presented in Figure 4. As can be observed, there is a slight decrease in the $\%R_{SPE}$ values (less than 5%) for all compounds, except TRI, when the volume of washing solvent is increased to 5 mL. This decrease in recovery was considered to be largely insignificant, so 5 mL of MeOH was selected as the washing step. Previous studies report the use of MeOH as washing solvent at different volumes (3 – 10 mL) when working

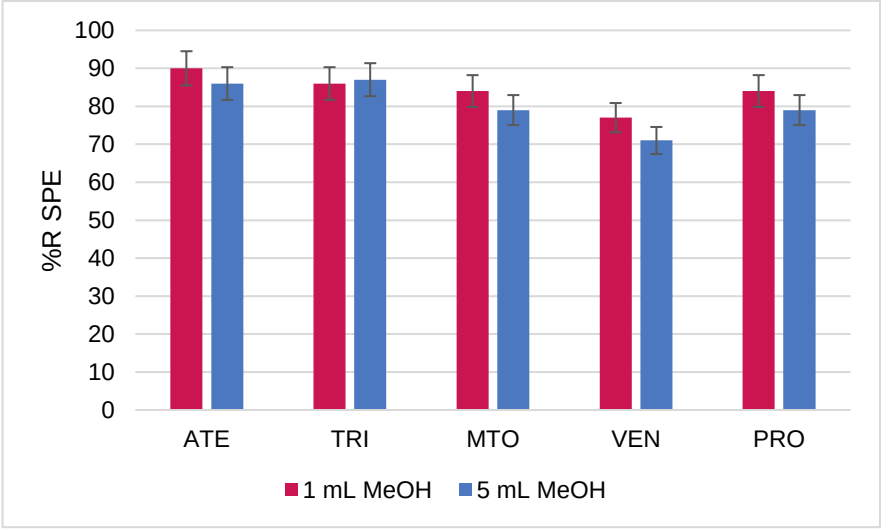

Figure 4. SPE recoveries when 10 mL samples were loaded at pH 3 with the inclusion of 1 mL and 5 mL of MeOH as washing step.

with SCX sorbents [13,14,16]. Regarding the elution step, 5 mL of 5% NH_4OH in MeOH gave good results and was consistent with other studies in which NH_4OH solutions at various concentrations were used with other SCX sorbents [14–17].

The loading volume was evaluated by increasing the loading volume from 25 to 100 mL. Since the recoveries remained higher than 70% when 100 mL of ultrapure water was percolated, this loading volume was selected going forward.

The selectivity of the sorbent for basic compounds through cation-exchange interactions was evaluated using a mixture of the five basic pharmaceuticals listed together with seven acidic pharmaceuticals (diclofenac, flurbiprofen, valsartan, clofibric acid, fenoprofen, bezafibrate and naproxen). It was observed that only the basic compounds were retained through ion-exchange interactions; the acidic compounds bound to the sorbent during the loading step through reversed-phase interactions but could be eliminated easily from the sorbent in the washing step with MeOH ($\%R_{SPE} < 25\%$).

3.4. Comparison of core-shell polymer microspheres with non-core-shell equivalents

Our hypothesis is that practically useful advantages will arise from having ion-exchange groups localized towards the surfaces of polymer microspheres. Whilst benchmarking the new core-shell construct materials against non-core-shell materials offering similar ion-exchange character is very difficult, given the range of variables (surface area, particle size, ion-exchange capacity, etc.), in a previous study reported by our group a series of polymeric SCX sorbents were synthesised by precipitation polymerisation and non-aqueous dispersion polymerisation [11]. The best performing sorbent in that study was a material prepared by non-aqueous dispersion polymerisation, which had a specific surface area of 1020 m^2/g and a sulfonic acid content of 0.6 mmol/g. Although this material did not have a core-shell format, the material performed well; for the SPE of several basic compounds (including ATE, TRI, MTO and PRO) from 50 mL of ultrapure water, with 5 mL of MeOH as washing step, the recoveries were higher than 70%. However, it was necessary to use 3x5 mL of 5% NH_4OH in MeOH in the elution step, compared to 1x5 mL in the present work. Furthermore, larger samples volumes can be accommodated by the new sorbent (100 mL). It is not unreasonable to suppose that the superior performance of the new sorbent arises, at least in part, because of its core-shell format. In addition, the cartridges packed with the core-shell sorbent worked well in an off-line SPE format – there were no flow through issues or problems with clogging/blocking of the cartridge – and it will be interesting to evaluate the sorbent in online SPE.

3.5. Method validation

The optimised method was validated in terms of apparent recovery ($\%R_{app}$) at two concentration levels, matrix effect (%ME), accuracy, detection and quantification limits, linear range, and precision for the determination of the basic pharmaceuticals in river water, effluent wastewater and influent wastewater samples. Bearing in mind our previous experiences, it was decided to reduce the loading volume to 50 mL for effluent wastewater samples, and to 25 mL for influent wastewater samples.

The apparent recoveries were determined by dividing the measured concentration of the spiked samples (with the blank concentration subtracted) by the theoretical concentration. The $\%R_{app}$ were obtained by spiking the water samples at two concentration levels: 0.5 and 5 µg/L for river water samples, 1 and 10 µg/L for effluent wastewater samples and 2 and 20 µg/L for influent wastewater samples. Table 1 shows that the $\%R_{app}$ for all compounds were higher than 39%. It can be observed that PRO provided very high recoveries with river water samples (84 and 91%) and that the values decrease significantly when the complexity of the sample is increased (67 and 58% for effluent, 45 and 50% for influent). This trend was not observed for any of the other compounds, moreover the recoveries of ATE, TRI and MTO for influent samples were slightly higher than for effluent samples. This can be explained by the fact that loading volume for effluent wastewater samples was 50 mL whereas the loading volume for influent wastewater samples was 25 mL. Gilart *et al.* [14] reported recoveries ranging from 39 to 97% for effluent samples and from 24 to 97% for influent samples when determining basic pharmaceuticals using a non-commercial polymeric SCX sorbent. Krizman *et al.* [18] extracted opiods from river water, effluent and influent wastewater samples by applying an Oasis MCX sorbent in the SPE. The recoveries obtained were 73 – 109% for river water, 78 – 116% for effluent wastewater and 44 – 95% for influent wastewater samples.

The matrix effect was calculated by comparing the concentration obtained upon spiking (0.5 µg/L for river water samples, 1 µg/L for effluent samples and 2 µg/L for influent samples) a non-spiked sample after SPE, subtracting the blank signal from the theoretical concentration. A negative value indicates signal suppression while a positive value indicates signal enhancement. It can be observed in Table 1 that, in most cases, there was signal suppression, and that low matrix effects were observed considering that values in the range of ±20% are considered acceptable. For river water samples, only PRO presented values higher than ±20% (+24%), for effluent samples TRI and VEN had values of -29 and -24%, respectively, and for influent samples VEN and PRO had values of -24 and +25%, respectively. Despite these values being higher than ±20%, they are still quite low. Prosen *et al.* [15] obtained %ME values ranging from -3 to -41% for river water samples, from -46 to +10% for effluent and from -49 to +16% for influent samples when determining drugs of abuse with Oasis MCX; even then, it should be noted that in the case of influent samples, a 1/5 dilution was required to reduce the matrix effect. When using Oasis HLB for the extraction of pharmaceuticals from wastewater, Mostafa *et al.* [19] observed matrix effects higher than ±20% for most of their compounds. When working with sorbents like Oasis HLB,

Table 1. Apparent recoveries at two concentration levels and matrix effects. Concentration values are reported in section 3.4.

	River			**Effluent wastewater**			**Influent wastewater**		
	$\%R_{app}$ high	$\%R_{app}$ low	%ME	$\%R_{app}$ high	$\%R_{app}$ low	%ME	$\%R_{app}$ high	$\%R_{app}$ low	%ME
ATE	58	55	-2	48	46	-17	63	69	-20
TRI	56	54	-18	39	45	-29	56	56	-19
MTO	65	58	+7	51	54	-18	62	56	-18
VEN	57	44	+1	61	69	-24	42	47	-24
PRO	84	91	+24	67	58	+20	45	50	+25

it is possible to retain a wide range of compounds, however it is not possible to introduce an exhaustive washing protocol and this leads to significantly high matrix effects.

Accuracy was assessed by determining four samples at 0.5 µg/L for river water samples, 1 for effluent wastewater samples and 2 µg/L for influent wastewater samples. Relative recovery was determined as the percentage of the mean experimental concentration and the actual spiked concentration. The results show good accuracy with relative recoveries ranging from 92 to 108%.

The method detection limit (MDL) and quantification limits (MQL) were estimated by applying the preconcentration factor and the apparent recoveries into the instrumental limits. More specifically, the instrumental detection limit is defined as the concentration at which the signal-to-noise ratio was higher than 3, with one of the fragments producing a signal exceeding 10^3. Furthermore, the instrumental quantification limit (MQL) was selected as the lowest point on the calibration curves, ensuring a signal-to-noise ratio of at least 10. Table 2 shows the MDL and MQL values for the selected basic pharmaceuticals; it can be seen that the values are in the low ng/L range, and in some cases are lower than 1 ng/L, demonstrating low detection limits.

Table 2. Method detection and method quantification limits

	River		Effluent		Influent	
	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)
ATE	0.2	1	1	3	2	6
TRI	0.1	0.5	3	5	5	10
MTO	0.1	0.5	2	4	4	10
VEN	0.3	1	2	4	3	8
PRO	0.1	0.3	1	3	3	8

The precision was evaluated as the repeatability (intra-day precision) and reproducibility between days (inter-day precision) (n=4 in both cases). The %RSD obtained was always lower than 13%, meaning that the precision was good.

3.6. Analysis of samples

The validated method was applied to the determination of the five basic pharmaceuticals in four samples of river water, effluent wastewater and influent wastewater. Regarding the low levels of matrix effects observed, it was decided to quantify the compounds through external calibration curves and apply the apparent recoveries calculated in the previous section. To confirm the presence of the compounds, three different parameters were used: the mass error of the protonated ion had to be lower than 5 ppm, the signal of at least one fragment ion had to be higher than 10^3 AU and at the ratio between one fragment ion and the protonated ion had to be within ± 40 % relative deviation (following the regulations listed in 2021/808/EC [20]).

Table 3 shows the natural occurrence of the analytes in the matrixes selected and the uncertainty. River water was the matrix where the lowest occurrence was found, and TRI was the compound found at the highest concentration (398 ng/L). It can be highlighted that all of the compounds were detected in all four samples, except for ATE, MTO and TRI which

were not detected in one of the samples. In several samples, the presence of the compounds could not be confirmed because the ratios between the fragment ions and the protonated ions did not meet the aforementioned requirements. The concentrations of ATE, MTO, PRO and TRI were slightly higher than the concentrations reported by Nadal *et al.* [21]. In that study, TRI was not quantified in any sample, meanwhile ATE, PRO and MTO were not found in concentrations above 45 ng/L. Lima *et al.* [22] also found lower concentrations of VEN in river water samples from Portugal, where VEN was not detected (LOD = 24 ng/L). The occurrence of VEN and MTO found by Egli *et al.* [23] in river water samples from Germany was similar to the data in the present study, ranging from 24 to 154 ng/L for VEN and from MQL to 284 ng/L for MTO.

Table 3. Range of concentrations and uncertainties (ng/L) obtained after the analysis of river water, effluent and influent wastewater samples.

	River	Effluent wastewater	Influent wastewater
ATE	<MDL – 178 ± 19	1092 ± 184 – 5453 ±643	332 ± 62 – 4031 ± 490
TRI	<MDL – 279 ± 33	282 ± 32 – 6730 ± 805	257 ± 32 – 8561 ± 705
MTO	<MDL – 165 ± 11	65 ± 9 – 6661 ± 768	272 ± 32 – 7995 ± 985
VEN	<MQL – 320 ± 34	357 ± 42 – 4525 ± 548	387 ± 43 – 7370 ± 888
PRO	<MQL – 268 ± 33	68 ± 8 – 3759 ± 321	139 ± 16 – 4279 ± 489

Effluent wastewater samples showed higher concentrations than river water samples, thus it was possible to quantify all five analytes in all four samples. It can be highlighted that one of the samples presented a concentration higher than 3,500 ng/L for all compounds, reaching levels higher than 6,500 ng/L for TRI and MTO. Meanwhile, the other samples did not present higher concentrations than 1,500 ng/L. Salas *et al.* [24] also determined ATE, TRI, MTO and PRO in effluent wastewater samples from Tarragona; similar concentrations were reported for ATE (1,037 – 2,551 ng/L), but lower concentrations were reported for the other compounds (ranging from 80 to 469 ng/L). ATE, VEN and PRO were also determined by Gómez-Canela *et al.* [25] in effluent wastewater samples from Barcelona. The concentrations of ATE were similar (MQL – 1510 ng/L), although PRO and VEN were determined at lower concentrations (<179 ng/L for PRO and 104 – 820 ng/L for VEN).

Influent samples presented the highest occurrences of the analytes, reaching levels higher than 5,000 ng/L for TRI, MTO or VEN. On the other hand, one of the samples presented concentrations for all the compounds lower than 400 ng/L. The occurrences found agree with data reported by Gilart *et al.* [14] when determining ATE, TRI and MTO in similar samples with concentrations ranging from 116 to 3,293 ng/L for these three compounds. Moreover, the occurrences reported by Vergeynst *et al.* [26] for TRI and VEN in influent samples from Belgium were close to the lower limits of the present study, reporting concentrations from 158 to 228 ng/L for TRI and from 119 to 480 ng/L for VEN. The concentrations found by Li *et al.* [27] in samples from Scotland were in the range from 155 to 2,100 ng/L for TRI, showing similar levels to the present study, however, PRO was one of the target compounds and was not detected.

4. Conclusions

In this study, hypercrosslinked core-shell polymer microspheres with SCX character have been prepared and applied successfully to the SPE of basic pharmaceuticals in environmental water samples, to very good effect thanks to the loading with 100 mL of water at pH 3, which enhanced the retention of the analytes through SCX interactions. The implementation of a clean-up step into the SPE protocol suppressed the matrix effects, even when very complex matrixes, such as influent wastewater samples, were used. Normally, core-shell materials are applied as stationary phases in LC, but the present work shows that they are promising materials for exploitation in SPE too.

Acknowledgements

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Suplementary data

Calculation of the effective surface area

$$V_{core} = \frac{4}{3}\pi r_{core}^3 = \frac{4}{3}\pi \cdot 1.27^3 = 8.48\ \mu m^3$$

$$V_{core-shell} = \frac{4}{3}\pi r_{core-shell}^3 = \frac{4}{3}\pi \cdot 1.49^3 = 14.10\ \mu m^3$$

$$V_{shell} = V_{core-shell} - V_{shell} = 14.10 - 8.48 = 5.62\ \mu m^3$$

$$Shell\ contribution\ to\ total\ volume = \frac{V_{shell}}{V_{core-shell}} = \frac{14.10}{5.62} = 0.40$$

$$Effective\ surface\ area = \frac{Surface\ area}{Shell\ contribution} = \frac{550\ m^2/g}{0.40} = 1383\ m^2/g$$

Calculation of the ion exchange capacity (IEC)

$$n_{NaOH} = n_{HCl} + n_{SO_3^-};\ V_{NaOH} \cdot M_{NaOH} = V_{HCl} \cdot M_{HCl} + n_{SO_3^-}$$

$$n_{SO_3^-} = V_{NaOH} \cdot M_{NaOH} - V_{HCl} \cdot M_{HCl}$$

$$n_{SO_3^-} = 0.015\ L \cdot 0.0984\ \frac{mol}{L} - 0.0129\ L \cdot 0.0995\ \frac{mol}{L} = 0.19\ mmol$$

$$IEC = \frac{n_{SO_3^-}}{m_{core-shells}} = \frac{0.19\ mmol}{0.0523\ g} = 3.68\ mmol/g$$

Tables and figures

Table S1.-: pK_a values and information about HRMS acquired ions and fragment ion/protonated ion ratios.

Compound	pK_a	Protonated ion	Fragment ion	Ratio (fragment ion/ protonated ion)
ATE	9.6	267.17020	190.08649	0.078
			145.06514	0.032
TRI	7.1	291.14517	245.10388	0.067
			261.09866	0.050
MTO	9.7	268.19061	116.10733	0.092
			191.10712	0.034
VEN	10.1	278.21133	260.20111	0.022
			58.06614	0.271
PRO	9.4	260.16418	116.10773	0.124
			183.08057	0.065

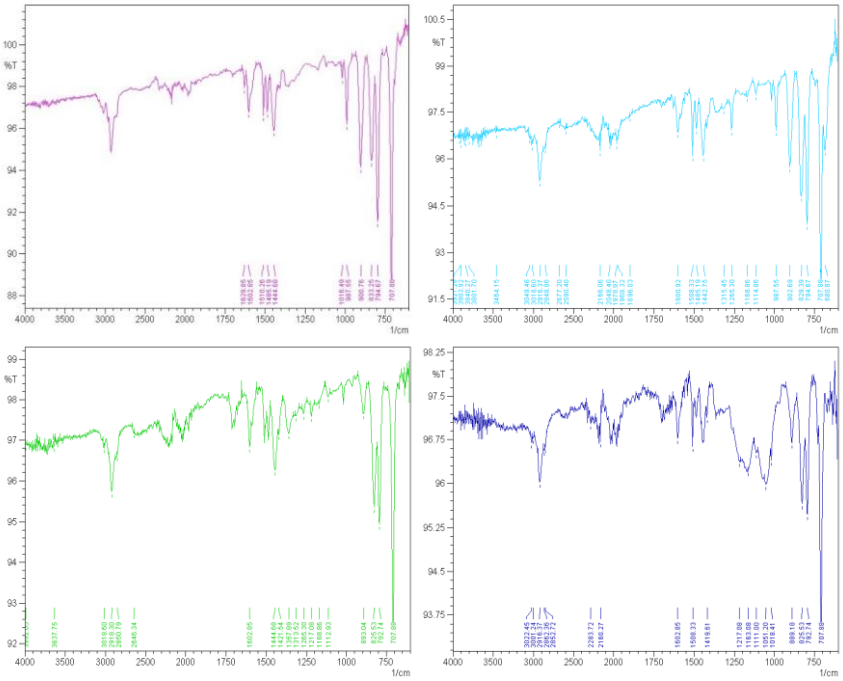

Figure S1. FTIR spectra: a) core polymer microspheres, b) core-shell polymer microspheres, c) hypercrosslinked core-shell polymer microspheres, and d) hypercrosslinked core-shell polymer microspheres with SCX character.

3.2.2. A facile route for the chemical functionalisation of polydivinylbenzenes and the application of an amphoteric polydivinylbenzene derivative to the simultaneous solid-phase extraction of acidic and basic drugs from water samples

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

Core-shell polymer microspheres with strong cation-exchange character for the extraction of basic pharmaceuticals from aqueous samples

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Abstract

Mixed-mode ion-exchange sorbents with amphoteric moieties are interesting as they can be applied to the simultaneous extraction of acidic and basic analytes. In the present study, an interesting route to obtain this type of functionalization is presented. A polydivinylbenzene material prepared through precipitation polymerization was easily modified through Diels-Alder cycloaddition with maleic anhydride, followed by the ring-opening reaction with ethylenediamine. The final material was functionalized with primary amines and carboxylic acid groups, being able to perform weak anion- (WAX) and weak cation-exchange (WCX) interactions.

The WAX/WCX material was evaluated for the simultaneous solid-phase extraction (SPE) of acidic and basic pharmaceuticals. The SPE parameters optimized were loading pH, loading volume, washing step and elution step. The optimized method was subjected to validation in terms of relative and apparent recoveries, detection and quantification limits, precision and matrix effect. It can be highlighted that the apparent recoveries were higher than 30% and the matrix effects were lower than ±25 in most cases. The developed method was applied to the simultaneous determination of the acidic and basic pharmaceuticals in river, effluent wastewater and influent wastewater samples.

Keywords: core-shell microparticles, polymer-based sorbent, strong cation-exchange interactions, pharmaceuticals, environmental water samples.

1. Introduction

In the recent years, the awareness surrounding contaminants of emerging concern (CECs) has been increasing due to their potential to harm human health and environmental species. This group includes compounds with wide diversity such as per-and polyfluoroalkyl substances (PFAs), endocrine disruptors or pharmaceuticals and personal care products, among others. All of them have in common the emerging interest on getting information about their occurrence and toxicological effects. [1,2]. In the recent years several studies have been performed to determine and evaluate the risk of the emerging contaminants in different areas around the world [1–5].

As have been mentioned, pharmaceuticals are considered CECs. Pharmaceuticals reach the environment mainly through wastewater disposal, since wastewater treatment plants cannot effectively remove them [6,7], becoming dangerous to environment and human health [8–10].

For these reasons, the development of selective and reliable methods for the determination of pharmaceuticals is necessary. Most of pharmaceuticals have acidic/basic properties, for instance, β-blockers, opioids, and antidepressants use to be basic, meanwhile fluoroquinolones, angiotensin II receptor blockers (“sartan drugs”) and non-steroidal anti-inflammatory drugs (NSAIDs) tend to be acidic. This acidic/basic character can be exploited to improve the extraction by using mixed-mode ion-exchange sorbents, sorbents that combine reversed-phase and ion-exchange interactions [11]. There are many studies where acidic or basic pharmaceuticals have been extracted using commercial mixed-mode ion-exchange sorbents [12–14]. The exploit of ion-exchange interactions allows to improve the selectivity of the analysis when a washing step with an organic solvent is included that removes the compounds retained through ion-exchange interactions. These studies applied cation-exchange or anion-exchange, being able to determine only acidic or basic compounds. To determine both types of compounds with commercial sorbents the combination of cation-exchange and anion-exchange sorbents in the same cartridge [15] or the connection in series [16] has been explored. In the recent years, the development of amphoteric in-house sorbents that combine both moieties has been reported [17–22] allowing the extraction of acidic and basic compounds with one material, being them silica-[18,20–24] or polymer-based [17,19]. Some silica-based sorbents that combined strong cation-exchange and strong anion-exchange moieties [20,23,24] have been evaluated in our group in the recent years. In those studies it was observed that acidic analytes were rinsed in the washing step with MeOH, fact that was attributed to the concentration of charges from the functional groups of the sorbent and the analytes may easier for MeOH to disrupt the interactions between acidic analytes and the sorbent. In another study, a polymeric sorbent combining weak moieties (WAX/WCX) was developed [25], in this case it was possible to introduce a washing step with 1 mL of MeOH. Regarding these results, the development of amphoteric sorbents can be better when weak exchange groups are included. In the mentioned study with WAX/WCX [25], the amino acid sarcosine was included on a divinylbenzene-vinylbenzene chloride polymer, so the distance between the

two groups was two carbon atoms. Another approach evaluated in the group was the preparation of polymeric SCX/WAX and WCX/SAX sorbents with quaternized sarcosine and taurine respectively [17]. Both studies used amino acids to include the amphoteric moieties, meaning that the distance between the two groups was two atoms. In the present study, a new WAX/WCX sorbent was developed using an unusual but widely-applicable approach which can most likely be applied to all polydivinylbenzenes and some other materials too. This approach involves the installation of polymer-bound anhydride units using a Diels-Alder cycloaddition, followed by ring-opening of the anhydride units using ethylenediamine. In related works, polymer-bound anhydride units have been ring-opened with water to give a WCX material [26].

The developed sorbent was applied in solid-phase extraction (SPE) followed by liquid chromatography with high resolution mass spectrometry detection (LC-HRMS) for the determination of acidic and basic pharmaceuticals in river, effluent wastewater and influent wastewater samples.

2. Experimental

2.1. Reagents and standards

Solid standards of five basic pharmaceuticals: venlafaxine (VEN), atenolol (ATE), metoprolol (MTO), propranolol (PRO) and trimethoprim (TRI) and four acidic pharmaceuticals: diclofenac (DIC), bezafibrate (BEZ), valsartan (VAL) and diclofenac (DIC) and the metabolite of clofibrate (clofibric acid (CLO)) were acquired from Sigma-Aldrich (Sant Louis, MO, USA) as pure standard (purity >96%). Solutions of 1000 µg/L were prepared in methanol (MeOH) and stored in the freezer in dark bottles (-20 ºC). Each week, working solutions with all analytes were prepared in a mixture of ultrapure water and MeOH (90/10, v/v) and stored in the refrigerator in dark bottles (4 ºC).

Acetonitrile (ACN) of HPLC and MS grade, methanol (MeOH) of HPLC grade and water of MS grade were purchased from Carlo Erba (Val de Reuil, France). HCOOH, HCl, acetic acid, and NH_4OH were purchased from Sigma-Aldrich. A Millipore purification system (Burlington, MA, USA) was used to obtain ultrapure water.

For the synthesis of the WAX/WCX sorbent, divinylbenzene-80 (DVB-80) (technical grade, 80 %), was supplied by Sigma-Aldrich. Prior to use, it was passed through a column containing aluminium oxide (neutral, activated, from Sigma-Aldrich) to remove the polymerisation inhibitor. The free radical initiator 2,2′-azo*bis*isobutyronitrile (AIBN) (98 %) was used as received from Sigma-Aldrich. Acetonitrile (ACN) (99.9 %, HPLC grade) was supplied by VWR Chemicals and toluene (≥ 99.3 %, lab reagent) was supplied by Sigma-Aldrich; both were used as received. For the chemical modification of the polydivinylbenzene microspheres, maleic anhydride (MA) (≥ 98 %), purchased from Fluka, was used as received. For the ring-opening of polymer-bound anhydride units, ethylenediamine (EDA) (≥ 99 %) was purchased from Sigma-Aldrich and used as received.

2.2. Synthesis of the WAX/WCX sorbent

The WAX/WCX sorbent was synthesized *via* a three-step synthetic protocol (presented in Figure 1): (i) Precipitation polymerisation (PP) of DVB-80 to give polyDVB-80 microspheres; (ii) Chemical modification of the polyDVB-80 microspheres *via* a Diels-Alder (DA) cycloaddition with MA; (iii) Ring-opening of polymer-bound anhydride units with EDA. Figure 1 shows the overall reaction scheme.

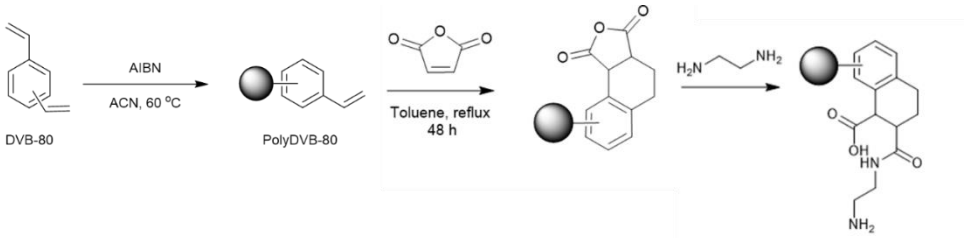

Figure 1. Reaction scheme outlining the synthesis of the WAX/WCX sorbent.

ATR-FTIR was carried out using a ThermoScientific Nicolet iS5 FTIR spectrometer. Samples were scanned 16 times each, over the range 400 – 4000 cm $^{-1}$. Spectra were recorded and analysed using Omnic Series Software from ThermoScientific. Scanning electron microscopy (SEM) was carried out using a Cambridge Stereoscan 90 Scanning Electron Microscope; samples were sputter-coated with gold prior to imaging using a Polaron SC500A Sputter Coater. Average particle sizes were determined (n = 100) using ImageJ (v1.54, National Institutes of Health) image processing software. Nitrogen sorption analysis was carried out using a Micrometrics ASAP 2020 Porosimeter.

2.2.1. Synthesis of polyDVB-80 microspheres

ACN (300 mL) and toluene (100 mL) (75/25 v/v) were added to a Nalgene bottle (500 mL). DVB-80 (8 mL, 7.312 g, 56.16 mmol, 2 vol % relative to solvent) was added to the solvent mixture and the monomer solution was ultrasonicated for 15 minutes. AIBN (0.146 g, 0.89 mmol, 2 wt. % relative to DVB-80) was added and the solution was sparged with nitrogen for a further 15 minutes. The bottle was sealed immediately under nitrogen and placed on a Stuart Scientific Low-Profile roller at approximately 10 rpm in a temperature-controlled incubator. The temperature was ramped from ambient temperature to 60 ºC over approximately 2 hours and then held at 60 ºC for 24 hours, during which time precipitation of the polymer particles occurred to give a white milky suspension of polymer particles. The product was collected *via* vacuum filtration on a 0.45 µm nylon membrane, washed with ACN and dried overnight *in vacuo* (40 ºC, ~ 0.01 bar). The product was isolated as a fine white powder (2.182 g, 29 %).

2.2.2. D-A reaction between poly-DVB-80 and MA

PolyDVB-80 microspheres (2.00 g) were suspended in toluene (200 mL) in a round-bottomed flask (500 mL). MA (5.90 g, 60 mmol) was dissolved in the suspension. The flask was sealed with a rubber septum and the mixture was sparged with nitrogen. The mixture

was heated under reflux and a nitrogen atmosphere for 48 hours, during which time the white solid changed to an off-white/orange colour. The product was collected *via* vacuum filtration on a 0.45 µm nylon membrane filter, washed with toluene and dried overnight *in vacuo* (40 ºC, ~ 0.01 bar). The product was isolated as a fine, off-white/orange powder (1.7453 g).

2.2.3. Ring-opening of polymer-bound anhydride units using EDA

EDA (2.493 g, 41.5 mmol) was dissolved in dry THF (50 mL) in a 100 mL three-necked, round-bottomed flask. The anhydride-containing polymer (1.50 g) was added and the mixture was stirred under nitrogen for 1 hour at ambient temperature. The temperature was increased to 70 ºC and the mixture was stirred for a further 12 hours, after which time the mixture was removed from the heat and cooled to ambient temperature. The product was collected *via* vacuum filtration, washed with methanol and dried overnight *in vacuo* (40 ºC, ~ 0.01 bar). The product was isolated as a yellow powder (1.390 g).

2.3. SPE procedure

150 mg of the in-house sorbent were packed in an empty 6 mL SPE cartridge (Symta, Madrid, Spain) where a 10 µm polyethylene frit (Symta) was placed at the bottom and another frit was put above the sorbent.

Before the loading of the sample, the sorbent was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted to pH 5. Then, a certain volume of sample adjusted to pH 5 (100 mL for river samples and 50 mL for effluent wastewater samples and 25 mL for influent wastewater samples) was loaded at an approximated flow rate of 5 mL/min. 1 mL of MeOH as cleaning step and the elution step consisted of 5 mL of 5% NH_4OH in MeOH. The eluate was evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1 mL of water/ACN (95/5, v/v). Prior to extraction, river samples were filtered with 0.45 µm nylon membrane filter (Scharlab), wastewater samples were filtered with 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK) prior to the filtration with the 0.45 µm filter.

2.4. Chromatographic conditions

Two liquid chromatographs were used. To optimize the SPE method, an Agilent 1200 (Agilent, Waldbronn, Germany) series equipped with a binary pump, an autosampler, an automatic injector and a diode array detector (DAD) was used. To analyze the samples the method was transferred to LC-HRMS using an Accela 1250 UHPLC, equipped with an Accela Autosampler and a quaternary pump, coupled to an Exactive Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a heated electrospray ionization and a high-energy collision dissociation (HCD) cell.

The chromatographic conditions were similar to a previous study [24] and are included in supplementary material. Instrumental calibration curves ($R^2 > 0.995$) were obtained through weighted calibration regression for the compounds: the lower limit was 0.05 µg/L for TRI, MTO and PRO; 0.1 µg/L for ATE, VEN, CLO, BEZ and VAL, 0.25 µg/L for DIC and 2.5 µg/L for FEN. The higher was 500 µg/L in all cases.

2.5. Validation parameters

The method was validated in terms of apparent recovery at two levels of concentration, matrix effect, accuracy, method limits of detection and quantification, and precision (repeatability and reproducibility between days).

To evaluate the efficiency of the extraction, the apparent recoveries (%R_{app}) were obtained as the ratio between the concentration obtained after SPE (subtracting the blank concentration) and the theoretical concentration.

The matrix effect (%ME) was obtained spiking a non-spiked sample after SPE and comparing the concentration obtained (subtracting the blank concentration) with the theoretical concentration using the following formula %ME = (C_{exp}/C_{the} x 100) – 100, where "C_{exp}" represents the concentration obtained by spiking a non-spiked sample after SPE, subtracting the blank signal, and "C_{the}" represents the theoretical concentration. A negative value indicates signal suppression meanwhile a positive value indicates signal enhancement.

Accuracy was evaluated as the relative recovery, calculated as the percentage of the mean experimental concentration (n=3) and the spiked concentration. Method detection (MDL) and quantification limits (MQL) were estimated by applying the preconcentration factor and the apparent recoveries to the instrumental limits. The instrumental detection limit was the concentration that presented a signal-to-noise ratio higher than 3 and one of the fragment ions had a signal higher than 10^3. The instrumental quantification limit was the lower point of the calibration curves.

Repeatability was obtained as the intra-day %RSD (relative standard deviation) (n=4) by analyzing samples spiked at the same concentration on the same day. The reproducibility between days was obtained as the inter-day %RSD (n=4) by analyzing samples (n=4) spiked at the same concentration on different days.

3. Results and discussion

3.1. Synthesis of the sorbent

PP was the synthetic route chosen to obtain particles in the low micron size range to be carried forward for chemical modification. Typical PP conditions for the synthesis of porous microspheres require the inclusion of a porogen in the solvent mixture. For this reason, toluene was included at 25 vol%. Under these conditions, good quality polyDVB-80 microspheres were obtained in an acceptable yield, and nitrogen sorption analysis showed that they were porous in the dry state.

To maximise the number of pendent (unreacted) styryl groups which could be exploited in D-A cycloadditions, DVB-80 was selected in preference to DVB-55. In a study of suspension polymerized polyDVB resins, Law *et al.* reported that approximately 45 % of the DVB-derived vinyl groups remain pendent and unreacted following polymerisation of DVB-80, compared to 32 % when using DVB-55 [27]. While the microspheres in the present work were synthesised *via* PP rather than by suspension polymerisation, it can be anticipated

that polyDVB-80 microspheres prepared by PP will contain a higher level of pendent vinyl groups than a polyDVB-55 prepared using a nominally identical PP method.

To install anhydride moieties into the polyDVB-80 microspheres, a DA cycloaddition with MA was employed. This is an attractive but underutilized approach with regards to functional polymer production as polydivinylbenzenes are used widely and there are several dienophiles which can be exploited as coupling partners in D-A reactions. The D-A cycloaddition was carried out in refluxing toluene for 48 hours to allow for a high conversion of styryl groups to anhydride units. Toluene is a good swelling solvent for such materials and allows the MA good access to the pores, however it was inevitable that some styryl groups deep within the pore network would remain inaccessible to the MA. For the subsequent ring-opening of the polymer bound anhydride units, EDA was used to produce the amphoteric WAX/WCX material. A 10-fold excess of EDA was used relative to the number of moles of anhydride residues, assuming 100 % conversion. This was done to maximise the conversion of anhydride units to amphoteric moieties.

3.3. Evaluation of the in-house sorbent

The extraction of the acidic and basic analytes with a weak cation- and anion- exchanger is a complex task. Both analytes and sorbent (Fig. 1) have ionizable groups that need to be charged to promote the ion-exchange interactions. In this sense and considering the pK_a values of the analytes (Table S1), ranging from 7.1 to 10.1 for the basic ones and from 3.2 to 4.5 for the acidic ones, and the previous experience in the group with polymer-based amphoteric exchangers [17,19], it was decided to carry out a pH screening. 10 mL of ultrapure water with pH adjusted to 3, 5, 6, 7 and 9 were loaded in the sorbent, 1 mL of MeOH was used as washing step and 5 mL of 5% NH_4OH in MeOH followed by 5 mL of 5% acetic acid in MeOH were used for elution.

To evaluate the results, SPE recoveries ($\%R_{SPE}$) were calculated as the ratio between the measured concentration after SPE and the theoretical concentration when working with ultrapure water. As expected, pH 3 and pH 9 provided the worst results due to the lack of charges in the sorbent. At pH 3, the basic analytes have $\%R_{SPE}$ lower than 40% due to the protonation of the carboxylic groups of the sorbent. Meanwhile at pH 9, the acidic analytes had $\%R_{SPE}$ lower than 45% due to the deprotonation of the amines in the sorbent. pH 5, 6 and 7 provided the most interesting results, as can be seen in Figure 2, the best results are obtained for pH 5 for all of the basic compounds ($\%R_{SPE}$ higher than 80%) and for most of the acidic ones, presenting the highest $\%R_{SPE}$ for DIC, BEZ and FEN. pH 6 presented the lowest recoveries of the three pH tested with recoveries ranging from 36 to 71%. In the case of pH 7, it provided the best results for some of the acidic analytes such as CLO or VAL and good results for the rest of analytes, this differences between CLO and VAL and the other acidic compounds could be explained since these compounds presented the lowest pK_a, BEZ who had a pK_a close to VAL also presented relatively high $\%R_{SPE}$ at pH 7. Since the best results for most of the compounds (all except for CLO and VAL) were obtained with pH 5, it was selected for the following experiments. Previous studies where WAX/WCX sorbents were applied used slightly acidic pH, for instance, Jin *et al.* [22] and Nadal *et al.* [19] selected

pH 6 to extract simultaneously acidic and basic compounds. However, in the present study it has been demonstrated that pH 5 provided better results.

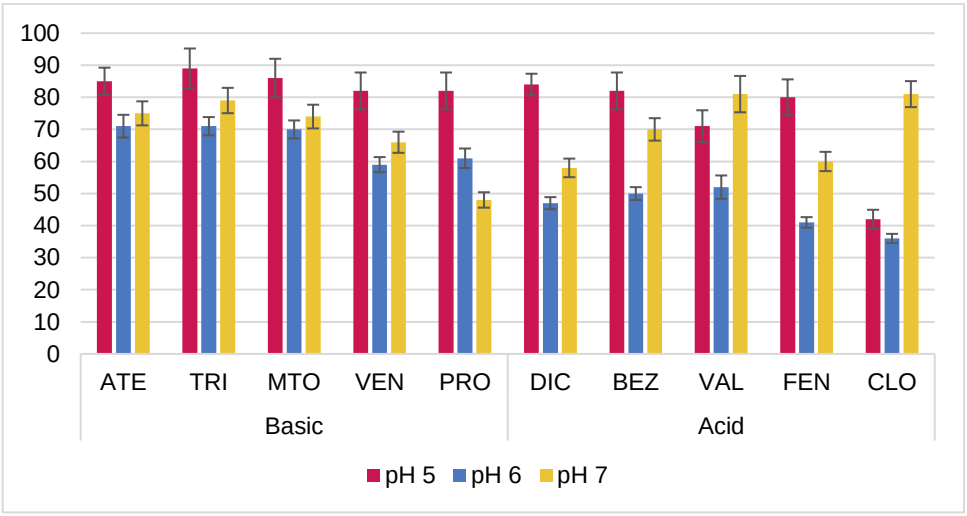

Figure 2. SPE recoveries when 10 mL of ultrapure water was loaded in a SPE cartridge with the in-house sorbent at pH 5, 6 and 7.

The initial elution step consisted of 5 mL of 5% NH_4OH in MeOH followed by 5 mL of 5% acetic acid in MeOH. It was observed during the pH optimization that both acidic and basic analytes were rinsed with 5 mL of 5% NH_4OH in MeOH, meaning that ammonium hydroxide is capable to disrupt the anion- and cation-exchange interactions. The anion-exchange interactions were disrupted by the deprotonation of the amine groups in the sorbent meanwhile the cation-exchange interactions are disrupted by the deprotonation of the basic analytes. For this reason, the acidic elution was removed. Moreover, the order of the elution was inverted, percolating firstly the acidic portion and it was observed that the acidic eluent only rinsed the acidic analytes. Other studies were cation-exchange and anion-exchange groups were combined in one sorbent also used solutions of NH_4OH in MeOH for the elution step [17,19,21].

The increase of MeOH volume used in washing step was also evaluated by using 3 mL of MeOH. It was observed a slight decrease in the $\%R_{SPE}$ of the basic compounds, ranging from 82 to 89% with 1 mL of MeOH and from 71% to 81% with 3 mL of MeOH. However, the decrease observed with the acidic analytes was steeper, ranging from 42 (CLO) to 84% (DIC) with 1 mL of MeOH and from 24% (CLO) to 59% (BEZ) with 3 mL. It was then decided to maintain the washing step with 1 mL of MeOH. The washing step was only 1 mL of MeOH, similar to the volume used in previous studies [17,19], but similarly to the mentioned studies, the use of higher volumes would have led to high losses in the recoveries

The loading volume was increased to 100 mL to obtain a higher preconcentration factor without spend long times during the SPE. No significant losses were observed in the $\%R_{SPE}$, therefore, 100 mL was selected as the loading volume for ultrapure water. Due to the

complexity of the environmental water samples, the volume with river samples was kept at 100 mL but it was reduced to 50 mL with effluent wastewater samples and to 25 mL with influent wastewater samples to prevent reaching the breakthrough volume.

The suitability of use maleic anhydride to introduce ion-exchange groups in polymeric sorbents through nucleophilic addition either with water to generate WCX [26] or with ethylenediamine to generate WAX/WCX. The performance of both sorbents to extract basic analytes can be compared, in the previous study, ATE, TRI, MTO and PRO were extracted, presenting recoveries for 100 mL of ultrapure water with 200 mg of sorbent ranging from 74 to 106%. In the present study, the recoveries ranged from 82 to 85%. This decrease in the WAX/WCX sorbent compared to the WCX sorbent can be explained by the electrostatic repulsions that occur between the negative charges of the carboxylic acid groups in the sorbent and the negative charges from the analytes. However, it seems that these repulsions between charges with the same sign, are not as intense as it was observed when sorbents that combined strong anion- and cation-exchange interactions [20,24] were evaluated by the group. In that studies it was observed that the inclusion of a washing step with MeOH induced the elution of the acidic analytes. One possible explanation was that the concentration of charges could generate electrostatic repulsions, making it possible for MeOH to rinse the acidic analytes. For this reason, it was considered that if amphoteric materials are used, working with at least one weak exchanger, would make it possible to retain both acidic and basic analytes and introduce a washing step with MeOH. Since the weak exchangers present an equilibrium between their protonated and deprotonated form, the electrostatic repulsions are lower than when strong exchangers are used.

3.4. Validation of the method

After the optimization of the method using LC-DAD, the method was transferred to LC-HRMS to apply it for the determination of the pharmaceuticals in river, effluent wastewater and influent wastewater samples. The method was validated with the parameters explained in section 2.6.

The apparent recoveries (%R_{app}) were obtained at two levels of concentration (Table 1 presents the concentration for each matrix). As can be observed, good recoveries were obtained for most compounds. Focusing on river samples, %R_{app} ranged from 32 to 69% except for VEN (27 and 29%) and VAL at high concentration (27%), being most values around 45-50%. Nadal *et al.* [19] obtained similar recoveries (25 – 87%) for acidic and basic analytes percolating 250 mL of river samples with a WAX/WCX polymeric sorbent, meanwhile Salas *et al.* [15] obtained higher recoveries (39 – 109%) when combining two commercial strong exchangers in one sorbent (loading 100 mL of river sample). Attending to the effluent wastewater samples, the recoveries ranged from 36 to 76%, slightly lower values than the obtained by Nadal *et al.* [17] when percolating 100 mL of effluent wastewater samples in a SAX/WCX sorbent (24 – 105%). Laven *et al.* [16] also obtained values slightly higher when loaded 50 mL of effluent wastewater sample in two cartridges with strong exchangers in series (45 – 141%). In the case of influent wastewater samples, the recoveries ranged from 34 to 77%, except the case of TRI (28 and 29%) and MTO (22 and

25%). The results are very similar to the ones obtained by Salas *et al.* [15] when 50 mL of influent wastewater were loaded, whose values ranged from 34 to 76%.

Table 1. Apparent recoveries at two levels of concentration, relative recoveries and matrix effects.

		River			Effluent wastewater			Influent wastewater		
		$\%R_{app}$ 5 µg/L	$\%R_{app}$ 0.5 µg/L	%ME 0.5 µg/L	R_{app} 10 µg/L	R_{app} 1 µg/L	%ME 1 µg/L	R_{app} 20 µg/L	R_{app} 2 µg/L	%ME 2 µg/L
Basic	ATE	61	69	-2	54	48	-48	35	34	-49
	TRI	43	39	-18	36	45	-24	29	28	-20
	MTO	33	32	-9	40	41	+10	25	22	+57
	VEN	27	29	+20	61	52	-17	45	34	+14
	PRO	46	44	+13	56	58	+46	35	38	-28
Acidic	CLO	54	57	-16	78	67	-2	77	73	-6
	BEZ	47	62	+3	67	66	+11	64	55	-38
	VAL	27	37	-6	55	46	+40	65	72	+38
	FEN	47	53	-24	76	75	+15	40	46	-28
	DIC	44	42	-31	37	53	-17	39	44	-34

Attending to the matrix effects (%ME) and considering that ±20% is considered acceptable in terms of matrix effect, the results for river are remarkably low, as can be observed in Table 1, only FEN and DIC had higher values with -24 and -31%, respectively. In the case of effluent wastewater samples, the results were also good, only ATE, PRO and VAL reported values higher than ±20%. Finally, regarding influent wastewater samples, the use of 1 mL of MeOH of washing step was not enough to reduce the matrix effects of the most complex matrix. Only TRI, VEN and CLO had low %ME ranging from -20 to +14%, however, only ATE and MTO presented high %ME values -49% and +57, respectively. The results with river and effluent samples are comparable to the ones obtained by Nadal *et al.* [17], who obtained matrix effects ranging from +17 to -30% for river samples and from +18 to -12% in effluent samples. Comparing with Laven *et al.* [16] study, the results of the present study were comparable with effluent wastewater samples, from -

Table S2 shows the MDL and MQL which were in low ng/L and in some cases below 1 ng/L. The exception was FEN, whose instrumental limits were high due to low ionization yield. In the case of accuracy, Table 1 shows the relative recoveries obtained for the compounds with $\%R_{app}$ higher than 30%, as it can be observed, results are acceptable since relative recoveries range from 88 to 114%. Attending to the precision, the %RSD values obtained for repeatability were lower than 12% and for reproducibility between days were lower than 18%, which was considered acceptable.

3.5. Analysis of samples

The five basic and five acidic pharmaceuticals were determined in four samples from river, effluent wastewater and influent wastewater collected in the province of Tarragona (Spain). The concentrations were obtained through external calibration curves and the apparent recoveries (section 3.4). Since the determination of samples was performed with LC-HRMS,

four confirmation parameters were used following the indications of 2021/808/EC [28]: less than 5 ppm in the mass error of the protonated or deprotonated ion, the retention time should be in the range of 0.1 min, one fragment ion with at least 10^3 AU signal and a relative deviation lower than 40% in the relative intensity of one fragment ion and the protonated or deprotonated ion.

Table 2. Concentration levels in ng/L of the selected analytes in river, effluent wastewater and influent wastewater samples.

		River	Effluent	Influent
Basic	ATE	<MDL – 92 ± 11	55 ± 6– 3444 ± 376	603 ± 7– 5424 ± 621
	TRI	<MDL – 398 ± 48	354 ± 35 – 6132 ± 734	322 ± 30 – 17167 ± 2087
	MTO	4 ± 1 – 117 ± 15	154 ± 18 – 8324 ± 1176	377 ± 44 – 34464 ± 4270
	VEN	<MQL – 146 ± 17	210 ± 24 – 3654 ± 438	196 ± 21– 8034 ± 967
	PRO	15 ± 2 – 139 ± 16	144 ± 16 – 2367 ± 292	98 ± 11– 6140 ± 723
Acidic	CLO	<MDL – 45 ± 6	34 ± 4 – 700 ± 86	60 ± 15 – 1257 ± 155
	BEZ	<MQL – 43 ± 5	44 ± 3 – 1227 ± 146	279 ± 34 –1532 ± 178
	VAL	<MQL – 123 ± 23	896 ± 165 – 5731 ± 1045	628 ± 102 – 21891 ± 2685
	FEN	<MDL	MQL – 557 ± 68	MQL ± 43 – 852 ± 122
	DIC	MQL – 155 ± 19	76 ± 4 – 789 ± 97	108 ± 11 – 597 ± 77

Table 2 presents the results obtained for the compounds in river, effluent and influent wastewater samples with the uncertainty. Starting with river samples, it was the sample where lowest occurrence was found. Only PRO and MTO were quantified in all samples, TRI was the compound with highest occurrence found having a sample with 398 ng/L and FEN was not even detected. It should be noted that for most of the compounds (all of them except TRI, PRO and VAL) presented peaks at the same retention time but the ratios between the fragment ions and the molecular ion did not match with the reference ratios in at least one of the samples. Zhang *et al.* [29] found similar concentration of MTO (156 ng/L) in samples from China, while the occurrence found for PRO, DIC and CLO was lower (<10 ng/L). In the case of Hilton *et al.* [30] determined DIC, PRO and TRI, among other pharmaceuticals in surface samples from UK with higher concentrations than the present study, 460, 180 and 270 ng/L respectively. Egli *et al.* [31] also determined several pharmaceuticals in river samples from Germany, being DIC, VAL, VEN, TRI and PRO among them. In that study, the concentrations found for DIC and VAL were higher (99 – 760 and 54 – 388 ng/L), similar for VEN (24 – 154 ng/L) and lower for PRO and TRI (<13 and <62 ng/L).

Attending to effluent wastewater samples, all the compounds were quantified, except for FEN. Three of the samples presented significantly lower concentrations than the other one, being the first three with concentrations lower than 1000 ng/L for all compounds meanwhile the other one reach concentrations as high as 6132 ng/L (TRI) or 8,324 ng/L (MTO). It should be highlighted that in some of the cases where low presence was found, the confirmation of the compounds was not possible since the relative deviation of the ratios was higher than 40%. Vergili *et al.* [32] determined TRI, VEN and DIC in effluent wastewater samples from Turkey, DIC was found in significantly higher concentrations, ranging from

300 to 3,400 ng/L meanwhile TRI and VEN were below the MQL (10 ng/L). In a previous study from our research group. [26] ATE and TRI were determined in samples from the same wastewater treatment plant from Tarragona (Spain), ATE and TRI were found at similar ranges of concentration (67 – 539 ng/L and 21 – 660 ng/L) considering the samples with low concentration of the present study. ATE, TRI, MTO and DIC (among others) were quantified in effluent wastewater samples from France in the study performed by Miossec *et al.* [33], only ATE and DIC were detected with concentrations higher than 1,000 ng/L, meanwhile the concentration of MTO was 192 ng/L and of TRI was 56 ng/L.

The samples with highest occurrence found were the influent wastewater. Similarly to effluent wastewater samples, it was possible to quantify all compounds in all samples (except for FEN). One sample presented high concentrations of some compounds such as TRI (17,167 ng/L), MTO (34,464 ng/L) or VAL (21,891 ng/L), on the other hand, one of the samples presented low concentrations of all compounds, being the compound with highest occurrence in this sample VAL (628 ng/L). Van Nuijs *et al.* [34] determined ATE, MTO and VEN in influent wastewater samples from Belgium, similar ATE levels were found, ranging from 41 to 2,118 ng/L meanwhile MTO and VEN were found with lower concentrations, 252 – 1,190 ng/L and 119 – 480 ng/L, respectively. ATE and MTO were also present in the study performed by Laven *et al.* [16], ATE concentration was 1,400 ng/L and MTO 790 ng/L. PRO was also determined, with an occurrence of 87 ng/L, significantly lower than the present study. Kot-Wasik *et al.* [35] quantified several pharmaceuticals in influent wastewater samples, in their study, ATE was found at a lower concentration, ranging from 108 to 503 ng/L. Moreover, DIC was found at higher concentration, ranging from 743 to 5,401 ng/L.

4. Conclusions

In this study, a novel WAX/WCX polymeric sorbent for SPE has been developed, characterized and evaluated. The functionalization of polyDVB microspheres with maleic anhydride and the subsequent opening of the polymer-bound anhydride rings is a novel and powerful way to introduce anion- and cation-exchange groups into a porous polymer.

The sorbent was used to determine five acidic and five basic pharmaceuticals in river, effluent wastewater and influent wastewater samples, showing its suitability to extract simultaneously both acidic and basic analytes with good validation parameters. Moreover, it was possible to introduce a washing step with methanol that helped to reduce the matrix effects. The results obtained exhibit the interest of using sorbents that combine weak exchange interactions for the extraction of acidic and basic pharmaceuticals.

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Suplementary data

Chromatographic conditions:

A Luna Omega Polar C_{18} 100 (150 x 3.0 mm, 5 µm particle size) equipped with a 3 mm precolumn (Phenomenex, Torrace, CA, United States) was selected as chromatographic column. The injection volume was set at 20 µL, the flow rate at 0.4 mL/min and the column oven at 30 ºC. The solvents for mobile phase were ultrapure water adjusted to pH 3 with HCl (solvent A) and ACN (solvent B). When working with LC-HRMS, solvent A was MS grade water with 0.1 % of HCOOH. The gradient program started with 5% of B, increasing the %B until 60% within 6 min, then the %B was increased until 100% within 22 min, where it was held for 3 min. 1 min was used to return to the initial conditions where it was maintained for 3 min.

When working with LC-DAD, two different wavelengths were used to quantify the analytes, 230 nm was used for ATE, MTO, DIC and BEZ and 210 nm was used for TRI, VEN, PRO, VAL and CLO. When working with LC-HRMS, two windows were used for quantification. The first one was used for basic analytes using positive ionization mode, from the beginning to min 13. The conditions were: 40 AU (arbitrary units) for sheath gas flow rate, 15 AU for auxiliary gas flow rate, 5 AU for sweep gas flow rate, 4 kV for spray voltage, 45 V for capillary voltage, 65 V for tube lens voltage and 22 V for skimmer voltage. The second window was used to quantify the acidic analytes using negative ionization mode, from min 13 to the end. The conditions for this window were 40 AU for sheath gas flow rate, 15 AU for auxiliary gas flow rate, 5 AU for sweep gas flow rate, 4 kV for spray voltage, -25 V for capillary voltage, -60 V for tube lens voltage and -22 V for skimmer voltage. In both ionization modes, the capillary temperature was set at 330 ºC and the heater temperature at 350 ºC. Each window had two alternative scan events with a range from 50 to 450 m/z. The first event consisted of a full scan at 50,000 FWHM (Full Width Half Maximum) with an injection time of 250 ms, meanwhile the second scan consisted of a fragmentation scan at 10,000 FWHM with an injection time of 50 ms and a collision voltage of 20 eV in both windows.

Table S1. Protonated and deprotonated ions and fragment ions for the analytes.

	Compound	pK_a	$[M+H]^+$ (basic) and $[M-H]^-$ (acidic)	Fragment ion
Basic	ATE	9.6	267.17020	190.08649 145.06514
	TRI	7.1	291.14517	245.10388 261.09866
	MTO	9.7	268.19061	116.10733 191.10712
	VEN	10.1	278.21133	260.20111 58.06614
	PRO	9.4	260.16418	116.10773 183.08057
Acidic	CLO	3.2	213.03151	126.99430 85.02819
	BEZ	3.8	360.10059	274.06412 154.00548
	VAL	3.6	434.21912	350.16254 179.08580
	FEN	4.5	241.08667	93.03340 211.07599
	DIC	4.1	294.00919	250.01923 250.01923

Table S2 . Method detection (MDL) and method quantification limits (MQL).

		River		Effluent		Influent	
		MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)
Basic	ATE	0.2	1	0.7	2	2	6
	TRI	0.2	1	0.4	1	1	4
	MTO	0.3	1	0.4	1	2	5
	VEN	0.6	2	0.6	2	2	6
	PRO	0.2	1	0.3	1	1	3
Acidic	CLO	0.6	2	1	3	2	5
	BEZ	0.5	2	1	3	2	7
	VAL	1	3	1	4	2	6
	FEN	16	47	22	67	73	217
	DIC	2	6	3	9	8	23

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Alberto Moral Ruiz

3.2.3. Discussion of results

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In this chapter, the development and evaluation of two novel mixed-mode ion-exchange sorbents are presented. Both sorbents are based on polydivinylbenzene (PDVB), which is a polymer commonly applied to synthesize sorbents to be applied in sorptive extraction techniques in our research group [1–3] due to the enhanced morphology that provided high surface areas and suitable particle shape delivered in precipitation polymerization (PP) conditions. Moreover, in the study of sulfonated core-shells, vinylbenzene chloride (VBC) was used to include the functional groups as has been done previously in other studies performed in our research group [4,5] since it provided consistent number of pending chlorine groups through which different ion-exchange groups could be introduced.

Starting with the hypercrosslinked sulfonated core-shells, which were presented in section 3.2.1. As it was pointed out in this section, the use of core-shell format can facilitate the interactions between the sorbent and the analytes thanks to the short diffusion paths. The aim was to obtain the advantages of using small particles but avoiding back pressure problems. However, one of the most relevant data to complete the study is obtaining TEM images that can justify the morphology of the core-shells. Although we were not able to obtain these images, the obtention of core-shells could be intuited by the data presented of size and specific surface area. The size of the DVB cores was in the range from 2.5 to 2.7 µm meanwhile the size of the DVB/VBC core-shells was in the range from 3.0 to 3.2 µm, meaning that the second polymerization reaction occurred in the surface of the DVB cores, increasing the size of them instead generating new particles. Moreover, the specific surface area measured through BET (Brunauer, Emmett and Teller) theory presented a specific surface area for the DVB cores of less than 5 m^2/g meanwhile the DVB/VBC core-shells presented a specific surface area of 200 m^2/g, meaning that there has been a modification in the surface of the polymer, significantly increasing the porosity.

Regarding the performance of the material for the extraction of basic compounds, in our group other materials functionalized with strong cation-exchange (SCX) moieties have been developed and evaluated for the extraction of similar compounds [3,6]. It should be pointed out that the materials were not obtained through PP but through non-aqueous dispersion polymerization (NADP). Moreover, the performance of the material presented in section 3.1.1. which was silica-based functionalized with SCX/SAX moieties but was exploited as a SCX material can be also compared with the sulfonated core-shells. In Table 1, some properties of the materials and the extraction procedures.

As can be observed in Table 1, different types of polymers were used, as it was highlighted previously, DVB is present in two of the three studies where polymeric sorbents were evaluated. Regarding the properties of the sorbents, despite being a core-shells format, the particles prepared in section 3.2.1. are smaller than the ones developed by Gilart *et al.* [3] and in section 3.1.1., and are in the range of the ones synthesized by Fontanals *et al.* [6]. Regarding the specific surface area, it might seem that 550 m^2/g is low, especially compared to the PVP/VBC particles [6]. However, as it was pointed out in section 3.2.1., the effective surface area of the core-shells is 1383 m^2/g, being a remarkable result. In addition, the core-shells presented the higher ion-exchange capacity (IEC) among the sorbents presented, being also higher than Oasis MCX (1 mmol/g).

Table 1. Properties and extraction procedures of cation exchange materials evaluated in our research group.

Study	Gilart *et al.* (2014) [3]	Fontanals et *al.* (2014) [6]	Section 3.1.1.	Section 3.2.1.
Description	Polymeric SCX	Polymeric SCX	Silica SCX/SAX	Polymeric SCX
Polymer	HEMA/DVB	PVP/VBC	-	DVB/VBC
Size (µm)	32 - 50	2 - 3	50 - 60	3 – 3.2
Specific surface area (m^2/g)	150	1020	-	550
IEC (mmol/g)	2.1	0.6	-	3.7
Loading pH	pH 3	pH 3	pH 3	pH 3
Washing step	5 mL MeOH	5 mL MeOH	5 mL MeOH	5 mL MeOH
Elution	3 mL 5% NH_4OH in MeOH	5 mL 5% NH_4OH in MeOH	5 mL 5% NH_4OH in MeOH	5 mL 5% NH_4OH in MeOH

Abbreviations: DVB: divinylbenzene, EWW: effluent wastewater, HEMA: 2-hydroxylethyl methacrylate, IEC: ion-exchange capacity, IWW: influent wastewater, PVP: poly(N-vinylpyrrolidone), RW: river water, VBC: vinylbenzene chloride

Regarding the extraction conditions, as presented in Table 1, in all studies the same loading pH (pH 3) and the same washing protocol (5 mL of methanol (MeOH)) were applied, meanwhile the elution step was mostly the same (3 or 5 mL of 5% NH_4OH in MeOH). The use of pH 3 is coherent since basic compounds were extracted and, in order to exploit cation-exchange interactions it is necessary to have them in ionic form. The use of 5 mL of MeOH enables the rinse of compounds which are not retained through ion-exchange interactions, reducing the matrix effect. In addition, the use of basic methanol causes the deprotonation of the basic analytes, disrupting the SCX interactions. Moreover, to compare the performance of the developed sorbents, Table 2 presents the results in terms of apparent recovery ($\%R_{app}$) and matrix effect (%ME) of four basic analytes when extracted from effluent wastewater samples. In addition, a polymeric sorbent functionalized with weak cation-exchange (WCX) moieties [7] is also presented because similar analytes were extracted.

Table 2. Apparent recoveries and matrix effect obtained for different studies where cation-exchange materials were applied for the extraction of ATE, TRI; MTO and PRO from effluent samples..

	Gilart *et al.* (2014) [3]		Fontanals et *al.* (2014) [6]		Fontanals *et al.* (2021) [7]		Section 3.1.1.		Section 3.2.1.	
	Polymeric SCX (50 mL)		Polymeric SCX (50 mL)		Polymeric WCX (100 mL)		Silica SCX/SAX (50 mL)		Polymeric SCX (50 mL)	
	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME
ATE	89	<±20%*	117	+12	62	-27	71	-25	48	-17
TRI	87		41	10	72	-32	66	-24	39	-29
MTO	93		12	6	80	-18	58	-19	51	-18
PRO	97		97	10	66	-15	40	-18	67	20

*In this study, the %ME was presented in a figure.

As can be seen in Table 2, the five sorbents developed show good performance for the extraction of basic analytes. In all cases (except for MTO in the study from Fontanals *et al.*

[6]), the recoveries were higher than 39% showing the suitability of the different sorbents evaluated. Moreover, the washing step based on 5 mL of MeOH make it possible to reduce the %ME since the highest value was -32% and, in most cases, lower than ±20%. The results of the study presented in section 3.2.1., who presented similar apparent recoveries (except for Gilart *et al.* [3]) and matrix effects to the previous studies demonstrating that the core-shell systems are suitable for its exploitation in SPE. In addition, as it was mentioned in section 3.2.1., one interesting study that can be performed in the future is the evaluation of these particles for on-line SPE, since the core-shells systems would present less back pressure problems.

Moving to the discussion of the amphoteric microparticles presented in section 3.2.2., some mixed-mode ion-exchange materials functionalized with both anion- and cation-exchange moieties have been developed in our group [8,9] and in other research groups [10,11]. Moreover, in chapter 3.1. some materials functionalized with both moieties were also presented. In Table 3, several of the mentioned materials are presented, all of them based on in-house sorbents except for the study by Salas *et al.* [12] where commercial mixed-mode ion-exchange materials with strong cation-exchange (Oasis MCX) and strong anion-exchange (Oasis MAX) functional groups were packed in the same cartridge.

Table 3. Analytes, loading pH, washing step and elution from different studies.

Description of the material	Type of functional group	Analytes	Matrix	Loading pH	Washing step	Elution	Ref.
Polymeric WAX/WCX	Maleic anhydride opened with EDA	Acidic and basic pharm.	RW, EWW, IWW	pH 5	1 mL MeOH	5 mL 5% NH_4OH in MeOH	3.2.2.
Polymeric SCX/SAX	Commercial Oasis MCX and MAX	Acidic and basic pharm.	RW, EWW, IWW	pH 7	15 mL MeOH	5 mL 5% HCOOH in MeOH + 5 mL 5% NH_4OH in MeOH	[12]
Polymeric WAX/WCX	Sarcosine	Acidic and basic sweeteners and pharm.	RW, EWW	pH 6	1 mL MeOH	5 mL 5% NH_4OH in MeOH	[8]
Polymeric SAX/WCX	Quaternized sarcosine	Sweeteners and pharm.	RW, EWW	pH 6	1 mL MeOH	5 mL 5% NH_4OH in MeOH	[9]
Silica SCX/SAX	Zwitterionic additive	Acidic and basic pharm.	RW, EWW, IWW	pH 5	-	5 mL 1% NH_4OH in MeOH	3.1.5
Silica WCX/WAX	Modification of azide groups	Acidic, neutral and basic Pharm.	Serum	pH 6	-	2 mL MeOH + 2 mL 5% HCOOH in MeOH	[10]
Silica WCX/WAX	Ampholine	Mix of acidic and basic analytes	Juice	pH 6	-	1 mL MeOH pH 11 + 1 mL MeOH pH 1	[11]

Abbreviations: EDA: ethylenediamine, EWW: effluent wastewater, IWW: influent wastewater, Pharm: pharmaceuticals, RW: river water,

As can be observed in Table 3, several mixed-mode ion-exchange materials functionalized with both cation-exchange and anion-exchange moieties to simultaneously extract acidic and basic analytes have been developed. The functional groups have been included with different synthetic routes, such as using reagents that already had both moieties like the zwitterionic additives presented in section 3.1.5., amino acids or ampholine [8,9,11]. Another option was the one presented in section 3.2.2., with the ring opening of maleic anhydride with ethylenediamine. Moreover, Jin *et al.* [10] included in silica azide groups which were modified through a click reaction to add the carboxylic acid group (WCX) and through a reduction to obtain the amine (WAX). Regarding the extraction conditions, in all studies the pH applied are in the range from pH 5 to 7 since it is needed an intermediate pH to exploit both ion-exchange interactions. High pH prevents the protonation of basic analytes meanwhile acidic ones prevent the deprotonation of acidic analytes. Moreover, it can be observed that in the studies performed in our group, a washing step based in MeOH is included, except in the case of the study presented in section 3.1.5., where this washing protocol was also evaluated. Regarding the elution, there are studies where an acidic and a basic elution is needed [11,12], meanwhile in others, the analytes are eluted in one step with basic MeOH. In the case of the study performed by Jin *et al.* [10], the elution steps were MeOH to elute the acidic and neutral analytes and acidic MeOH to elute the basic ones. Since the acidic analytes were rinsed with MeOH, it might indicate that these analytes were retained through reversed-phase interactions rather than ion-exchange.

As it was pointed out in section 3.1.5., when sorbent CW20/ZW-1 was applied, it was not possible to include a washing step based in MeOH. On the other hand, with the WAX/WCX material developed in section 3.2.2., 1 mL of MeOH was used as washing protocol. In this sense, Table 4 and Table 5 present the $\%R_{app}$ and %ME of these studies when they were applied to extract acidic and basic pharmaceuticals from river, effluent and influent wastewater samples.

Table 4. Apparent recoveries obtained for WAX/WCX and CW20/ZW-1.

		River		**Effluent WW**		**Influent WW**	
		WAX/WCX	CW20/ZW-1	WAX/WCX	CW20/ZW-1	WAX/WCX	CW20/ZW-1
Basic	ATE	69	11	48	18	34	14
	TRI	39	50	45	61	28	33
	MTO	32	68	41	81	22	48
	VEN	29	64	52	64	34	38
	PRO	44	48	58	59	38	52
Acidic	DIC	57	9	67	9	73	14
	BEZ	62	75	66	68	55	64
	VAL	37	69	46	65	72	-*
	FEN	53	69	75	83	46	45
	CLO	42	33	53	33	44	16

Table 5. Matrix effects obtained for WAX/WCX and CW20/ZW-1..

		River		Effluent WW		Influent WW	
		WAX/WCX	CW20/ZW-1	WAX/WCX	CW20/ZW-1	WAX/WCX	CW20/ZW-1
Basic	ATE	-2	-31	-48	-41	-49	-53
	TRI	-18	-46	-24	-45	-20	-58
	MTO	-9	-25	10	-41	57	-54
	VEN	20	-21	-17	-49	14	-62
	PRO	13	14	46	-12	-28	-31
Acidic	DIC	-16	4	-2	-3	-6	-16
	BEZ	3	-13	11	-30	-38	-31
	VAL	-6	13	40	-7	38	-*
	FEN	-24	14	15	-26	-28	-51
	CLO	-31	-4	-17	-24	-34	-84

As can be observed in Table 4, in general the recoveries of both studies are similar, being in some cases higher with WAX/WCX and in other with CW20/ZW-1. Moreover, it should be highlighted that with some analytes such as ATE or DIC, CW20/ZW-1 performed poorly with $\%R_{app}$ lower than 15% meanwhile WAX/WCX only presented recoveries lower than 30% in three cases (VEN in river, TRI and MTO in influent wastewater). Attending to the %ME, the results are quite similar in river samples. However, when the complexity of the samples increased, the influence of the washing step with MeOH was more relevant. For instance, in influent samples, CLO presented -34% with WAX/WCX and -84% with CW20/ZW-1 or VEN presented +14% with WAX/WCX and -62% with CW20/ZW-1, proving that with complex samples a washing step is needed to reduce the matrix effects.

In summary, the studies performed in this chapter delivered promising results that we hope that can publish when the manuscripts are prepared. Both sorbents developed showed great performance for the SPE of pharmaceuticals from environmental water samples. The sulfonated core-shells systems proved to be a good format to extract basic analytes. In the case of the amphoteric particles with WAX/WCX moieties, we can confirm what was pointed out in section 3.1.6., when we hypothesized that the combination of SCX/SAX moieties was not suitable for the extraction of acidic and basic analytes including a washing step. Considering the previous results with WAX/WCX [8] and SAX/WCX [9], and the results from the study in section 3.2.2., the use of at least one weak ion-exchange moiety is recommended if a selective extraction applying a protocol with a washing step based on an organic solvent is wanted to apply.

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4. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
IN-HOUSE MIXED-MODE ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF CONTAMINANTS FROM ENVIRONMENTAL SAMPLES
Alberto Moral Ruiz

The main conclusions that can be drawn from the studies in the present Doctoral Thesis are the following:

1. The development of novel materials with enhanced features is an evolving field of research that enables the selectivity and sensitivity of analytical methods to be improved.
2. Silica-based sorbents modified using the sol-gel approach makes it possible to introduce several moieties. This is a novel approach to obtaining mixed-mode ion-exchange sorbents. The ionic moieties can be introduced either by adding the ionic groups individually or with additives that contain them both.
3. The SPE protocol has to be carefully optimized so that either acidic and basic analytes are selectively and simultaneously extracted or one group of analytes is extracted when there is a washing step.
4. The silica-based sorbent with strong cationic and anionic moieties (SiO_2-SAX/SCX) was successfully applied to the selective extraction of basic pharmaceuticals from environmental water samples thanks to a washing step based on methanol.
5. Various zwitterionic additives that combine strong cationic and anionic moieties were successfully integrated into silica-based sorbents and used to simultaneously extract acidic and basic pharmaceuticals after the protocol had been optimized.
6. The integration of graphene particles into silica-based mixed-mode zwitterionic sorbents increased the ability of the material to extract analytes with high delocalized π-density. The integration of activated carbon, however, did not present such advantages. These features meant that the SiO_2-G-SAX/SCX sorbent could be successfully applied to extract benzothiazoles, benzotriazoles and benzenesulfonamides and selectively extract NH_2BT, so it could be extracted from organic extracts in fish and dust samples with significantly low matrix effects.
7. The results of using core-shell microparticles showed that this type of material is exploitable for solid-phase extraction.
8. The nucleophilic ring opening of maleic anhydride with ethylenediamine proved to be a good approach to prepare mixed-mode amphoteric ion-exchange materials with weak anion- and cation-exchange moieties. This material allowed the simultaneous selective extraction of acidic and basic analytes when there was a methanol washing step.
9. The combination of SCX/SAX moieties with the analytes in ionic generate high concentration of charges that might result in the acidic analytes being rinsed with methanol. To prevent this, it has been learnt that at least one of the ion-exchange moieties should be weak
10. The analytical methods developed using SPE with the novel sorbents followed by liquid chromatography with mass spectrometry were effectively applied to determine the studied contaminants in environmental samples.
11. Most of the studied contaminants were found at such low levels of concentration as ng/L in river samples, ng/L - µg/L in wastewater samples, ng/g in dust or ng/g dried weight in fish, which indicates the potential of the sorbents developed for

analytical methods.dried weight in fish, which indicate the potential of the developed sorbents in the analytical methods.

APPENDIX

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List of publications

1. “Novel sol-gel silica-based mixed-mode zwitterionic sorbents for determining drugs in environmental waters samples”. A. Moral., F. Borrull, K.G. Furton, A. Kabir, N. Fontanals, R.M. Marcé. Journal of Chromatography A 1676 (2022) 463237.

2. “Extraction of selected benzothiazoles, benzotriazoles and benzenesulfonamides from environmental water samples using a home-made sol-gel silica-based mixed-mode zwitterionic sorbent modified with graphene”. A. Moral., F. Borrull, K.G. Furton, A. Kabir, R.M. Marcé, N. Fontanals. Talanta 256 (2023) 124315.

3. “Application of a homemade silica-based mixed-mode ion-exchange sorbent for the determination of drugs in environmental water samples”. A. Moral., P. Clivillé-Cabré, F. Borrull, K.G. Furton, A. Kabir, R.M. Marcé, N. Fontanals. Advances in Sample Preparation 7 (2023) 100074.

4. “Selective determination of 2-aminobenzothiazole in environmental water and organic extracts from fish and dust samples”. A. Moral., F. Borrull, K.G. Furton, A. Kabir, N. Fontanals, R.M. Marcé. Analytical and Bioanalytical Chemistry 416 (2024) 439-448.

5. “Introduction of zwitterionic functional groups in silica sorbent for the simultaneous determination of acidic and basic pharmaceuticals”. A. Moral., F. Borrull, K.G. Furton, A. Kabir, N. Fontanals, R.M. Marcé. Journal of Chromatography A (submitted)

6. “Novel materials for sorptive techniques for the analysis of environmental water samples” A. Moral., F. Borrull, R.M. Marcé, N. Fontanals. Trends In Analytical Chemistry (submitted)

7. "Core-shell polymer microspheres with strong cation-exchange character for the extraction of basic pharmaceuticals from aqueous samples". A. Moral., A. Corrigan, F. Borrull, P.A.G. Cormack, N. Fontanals, R.M. Marcé. (in preparation)

8. “A facile route for the chemical functionalisation of polydivinylbenzenes and the application of an amphoteric polydivinylbenzene derivative to the simultaneous solid-phase extraction of acidic and basic drugs from water samples". A. Moral., C. Craig, A. Corrigan, F. Borrull, P.A.G. Cormack, N. Fontanals, R.M. Marcé. (in preparation).

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