

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

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34 ABSTRACT

35

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

62

63 1. Introduction

64 Solid-phase extraction (SPE) is a widely used sample preparation technique for liquid
65 samples by virtue of its ability to enrich analytes and eliminate matrix interferences, with
66 the popularity of this technique being influenced positively by the range of SPE sorbents
67 available among other advantages [1,2]. Although a number of sorbents have been
68 available commercially for several years, a recent trend in the field concerns the
69 development of new and improved sorbents [1–4]. These sorbents aim to enhance the
70 sensitivity and/or the selectivity of SPE based methods, thereby enabling increasingly
71 complex matrices to be handled. Polymer-based sorbents having high-specific surface
72 areas and/or hydrophilic character, such as hypercrosslinked polymers and hydrophilic
73 macroporous polymers, respectively, address the demand for improved sensitivity [5–
74 7], whilst immunosorbents [8,9] and molecularly imprinted polymers were developed to
75 improve selectivity [2,10,11].

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

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

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

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

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

121 Of course, there is no chemical reason for why cation-exchange and anion-exchange
122 cannot be combined within a single sorbent material, however this is a relatively
123 unexplored area to date. Thus far, this strategy has been adopted only for silica-based
124 mixed-mode ion-exchange materials for use as either mixed-mode LC stationary phases
125 [28,29] or as mixed-mode SPE sorbents [30]. In the study by Jin *et al.* [30], a ternary
126 mixed-mode silica sorbent bearing C₈ groups, a primary amine and a carboxylic acid, was
127 used to retain acidic, neutral and basic compounds. The results from the latter study
128 showed that electrostatic repulsions and hydrophobic interactions between the sorbent
129 and the analytes influenced the retention behaviour. To the best of our knowledge, organic
130 polymers bearing amphoteric moieties, optimised for use as mixed-mode SPE sorbents,
131 have not yet been described in the literature, although a methacrylate-based material
132 bearing zwitterionic sulfoethylbetaine groups was used as an SPE sorbent to retain
133 hydrophilic solutes by a mechanism which relies upon the water retention capacity of the
134 sorbent surface [31] rather than ionic exchange interaction mechanisms.

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

145

146 2. Experimental

147 2.1. Reagents and standards

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

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

171 Stock solutions of individual standards were prepared in MeOH at a concentration of
172 1000 mg L^{-1} and stored at $-20\text{ }^\circ\text{C}$. Working solutions of a mixture of all compounds were
173 prepared weekly in a mixture of ultrapure water and MeOH (50/50) and were stored at 4
174 $^\circ\text{C}$ in the dark. Ultrapure water was provided by a water purification system (Veolia, Sant
175 Cugat del Vallès, Spain) and HPLC grade MeOH and ACN were purchased from J. T.
176 Baker (Deventer, The Netherlands). Formic acid (HCOOH) and ammonium hydroxide
177 (NH_4OH) from Sigma-Aldrich, and hydrochloric acid (HCl) from Scharlab (Barcelona,
178 Spain), were used to prepare the mobile phase and the solutions for SPE.

179

180 2.2. Synthesis and characterisation of HXLPP-WAX/WCX sorbent

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

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

187

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

199

200 A published synthesis protocol was followed for the preparation of the hypercrosslinked
201 polymer microspheres [32]. For the synthesis of poly(DVB-*co*-VBC) microspheres by
202 PP, DVB (5.033 g, 38.7 mmol) and VBC (15.021 g, 98.4 mmol) (25/75, w/w), were
203 dissolved in ACN (500 mL) in a Nalgene bottle (1 L). The monomer solution was
204 ultrasonicated at r.t. for 15 min. and then sparged with oxygen-free N_2 for a further 15
205 min. at ice-bath temperature. AIBN (0.560 g, 3.4 mmol, 2 mol% relative to the number
206 of moles of polymerisable double bonds) was then added, the bottle sealed immediately
207 under nitrogen and placed on a low-profile roller housed inside a temperature controlled
208 incubator. The incubator temperature was ramped from ambient to 60 °C over a period of
209 around 2 h and then held at 60 °C for 48 h, after which time a milky suspension of polymer
210 particles had formed. The product was isolated by vacuum filtration on a 0.45 μm nylon
211 membrane filter, washed with ACN and acetone, and dried overnight *in vacuo* (70 °C, 60
212 mbar). The product was isolated in the form of a free-flowing, white powder (3.268 g, 16
213 %). The characterisation data for the poly(DVB-*co*-VBC) microspheres can be found in
214 the Supplementary Information.

215

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

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

230

231 For the chemical functionalisation of HXLPP with sarcosine ethyl ester hydrochloride to
232 give HXLPP-WAX, HXLPP (2.907 g, 3.2 mmol Cl g⁻¹) and EtOH (70 mL) were
233 introduced into a three-necked, round-bottomed flask equipped with a reflux condenser
234 and an overhead stirrer, and the flask immersed in a temperature-controllable oil bath.
235 The suspension of microspheres was stirred gently at 100 rpm for 1.5 h, then K₂CO₃
236 (3.883 g, 28.1 mmol) and sarcosine ethyl ester hydrochloride (4.388 g, 28.6 mmol)
237 dissolved in H₂O (70 mL) added. The flask contents were heated at 75 °C for 18 h. After
238 cooling, the product was isolated by vacuum filtration on a 0.45 µm nylon membrane
239 filter and washed sequentially with EtOH, MeOH, 1:1 MeOH:H₂O, 0.01 M aqueous
240 NaHCO₃, H₂O and acetone, prior to drying for 24 h *in vacuo* (70 °C, 60 mbar). HXLPP-
241 WAX was isolated as a free-flowing, orange-coloured powder (2.958 g). See
242 Supplementary Information for the characterisation data.

243

244 The ester groups in HXLPP-WAX were hydrolysed under basic conditions to give
245 HXLPP-WAX/WCX. For this, HXLPP-WAX (2.920 g, 2.3 mmol of sarcosine ethyl ester
246 groups) was placed into a glass Duran bottle together with a solution of KOH (4.353 g,
247 77.6 mmol) in ethanol (100 mL). The tube was sealed and a low-profile roller used to mix
248 the contents at r.t. for 24 h. The product was filtered by vacuum on a 0.45 µm nylon
249 membrane filter, washed with large volumes of EtOH and then dried overnight *in vacuo*
250 (70 °C, 60 mbar). HXLPP-WAX/WCX was isolated as its potassium carboxylate and in
251 the form of a free-flowing, orange-coloured powder (3.274 g). FT-IR $\bar{\nu}/\text{cm}^{-1}$: 3014
252 (aromatic C-H stretch), 2914 (aliphatic C-H stretch), 1587 (carboxylate (CO₂)⁻
253 asymmetric stretch), 1400 (carboxylate (CO₂)⁻ symmetric stretch), 1040 (amine C-N
254 stretch), 827 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend),
255 796 (1,4-disubstituted aromatic out-of-plane C-H bend), 682 (aromatic ring bend).
256 Elemental microanalysis: Found for HXLPP-WAX/WCX: C, 73.4 %; H, 8.5 %; N, 1.2
257 %; Cl, 1.9 %; Sarcosine loading level = 0.9 mmol g⁻¹. SEM microscopy: mean particle
258 diameter = 3.02 µm; C_v = 18.2 %. N₂ sorption analysis: Langmuir SSA = 1,140 m² g⁻¹;
259 specific pore volume = 0.45 cm³ g⁻¹; mean pore width = 2.7 nm.

260

261 2.3. Solid-phase extraction procedure

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

268 The SPE protocol started with the conditioning of the sorbents, and this involved passing
269 10 mL of MeOH through the cartridges followed by 10 mL of ultrapure water adjusted to

270 the same pH as the sample. The volumes of river water samples and effluent wastewater
271 samples used for the loading step was 100 mL, with all samples being adjusted to pH 6
272 with HCl. After loading of the samples onto the sorbent, the washing step involved
273 washing with 1 mL of MeOH, and the elution step involved washing with 5 mL MeOH
274 containing 5% NH₄OH. A miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) was
275 used to evaporate the extracts to dryness prior to reconstitution with 1 mL of mobile phase
276 (H₂O/ACN, 90/10, v/v). All reconstituted extracts were filtered using 0.45 μm PTFE
277 syringe filters (Scharlab) before injection into the chromatographic system. The SPE
278 cartridges were reused more than 20 times when analysing environmental waters.

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

282

283 **2.4. Instrumentation and chromatographic conditions**

284 The chromatographic system was an Agilent 1200 UHPLC equipped with a binary pump,
285 an autosampler, an automatic injector and a DAD detector (Agilent, Waldbronn,
286 Germany). The chromatographic column used was a Tracer Excel 120 C₈ (150 mm × 4.6
287 mm i.d., 5 μm particle size) supplied by Teknokroma (Sant Cugat del Vallès, Spain). The
288 mobile phase was a mixture of ultrapure water (adjusted to pH 2.8 with HCl) (solvent A)
289 and ACN (solvent B). The gradient profile started with 10% of B, which was raised to
290 40% B within 12 min. and then to 100% B within 16 min. Subsequently, it was held at
291 100% B for 3 min. before returning to the initial conditions in 3 min. The column was
292 kept at 30 °C and the flow rate was 600 μL min⁻¹. The injection volume was 20 μL.

293

294 **3. Results and discussion**

295 **3.1. Synthesis of the HXLPP-WAX/WCX sorbent**

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

305

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

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

313

314 The very fast hypercrosslinking reaction was not taken to full conversion of chloromethyl
315 groups into methylene bridges – for this, the hypercrosslinking reaction time was
316 restricted to 10 minutes – and this left a good number of pendent chloromethyl groups
317 (1.1 mmol g^{-1}) unreacted and available for use as chemical handles in subsequent
318 polymer-analogous reactions. The synthetic strategy taken to install amphoteric moieties
319 into the polymers was to use a sarcosine derivative as a nucleophile to displace chloride
320 in a nucleophilic aliphatic substitution reaction. This approach is very attractive with
321 regards to the future development and elaboration of a family of amphoteric polymers,
322 since many α -amino acids are available and the polymer-analogous reactions are
323 efficient. Of course, the methodology is not restricted to α -amino acids, although having
324 the amino groups in close proximity to the carboxylic acid groups, as is the case for α -
325 amino acids, may allow for fine-tuning of sorbent selectivity. In the present case,
326 sarcosine residues were installed into the hypercrosslinked microspheres by treatment of
327 the microspheres with sarcosine ethyl ester hydrochloride under basic conditions (to give
328 HXLPP-WAX, which is a new weak ion-exchanger in its own right), followed by ester
329 hydrolysis to give the amphoteric polymer target, HXLPP-WAX/WCX. This high quality
330 polymer was isolated in a good yield (close to full recovery of the microsphere product
331 across the two polymer-analogous reactions) in a convenient beaded format (mean
332 particle diameter = $3 \mu\text{m}$; CV = 18%). Fig. 2 shows an SEM image of the HXLPP-
333 WAX/WCX microspheres. HXLPP-WAX/WCX had a well-developed porous
334 morphology (Langmuir SSA = $1,140 \text{ m}^2 \text{ g}^{-1}$; mean pore diameter = 2.7 nm); the high SSA
335 and low mean pore diameter arise from the hypercrosslinking process, and the values are
336 consistent with a permanently porous polymeric material which contains a significant
337 proportion of micropores. The loading level of sarcosine residues was calculated from the
338 elemental microanalytical data to be 0.9 mmol g^{-1} .

339

340 **3.2. Optimisation of the SPE protocol**

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

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

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

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

372

373 **3.2.1. Sample pH**

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

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

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

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

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

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

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

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

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

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

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

436 In the study by Jin *et al.* [30] where a ternary mixed-mode silica SPE sorbent bearing C₈
437 groups, a primary amine and a carboxylic acid was developed, at pH 6 only the basic
438 compounds were retained by ion-exchange mechanisms, whereas the acidic and neutral
439 compounds were retained primarily by hydrophobic interactions.

440

441 **3.2.2. Volume of washing solvent**

442 The next parameter optimised for the SPE protocol was the volume of MeOH used in the
443 washing step. Since the weakly acidic compounds, as well as the amphoteric compounds
444 (ALI, NEO), were lost during the washing step, the volume of MeOH was reduced from
445 2 x 2 mL to 1 mL. Simultaneously, the mass of sorbent per cartridge was increased from
446 100 mg to 200 mg (all the following experiments were performed using 200 mg sorbent).
447 As can be seen in Fig. 3, the effect of these two changes to the SPE method was to reduce
448 the loss of analytes in the wash, leading to better recoveries for all analytes. All of the
449 basic compounds (TRI, MEP, PROP, and MET) were retained and eluted successfully,
450 with %R between 93% and 111%. Interestingly, the recoveries of weakly acidic
451 compounds improved considerably too (the recoveries of FEN and DICLO were 73% and
452 74%, respectively, whereas the recovery of CLO AC was 48%). Moving forward, the
453 volume of washing solvent was fixed at 1 mL for all subsequent experiments.

454

455 **3.2.3. Elution conditions**

456 As was outlined earlier, two different elution solvents were applied in series: firstly, an
457 acidic elution solvent (5 mL MeOH containing 5% HCOOH) to disrupt cation-exchange
458 interactions and, secondly, a basic elution solvent (5 mL MeOH containing 5% NH₄OH)
459 to disrupt anion-exchange interactions. Accordingly, the elution recoveries are reported
460 as the recovery from the acidic elution followed by the elution from the basic elution, or
461 *vice versa*, with the overall recovery value for any given analyte being the sum of both
462 recovery values. The data is presented in Table S1.

463 For acidic elution followed by basic elution, with the exception of ACE and SAC (which
464 appeared in both elution fractions) the analytes were collected in the acidic fraction only.
465 This is because formic acid switches off cation-exchange interactions between basic
466 analytes and polymer-bound carboxylates by protonating the carboxylate residues and
467 protonating any weakly acidic analytes bound through anion-exchange. For basic elution
468 followed by acidic elution, all of the analytes were eluted quantitatively by the base. This
469 is because ammonium hydroxide neutralises any polymer-bound amines, thereby turning
470 off WAX behaviour, and deprotonates and neutralises any basic analytes, which means
471 that they can no longer be retained on the sorbent by a WCX mechanism. Consequently,
472 for the optimised SPE protocol an acidic elution step was not required; going forward,
473 the optimised elution step used 5 mL MeOH containing 5% NH₄OH.

474

475 **3.2.4. Sample volume**

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

487 Once all the parameters had been optimised, the optimal conditions for the SPE protocol
488 were fixed as follows: 200 mg of sorbent; 100 mL of sample adjusted to pH 6; 1 mL
489 MeOH as washing solution; elution with 5 mL MeOH containing 5% NH₄OH. Moreover,
490 in order to increase the sensitivity of the method, the elution extract was evaporated to
491 dryness and reconstituted with 1 mL of mobile phase. It should be noted that no losses of
492 analytes were observed during the evaporation step.

493

494 **3.3. Analysis of environmental samples**

495

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

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

511 Similar recoveries were obtained in other studies that use weak cation- or weak anion-
512 exchange mixed-mode sorbents to determine these compounds in environmental waters
513 [14,18]. In Fontanals' study [14], where an HXLPP-WAX polymer was used as SPE

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

528

529

530 **4. Conclusions**

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

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

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

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

557

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564

565 **References**

- 566 [1] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase
567 extraction of organic compounds : A critical review. part ii, *Trends Anal. Chem.*
568 80 (2016) 655–667. <https://doi.org/10.1016/j.trac.2015.08.014>.
- 569 [2] L.M. Madikizela, S. Ncube, L. Chimuka, Recent Developments in Selective
570 Materials for Solid Phase Extraction, *Chromatographia.* 82 (2019) 1171–1189.
571 <https://doi.org/10.1007/s10337-018-3644-8>.
- 572 [3] Y. Li, C. Huang, J. Yang, J. Peng, J. Jin, H. Ma, J. Chen, Multifunctionalized
573 mesoporous silica as an efficient reversed-phase/anion exchange mixed-mode
574 sorbent for solid-phase extraction of four acidic nonsteroidal anti-inflammatory
575 drugs in environmental water samples, *J. Chromatogr. A.* 1527 (2017) 10–17.
576 <https://doi.org/10.1016/j.chroma.2017.10.051>.
- 577 [4] N. Fontanals, F. Borrull, R.M. Marcé, Mixed-mode ion-exchange polymeric
578 sorbents in environmental analysis, *J. Chromatogr. A.* 1609 (2020) art. 460531.
579 <https://doi.org/10.1016/j.chroma.2019>.
- 580 [5] D. Bratkowska, N. Fontanals, F. Borrull, P.A.G. Cormack, D.C. Sherrington,
581 R.M. Marcé, Hydrophilic hypercrosslinked polymeric sorbents for the solid-
582 phase extraction of polar contaminants from water, *J. Chromatogr. A.* 1217
583 (2010) 3238–3243. <https://doi.org/10.1016/j.chroma.2009.08.091>.
- 584 [6] L. Tan, B. Tan, Hypercrosslinked porous polymer materials: Design, synthesis,
585 and applications, *Chem. Soc. Rev.* 46 (2017) 3322–3356.
586 <https://doi.org/10.1039/c6cs00851h>.
- 587 [7] N. Fontanals, R.M. Marcé, F. Borrull, P.A.G. Cormack, Hypercrosslinked
588 materials: Preparation, characterisation and applications, *Polym. Chem.* 6 (2015)
589 7231–7244. <https://doi.org/10.1039/c5py00771b>.
- 590 [8] J. Plotka-Wasyłka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Modern
591 trends in solid phase extraction: New sorbent media, *TrAC - Trends Anal. Chem.*
592 77 (2016) 23–43. <https://doi.org/10.1016/j.trac.2015.10.010>.
- 593 [9] A. Orellana-Silla, S. Armenta, M. de la Guardia, J. V. Mercader, F.A. Esteve-
594 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 from urine 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 their metabolites in surface and wastewater using solid-phase
606 extraction coupled to liquid chromatography with high-resolution mass
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-tunable, 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>.

- 639 [19] D. Bratkowska, A. Davies, N. Fontanals, P.A.G. Cormack, F. Borrull, D.C.
640 Sherrington, R.M. Marcé, Hypercrosslinked strong anion-exchange resin for
641 extraction of acidic pharmaceuticals from environmental water, *J. Sep. Sci.* 35
642 (2012) 2621–2628. <https://doi.org/10.1002/jssc.201200451>.
- 643 [20] C. Kim, H. Ryu, E.G. Chung, Y. Kim, J. Lee, A review of analytical procedures
644 for the simultaneous determination of medically important veterinary antibiotics
645 in environmental water : Sample preparation , liquid chromatography , and mass
646 spectrometry, *J. Environ. Manage.* 217 (2018) 629–645.
647 <https://doi.org/10.1016/j.jenvman.2018.04.006>.
- 648 [21] L. Vidal, O. Robin, J. Parshintsev, J.P. Mikkola, M.L. Riekkola, Quaternary
649 ammonium-functionalized silica sorbents for the solid-phase extraction of
650 aromatic amines under normal phase conditions, *J. Chromatogr. A.* 1285 (2013)
651 7–14. <https://doi.org/10.1016/j.chroma.2013.02.003>.
- 652 [22] J. Wang, P.R. Gardinali, Analysis of selected pharmaceuticals in fish and the
653 fresh water bodies directly affected by reclaimed water using liquid
654 chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.* 404 (2012)
655 2711–2720. <https://doi.org/10.1007/s00216-012-6139-8>.
- 656 [23] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the
657 analysis of pharmaceuticals, personal care products and illicit drugs in surface
658 water and wastewater by solid-phase extraction and ultra performance liquid
659 chromatography-electrospray tandem mass spectrometry, *Anal. Bioanal. Chem.*
660 391 (2008) 1293–1308. <https://doi.org/10.1007/s00216-008-1854-x>.
- 661 [24] L. Martín-Pozo, B. De Alarcón-Gómez, R. Rodríguez-Gómez, M.T. García-
662 Córcoles, M. Çipa, A. Zafra-Gómez, Analytical methods for the determination of
663 emerging contaminants in sewage sludge samples . A review, *Talanta.* 192
664 (2019) 508–533. <https://doi.org/10.1016/j.talanta.2018.09.056>.
- 665 [25] M. Lavén, T. Alsberg, Y. Yu, M. Adolfsson-Erici, H. Sun, Serial mixed-mode
666 cation- and anion-exchange solid-phase extraction for separation of basic, neutral
667 and acidic pharmaceuticals in wastewater and analysis by high-performance
668 liquid chromatography-quadrupole time-of-flight mass spectrometry, *J.*
669 *Chromatogr. A.* 1216 (2009) 49–62.
670 <https://doi.org/10.1016/j.chroma.2008.11.014>.
- 671 [26] N. Köke, D. Zahn, T.P. Knepper, T. Frömel, Multi-layer solid-phase extraction
672 and evaporation—enrichment methods for polar organic chemicals from aqueous
673 matrices, *Anal. Bioanal. Chem.* 410 (2018) 2403–2411.
674 <https://doi.org/10.1007/s00216-018-0921-1>.
- 675 [27] D. Salas, F. Borrull, N. Fontanals, R.M. Marcé, Combining cationic and anionic
676 mixed-mode sorbents in a single cartridge to extract basic and acidic
677 pharmaceuticals simultaneously from environmental waters, *Anal. Bioanal.*
678 *Chem.* 410 (2018) 459–469. <https://doi.org/10.1007/s00216-017-0736-5>.
- 679 [28] A.A. Kazarian, M.R. Taylor, P.R. Haddad, P.N. Nesterenko, B. Paull, Ion-
680 exchange and hydrophobic interactions affecting selectivity for neutral and
681 charged solutes on three structurally similar agglomerated ion-exchange and
682 mixed-mode stationary phases, *Anal. Chim. Acta.* 803 (2013) 143–153.
683 <https://doi.org/10.1016/j.aca.2013.03.063>.

- 684 [29] L. Qiao, A. Dou, X. Shi, H. Li, Y. Shan, X. Lu, G. Xu, Development and
685 evaluation of new imidazolium-based zwitterionic stationary phases for
686 hydrophilic interaction chromatography, *J. Chromatogr. A.* 1286 (2013) 137–
687 145. <https://doi.org/10.1016/j.chroma.2013.02.066>.
- 688 [30] S. Jin, Y. Qiao, J. Xing, Ternary mixed-mode silica sorbent of solid-phase
689 extraction for determination of basic, neutral and acidic drugs in human serum,
690 *Anal. Bioanal. Chem.* 410 (2018) 3731–3742. [https://doi.org/10.1007/s00216-](https://doi.org/10.1007/s00216-018-1037-3)
691 [018-1037-3](https://doi.org/10.1007/s00216-018-1037-3).
- 692 [31] T. Tsukamoto, A. Yamamoto, W. Kamichatani, Y. Inoue, Synthesis of novel
693 sulfobetaine-type adsorbents and characteristics of their adsorption of polar
694 solutes in hydrophilic SPE, *Chromatographia.* 70 (2009) 1525–1530.
695 <https://doi.org/10.1365/s10337-009-1378-3>.
- 696 [32] N. Fontanals, P. Manesiotis, D.C. Sherrington, P.A.G. Cormack, Synthesis of
697 Spherical Ultra-High-Surface-Area Monodisperse Amphipathic Polymer Sponges
698 in the Low-Micrometer Size Range, *Adv. Mater.* 20 (2008) 1298–1302.
699 <https://doi.org/10.1002/adma.200702237>.
- 700 [33] N. Fontanals, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull,
701 Monodisperse, hypercrosslinked polymer microspheres as tailor-made sorbents
702 for highly efficient solid-phase extractions of polar pollutants from water
703 samples, *J. Chromatogr. A.* 1191 (2008) 118–124.
704 <https://doi.org/10.1016/j.chroma.2007.11.061>.
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711

712 **Figure captions**

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

714

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

717

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

720

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

726

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
Basic		NEO	38	39
		MEP	87	88
		MET	58	61
		PROP	75	79
		TRI	85	86

735

% RSD (n=3) < 20%