Microporous Polymer Microspheres with Amphoteric Character for the Solid-Phase Extraction of Acidic and Basic Analytes

Joan Carles Nadal[(1)], Kimberley L. Anderson[(2)], Stuart Dargo[(2)], Irvin Joas[(2)], Daniela Salas[(1)], Francesc Borrull[(1)], Peter A. G. Cormack[(2)*], Rosa Maria Marcé[(1)*] and Núria Fontanals[(1)]

[(1)]Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira I Virgili, Sescelades Campus, Marcel·lí Domingo s/n, 43007 Tarragona, Spain.

[(2)]WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, United Kingdom.

**corresponding authors: rosamaria.marce@urv.cat; Peter.Cormack@strath.ac.uk*

Keywords:

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

ABSTRACT

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

1. Introduction

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

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

Mixed-mode ion-exchange sorbents are classified into four distinct groups depending upon whether they are cation- or anion-exchangers and whether they offer strong or weak exchange properties. Strong ion-exchangers bear functional groups that are charged across the entire pH range of operation, whereas weak ion-exchangers bear functional groups that are ionised over a narrower pH window depending on their pK_a. Strong cation-exchangers (SCX) normally bear sulfonic acids (strong acids), whereas weak cation-exchangers (WCX) are decorated with carboxylic acids (weak acids). In contrast, sorbents with strong anion-exchange (SAX) character are normally based on immobilised quaternary ammonium species, whereas sorbents with weak anion-exchange (WAX) properties are functionalised with tertiary, secondary or primary amines [4,15]. Commercial offerings of mixed-mode ion-exchange sorbents and research works focused on the development of new materials have been reported [4,16]. Hypercrosslinked mixed-mode ion-exchange sorbents were developed and reported by our group [14,17–19]; these materials offer high capacity, which is ascribed to their high specific surface area (SSA) (typically >1000 $m^2\ g^{-1}$), in combination with tuneable ion-selectivity (which is governed by the chemical nature of the ion-exchange groups present). Published examples of hypercrosslinked mixed-mode ion-exchange sorbents include SAX materials decorated with quaternary ammonium species [19] and WCX materials which derive their selectivity from carboxylic acid groups installed during polymer synthesis involving a functional monomer [18].

Mixed-mode ion-exchange sorbents have been applied successfully within various analytical chemistry fields for the extraction of a variety of ionic analytes, including

pharmaceuticals, drugs of abuse and compounds of biological interest, among others. They are well-accepted by the analytical chemistry community [4,12,14,16–24]..

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

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

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

2. Experimental

2.1. Reagents and standards

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

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

Stock solutions of individual standards were prepared in MeOH at a concentration of 1000 mg L^{-1} and stored at −20 °C. Working solutions of a mixture of all compounds were prepared weekly in a mixture of ultrapure water and MeOH (50/50) and were stored at 4 ^{o}C in the dark. Ultrapure water was provided by a water purification system (Veolia, Sant Cugat del Vallès, Spain) and HPLC grade MeOH and ACN were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid (HCOOH) and ammonium hydroxide (NH_4OH) from Sigma-Aldrich, and hydrochloric acid (HCl) from Scharlab (Barcelona, Spain), were used to prepare the mobile phase and the solutions for SPE.

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

The HXLPP-WAX/WCX sorbent was synthesised *via* a three-step procedure: i) Precipitation polymerisation (PP) of DVB with VBC to give poly(DVB-*co*-VBC) microspheres; ii) Hypercrosslinking of poly(DVB-*co*-VBC) microspheres to give hypercrosslinked poly(DVB-*co*-VBC) microspheres, HXLPP; iii) Chemical treatment of

HXLPP with sarcosine ethyl ester hydrochloride *via* a polymer-analogous reaction (to give HXLPP-WAX), followed by ester hydrolysis, to give HXLPP-WAX/WCX.

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

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

For the hypercrosslinking of the poly(DVB-*co*-VBC) microspheres, poly(DVB-*co*-VBC) microspheres (3.210 g, 9.0 mmol of Cl) and DCE (50 mL) were placed into a three-necked, round-bottomed flask equipped with a reflux condenser and an overhead stirrer, and the flask immersed in an temperature-controllable oil bath. The suspension of microspheres was stirred gently at 100 rpm, for 1 h at room temperature and under N_2. Iron (III) chloride (2.067 g, 12.7 mmol) and a second portion of DCE (40 mL) were added, the mixture heated to 80 °C and then left to react for a further 10 min. Thereafter, the flask contents (which were dark purple in colour) were cooled to r.t. and filtered by vacuum on a 0.2 μm nylon membrane filter. The product on the filter was washed sequentially with MeOH, 2 M aqueous HNO_3, MeOH and acetone, and then washed with acetone *via* Soxhlet extraction for 24 h. The product was re-filtered by vacuum on a 0.45 μm nylon membrane filter and re-washed with MeOH and diethyl ether prior to drying

overnight *in vacuo* (70 °C, 60 mbar) to give HXLPP as a brown-coloured, free-flowing powder (2.945 g, 92 %). See Supplementary Information for the characterisation data.

For the chemical functionalisation of HXLPP with sarcosine ethyl ester hydrochloride to give HXLPP-WAX, HXLPP (2.907 g, 3.2 mmol Cl g^{-1}) and EtOH (70 mL) were introduced into a three-necked, round-bottomed flask equipped with a reflux condenser and an overhead stirrer, and the flask immersed in a temperature-controllable oil bath. The suspension of microspheres was stirred gently at 100 rpm for 1.5 h, then K_2CO_3 (3.883 g, 28.1 mmol) and sarcosine ethyl ester hydrochloride (4.388 g, 28.6 mmol) dissolved in H_2O (70 mL) added. The flask contents were heated at 75 °C for 18 h. After cooling, the product was isolated by vacuum filtration on a 0.45 μm nylon membrane filter and washed sequentially with EtOH, MeOH, 1:1 MeOH:H_2O, 0.01 M aqueous $NaHCO_3$, H_2O and acetone, prior to drying for 24 h *in vacuo* (70 °C, 60 mbar). HXLPP-WAX was isolated as a free-flowing, orange-coloured powder (2.958 g). See Supplementary Information for the characterisation data.

The ester groups in HXLPP-WAX were hydrolysed under basic conditions to give HXLPP-WAX/WCX. For this, HXLPP-WAX (2.920 g, 2.3 mmol of sarcosine ethyl ester groups) was placed into a glass Duran bottle together with a solution of KOH (4.353 g, 77.6 mmol) in ethanol (100 mL). The tube was sealed and a low-profile roller used to mix the contents at r.t. for 24 h. The product was filtered by vacuum on a 0.45 μm nylon membrane filter, washed with large volumes of EtOH and then dried overnight *in vacuo* (70 °C, 60 mbar). HXLPP-WAX/WCX was isolated as its potassium carboxylate and in the form of a free-flowing, orange-coloured powder (3.274 g). FT-IR $\bar{\nu}$/cm^{-1}: 3014 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1587 (carboxylate $(CO_2)^-$ asymmetric stretch), 1400 (carboxylate $(CO_2)^-$ symmetric stretch), 1040 (amine C-N stretch), 827 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend). Elemental microanalysis: Found for HXLPP-WAX/WCX: C, 73.4 %; H, 8.5 %; N, 1.2 %; Cl, 1.9 %; Sarcosine loading level = 0.9 mmol g^{-1}. SEM microscopy: mean particle diameter = 3.02 μm; C_v = 18.2 %. N_2 sorption analysis: Langmuir SSA = 1,140 m^2 g^{-1}; specific pore volume = 0.45 cm^3 g^{-1}; mean pore width = 2.7 nm.

2.3. Solid-phase extraction procedure

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

The SPE protocol started with the conditioning of the sorbents, and this involved passing 10 mL of MeOH through the cartridges followed by 10 mL of ultrapure water adjusted to

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

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

2.4. Instrumentation and chromatographic conditions

The chromatographic system was an Agilent 1200 UHPLC equipped with a binary pump, an autosampler, an automatic injector and a DAD detector (Agilent, Waldbronn, Germany). The chromatographic column used was a Tracer Excel 120 C_8 (150 mm × 4.6 mm i.d., 5 μm particle size) supplied by Teknokroma (Sant Cugat del Vallès, Spain). The mobile phase was a mixture of ultrapure water (adjusted to pH 2.8 with HCl) (solvent A) and ACN (solvent B). The gradient profile started with 10% of B, which was raised to 40% B within 12 min. and then to 100% B within 16 min. Subsequently, it was held at 100% B for 3 min. before returning to the initial conditions in 3 min. The column was kept at 30 °C and the flow rate was 600 μL min^{-1}. The injection volume was 20 μL.

3. Results and discussion

3.1. Synthesis of the HXLPP-WAX/WCX sorbent

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

For the PP, DVB was copolymerised with VBC, under textbook PP conditions, to give an acceptable yield of high quality poly(DVB-*co*-VBC) microspheres. The microspheres were gel-type, thus non-porous in the dry state but swellable in compatible solvents such as DCE. The polymer microspheres were hypercrosslinked in a DCE-swollen state, with

the pendent chloromethyl groups providing the source of internal electrophiles for methylene bridge formation. As anticipated, the hypercrosslinking led to a dramatic increase in SSA and retention of the high quality of the microspheres.

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

3.2. Optimisation of the SPE protocol

The HXLPP-WAX/WCX polymer was evaluated as an SPE sorbent by using a complex aqueous mixture of three different groups of test compounds as a sample. The test compounds comprised artificial sweeteners, illicit drugs and pharmaceuticals, and included compounds with acidic, basic and amphoteric properties, chosen to allow the selectivity of the sorbent to be evaluated in detail. Care was taken to elucidate the retention mechanism of each analyte, taking into account the pK_a values of the analytes (listed in Table 1) and the ionisation state of the sorbent as a function of pH. Control of the pH in the loading and washing steps is crucial, since the analytes and the sarcosine residues on the sorbent must be in appropriate ionisation states for the analytes to be retained. The sorbent is expected to function as an anion-exchanger at low pH and as a cation-exchanger at high pH, however at intermediate pH values the sarcosine residues are expected to be in a zwitterionic form and this opens up the possibility of analyte

retention by the sorbent *via* both ion-exchange mechanisms. Estimates of the pK_a values of polymer-bound Brønsted acids and bases can be made by consideration of small molecule analogues, however such estimates are complicated when the polymers are heterogeneous and micro-environmental effects are likely, as in the case for HXLPP-WAX/WCX.

The initial SPE conditions evaluated used 100 mg of sorbent and, for the loading step, 25 mL of a standard solution of the analytes at a range of different pH values. The washing step comprised 2 x 2 mL MeOH, and was included to remove non-selectively bound analytes, *i.e.*, analytes bound by hydrophobic interactions rather than by ionic interactions. Based on results from previous studies [14,18], two different elution solvents were applied: firstly, an acidic elution solvent (5 mL MeOH containing 5% HCOOH) to disrupt cation-exchange interactions and, secondly, a basic elution solvent (5 mL MeOH containing 5% NH_4OH) to disrupt anion-exchange interactions.

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

3.2.1. Sample pH

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

Thereafter, the sorbent was washed with MeOH. Unsurprisingly, it was found that the retention (or loss) or analytes in the washing step depended upon the pH of the loading solution. For loading at pH 3, it was found that all of the analytes were lost in the washing step, except for the strongly acidic ACE ($pK_a = -0.3$) and SAC ($pK_a = -1.6$). For loading at pH 6, the basic compounds TRI, PROP and MET were retained successfully and eluted subsequently with recoveries ranging from 71% to 97%, although the recovery of MEP (marginally less basic) was lower at 40%, whereas the acidic and amphoteric compounds (ACE, SAC, ALI, NEO, CLO AC, FEN and DICLO) were lost completely to the washing step. For loading at pH 9, only TRI, PROP, MET were recovered effectively, with recoveries ranging from 55 % to 88 %; the rest of the compounds were lost to the wash.

To provide more insight into the pH-dependent retention behaviour, samples were loaded at three more pH values (pH 2, 5 and 7) and the sorbents washed, as before, with MeOH. For loading at pH 2, it was observed, yet again, that only the most acidic analytes (ACE and SAC) were retained after washing with MeOH; this is because the sorbent displays WAX character but not WCX character at low pH, and ACE and SAC are the only two

analytes in anionic form at this pH. The other analytes were not retained at pH 2, either because they were acidic analytes in a neutral state (CLO AC, FEN and DICLO) or else because they were amphoteric/basic analytes in a cationic state. ACE and SAC were lost during the loading/washing steps when the loading pH was pH 5 or above since the amine moieties in the sorbent are not in an ionic form.

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

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

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

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

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

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

this finding is that the tertiary amine groups in the sorbent are influenced by the adjacent carboxylic acid groups such that they are not in their ionic form at pH 5.

In the study by Jin *et al.* [30] where a ternary mixed-mode silica SPE sorbent bearing C_8 groups, a primary amine and a carboxylic acid was developed, at pH 6 only the basic compounds were retained by ion-exchange mechanisms, whereas the acidic and neutral compounds were retained primarily by hydrophobic interactions.

3.2.2. Volume of washing solvent

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

3.2.3. Elution conditions

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

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

3.2.4. Sample volume

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

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

3.3. Analysis of environmental samples

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

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

Similar recoveries were obtained in other studies that use weak cation- or weak anion-exchange mixed-mode sorbents to determine these compounds in environmental waters [14,18]. In Fontanals' study [14], where an HXLPP-WAX polymer was used as SPE

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

4. Conclusions

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

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

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

for challenging chemical separations and applications where high capacity and selectivity is essential.

Acknowledgments

The authors would like to thank the *Ministerio de Economía, Industria y Competitividad, the Agencia Estatal de Investigación* and the European Regional Development Fund (ERDF) (Project: CTQ2017-88548-P) for the financial support given. J.C. Nadal is also grateful to Universitat Rovira i Virgili (URV) for his PhD grant (2017PMF-PIPF-49). I. Joas is grateful to the Erasmus+ mobility programme for financial support.

References

[1] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds : A critical review. part ii, Trends Anal. Chem. 80 (2016) 655–667. https://doi.org/10.1016/j.trac.2015.08.014.

[2] L.M. Madikizela, S. Ncube, L. Chimuka, Recent Developments in Selective Materials for Solid Phase Extraction, Chromatographia. 82 (2019) 1171–1189. https://doi.org/10.1007/s10337-018-3644-8.

[3] Y. Li, C. Huang, J. Yang, J. Peng, J. Jin, H. Ma, J. Chen, Multifunctionalized mesoporous silica as an efficient reversed-phase/anion exchange mixed-mode sorbent for solid-phase extraction of four acidic nonsteroidal anti-inflammatory drugs in environmental water samples, J. Chromatogr. A. 1527 (2017) 10–17. https://doi.org/10.1016/j.chroma.2017.10.051.

[4] N. Fontanals, F. Borrull, R.M. Marcé, Mixed-mode ion-exchange polymeric sorbents in environmental analysis, J. Chromatogr. A. 1609 (2020) art. 460531. https://doi.org/10.1016/j.chroma.2019.

[5] D. Bratkowska, N. Fontanals, F. Borrull, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, Hydrophilic hypercrosslinked polymeric sorbents for the solid-phase extraction of polar contaminants from water, J. Chromatogr. A. 1217 (2010) 3238–3243. https://doi.org/10.1016/j.chroma.2009.08.091.

[6] L. Tan, B. Tan, Hypercrosslinked porous polymer materials: Design, synthesis, and applications, Chem. Soc. Rev. 46 (2017) 3322–3356. https://doi.org/10.1039/c6cs00851h.

[7] N. Fontanals, R.M. Marcé, F. Borrull, P.A.G. Cormack, Hypercrosslinked materials: Preparation, characterisation and applications, Polym. Chem. 6 (2015) 7231–7244. https://doi.org/10.1039/c5py00771b.

[8] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Modern trends in solid phase extraction: New sorbent media, TrAC - Trends Anal. Chem. 77 (2016) 23–43. https://doi.org/10.1016/j.trac.2015.10.010.

[9] A. Orellana-Silla, S. Armenta, M. de la Guardia, J. V. Mercader, F.A. Esteve-Turrillas, Development of immunosorbents for the analysis of forchlorfenuron in

595 fruit juices by ion mobility spectrometry, Anal. Bioanal. Chem. 410 (2018)
596 5961–5967. https://doi.org/10.1007/s00216-018-1213-5.

597 [10] T. Murakami, Y. Iwamuro, R. Ishimaru, S. Chinaka, Molecularly imprinted
598 polymer solid-phase extraction of synthetic cathinones fromurine and whole
599 blood samples, J. Sep. Sci. 41 (2018) 4403–4566.
600 https://doi.org/10.1002/jssc.201800874.

601 [11] T. Zhou, G. Che, L. Ding, D. Sun, Y. Li, Recent progress of selective adsorbents:
602 From preparation to complex sample pretreatment, TrAC Trends Anal. Chem.
603 121 (2019) 115678. https://doi.org/10.1016/j.trac.2019.115678.

604 [12] H. Prosen, N. Fontanals, F. Borrull, R.M. Marcé, Determination of seven drugs
605 of abuse and theirmetabolites in surface and wastewater using solid-phase
606 extraction coupled to liquid chromatography with high-resolutionmass
607 spectrometry, J. Sep. Sci. 40 (2017). https://doi.org/10.1002/jssc.201700287.

608 [13] J. Valimaña-Traverso, S. Morante-Zarcero, D. Pérez-Quintanilla, M.Á. García, I.
609 Sierra, M.L. Marina, Cationic amine-bridged periodic mesoporous organosilica
610 materials for off-line solid-phase extraction of phenoxy acid herbicides from
611 water samples prior to their simultaneous enantiomeric determination by
612 capillary electrophoresis, J. Chromatogr. A. 1566 (2018) 146–157.
613 https://doi.org/10.1016/j.chroma.2018.06.042.

614 [14] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, Hypercrosslinked polymer
615 microspheres with weak anion-exchange character. Preparation of the
616 microspheres and their applications in pH-tuneable, selective extractions of
617 analytes from complex environmental samples, J. Chromatogr. A. 1215 (2008)
618 21–29. https://doi.org/10.1016/j.chroma.2008.10.124.

619 [15] D.W. Brousmiche, J.E. O'Gara, D.P. Walsh, P.J. Lee, P.C. Iraneta, B.C.
620 Trammell, Y. Xu, C.R. Mallet, Functionalization of divinylbenzene/N-
621 vinylpyrrolidone copolymer particles: Ion exchangers for solid phase extraction,
622 J. Chromatogr. A. 1191 (2008) 108–117.
623 https://doi.org/10.1016/j.chroma.2008.01.076.

624 [16] C. Huang, Y. Li, J. Yang, J. Peng, J. Tan, Y. Fan, L. Wang, J. Chen,
625 Hyperbranched mixed-mode anion-exchange polymeric sorbent for highly
626 selective extraction of nine acidic non-steroidal anti-inflammatory drugs from
627 human urine, Talanta. 190 (2018) 15–22.
628 https://doi.org/10.1016/j.talanta.2018.07.033.

629 [17] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, F. Borrull, Weak
630 anion-exchange hypercrosslinked sorbent in on-line solid-phase extraction-liquid
631 chromatography coupling to achieve automated determination with an effective
632 clean-up, J. Chromatogr. A. 1217 (2010) 2855–2861.
633 https://doi.org/10.1016/j.chroma.2010.02.064.

634 [18] D. Bratkowska, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, N.
635 Fontanals, Synthesis and application of hypercrosslinked polymers with weak
636 cation-exchange character for the selective extraction of basic pharmaceuticals
637 from complex environmental water samples, J. Chromatogr. A. 1217 (2010)
638 1575–1582. https://doi.org/10.1016/j.chroma.2010.01.037.

[19] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C. Sherrington, R.M. Marcé, Hypercrosslinked strong anion-exchange resin for extraction of acidic pharmaceuticals from environmental water, J. Sep. Sci. 35 (2012) 2621–2628. https://doi.org/10.1002/jssc.201200451.

[20] C. Kim, H. Ryu, E.G. Chung, Y. Kim, J. Lee, A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water : Sample preparation , liquid chromatography , and mass spectrometry, J. Environ. Manage. 217 (2018) 629–645. https://doi.org/10.1016/j.jenvman.2018.04.006.

[21] L. Vidal, O. Robin, J. Parshintsev, J.P. Mikkola, M.L. Riekkola, Quaternary ammonium-functionalized silica sorbents for the solid-phase extraction of aromatic amines under normal phase conditions, J. Chromatogr. A. 1285 (2013) 7–14. https://doi.org/10.1016/j.chroma.2013.02.003.

[22] J. Wang, P.R. Gardinali, Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 404 (2012) 2711–2720. https://doi.org/10.1007/s00216-012-6139-8.

[23] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography-electrospray tandem mass spectrometry, Anal. Bioanal. Chem. 391 (2008) 1293–1308. https://doi.org/10.1007/s00216-008-1854-x.

[24] L. Martín-Pozo, B. De Alarcón-Gómez, R. Rodríguez-Gómez, M.T. García-Córcoles, M. Çipa, A. Zafra-Gómez, Analytical methods for the determination of emerging contaminants in sewage sludge samples . A review, Talanta. 192 (2019) 508–533. https://doi.org/10.1016/j.talanta.2018.09.056.

[25] M. Lavén, T. Alsberg, Y. Yu, M. Adolfsson-Erici, H. Sun, Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry, J. Chromatogr. A. 1216 (2009) 49–62. https://doi.org/10.1016/j.chroma.2008.11.014.

[26] N. Köke, D. Zahn, T.P. Knepper, T. Frömel, Multi-layer solid-phase extraction and evaporation—enrichment methods for polar organic chemicals from aqueous matrices, Anal. Bioanal. Chem. 410 (2018) 2403–2411. https://doi.org/10.1007/s00216-018-0921-1.

[27] D. Salas, F. Borrull, N. Fontanals, R.M. Marcé, Combining cationic and anionic mixed-mode sorbents in a single cartridge to extract basic and acidic pharmaceuticals simultaneously from environmental waters, Anal. Bioanal. Chem. 410 (2018) 459–469. https://doi.org/10.1007/s00216-017-0736-5.

[28] A.A. Kazarian, M.R. Taylor, P.R. Haddad, P.N. Nesterenko, B. Paull, Ion-exchange and hydrophobic interactions affecting selectivity for neutral and charged solutes on three structurally similar agglomerated ion-exchange and mixed-mode stationary phases, Anal. Chim. Acta. 803 (2013) 143–153. https://doi.org/10.1016/j.aca.2013.03.063.

[29] L. Qiao, A. Dou, X. Shi, H. Li, Y. Shan, X. Lu, G. Xu, Development and evaluation of new imidazolium-based zwitterionic stationary phases for hydrophilic interaction chromatography, J. Chromatogr. A. 1286 (2013) 137–145. https://doi.org/10.1016/j.chroma.2013.02.066.

[30] S. Jin, Y. Qiao, J. Xing, Ternary mixed-mode silica sorbent of solid-phase extraction for determination of basic, neutral and acidic drugs in human serum, Anal. Bioanal. Chem. 410 (2018) 3731–3742. https://doi.org/10.1007/s00216-018-1037-3.

[31] T. Tsukamoto, A. Yamamoto, W. Kamichatani, Y. Inoue, Synthesis of novel sulfobetaine-type adsorbents and characteristics of their adsorption of polar solutes in hydrophilic SPE, Chromatographia. 70 (2009) 1525–1530. https://doi.org/10.1365/s10337-009-1378-3.

[32] N. Fontanals, P. Manesiotis, D.C. Sherrington, P.A.G. Cormack, Synthesis of Spherical Ultra-High-Surface-Area Monodisperse Amphipathic Polymer Sponges in the Low-Micrometer Size Range, Adv. Mater. 20 (2008) 1298–1302. https://doi.org/10.1002/adma.200702237.

[33] N. Fontanals, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Monodisperse, hypercrosslinked polymer microspheres as tailor-made sorbents for highly efficient solid-phase extractions of polar pollutants from water samples, J. Chromatogr. A. 1191 (2008) 118–124. https://doi.org/10.1016/j.chroma.2007.11.061.

Figure captions

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

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

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

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

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

729

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

730 % RSD (n=3) <18%

731

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

734

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

735 % RSD (n=3) < 20%