Synthesis and application of hypercrosslinked polymers with weak cation-exchange character for the selective extraction of basic pharmaceuticals from complex environmental water samples

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Abstract

The synthesis of high specific surface area sorbents (HXLPP-WCX) in the form of hypercrosslinked polymer microspheres with narrow particle size distributions, average particle diameters around 6 *μ*m, and weak cation exchange (WCX) character, is described. The WCX character arises from carboxylic acid moieties in the polymers, derived from the comonomer methacrylic acid. A novel HXLPP-WCX sorbent with an attractive set of chemical and physical properties was then used in an off-line solid-phase extraction (SPE) protocol for the selective extraction of a group of basic compounds from complex environmental samples, a priority being the clean separation of the basic compounds of interest from acidic compounds and interferences. The separation power of the new sorbent for basic pharmaceuticals was compared to two commercially available, mixed-mode sorbents, namely Oasis WCX and Strata X-CW. Under identical experimental conditions, HXLPP-WCX was found to deliver both higher capacity and better selectivity in SPE than either of the two commercially available materials. In an optimised SPE protocol, the HXLPP-WCX sorbent gave rise to quantitative and selective extractions of low μg l^{-1} levels of basic pharmaceuticals present in 500 ml of river water and 250 ml of effluent waste water.

Introduction

Solid-phase extraction (SPE) is a powerful analytical tool used widely for pre-concentration, fractionation and purification of analytes of interest from complex environmental [1-4] and biological samples (urine, blood and plasma) [5,6]. In recent years, a group of analytes of increasing interest is pharmaceuticals, since they are dispersed continuously into the environment as a result of human use [5,7], which can give rise to problems including health concerns for humans, therefore there is a demand for analytical methods which enable the accurate determination of pharmaceuticals in the environment, even when the pharmaceuticals are present at low levels. To satisfy this demand, and meet the appropriate detection limits, there is a requirement for suitable pre-concentration techniques which can both concentrate and clean-up the analytes present in the complex environmental matrices. SPE is excellent choice in this regard since it can provide high enrichment factors of the target compounds and eliminate interferences from the sample to be analysed. Polymeric materials are the most important group of sorbent used in SPE, since they offer attractive advantages such as good retention of analytes and sorbent stability under a much broader range of analysis conditions than for sorbents of other types (*i.e.*, silica- and carbon-based sorbents). Several polymeric sorbents have been developed and applied to the extraction of pharmaceuticals [8-10].

In recent years, SPE technology has expanded to offer the use of mixed-mode, polymeric ion-exchange media, which combines the attributes of reversed-phase chemistry and ion-exchange interactions into one single material [11]. Mixed-mode ion-exchange sorbents are designed to interact with

ionic species, but they can also retain non-charged species effectively through hydrophobic or hydrophilic interactions [11,12]. Mixed-mode sorbents are classified as either strong or weak ion-exchange, depending on the ionic groups tethered to the sorbent. An important advantage of weak ion-exchange sorbents is that the ionisation state of the resin may be tuned easily by pH, thus adding more versatility and power to SPE applications [12]. Amongst the most popular, commercially available mixed-mode sorbents are Oasis MCX, Oasis MAX, Oasis WCX, and Oasis WAX (all from Waters), which are classified as strong (MCX, MAX) or weak (WCX, WAX) cation/anion-exchange resins, respectively. All four of these interesting sorbents are derived from an Oasis HLB polymeric skeleton [poly(vinylpyrrolidone-co-divinylbenzene), ~800 $m^2 g^{-1}$] which has been modified chemically with sulfonic acid and quaternary ammonium groups in the case of the strong ion-exchangers, and carboxylic acid and piperazine groups in the case of the weak ion-exchangers. In an analogous fashion, Strata-X (a Phenomenex sorbent), which is based on a poly(styrene-co-divinylbenzene) skeleton bearing polar vinylpyrrolidone residues, can be modified chemically to give related sorbents bearing sulfonic acid groups (Strata-X-C), carboxylic acid groups (Strata-X-CW) or ethylene diamine groups (Strata-X-AW).

All of these commercial sorbents have macroreticular structures which give rise to weaker reversed-phase interactions with analytes than do hypercrosslinked polymer resins. Hypercrosslinked polymers are a new generation of permanently porous, polymeric resins with enhanced analyte retention characteristics arising from their high micropore contents and correspondingly high specific surface areas (>1000 $m^2 g^{-1}$) [13]. Recently, we disclosed the synthesis of mixed-mode hypercrosslinked sorbents with weak

112 anion exchange (WAX) character, and the application of these novel sorbents to
113 the SPE of acidic pharmaceuticals from aqueous samples [14].

114 The present study describes the synthesis of hypercrosslinked polymer
115 resins with weak cation-exchange (WCX) character, where the WCX properties
116 are derived from the presence of carboxylic acid moieties, and the application of
117 these sorbents to the SPE of basic pharmaceuticals from complex
118 environmental samples. The new materials have been bench-marked against
119 Strata-X-CW and Oasis WCX. Although previously porous polymer containing
120 methacrylic acid (MAA) in monolith format has been applied to in-tube-solid-
121 phase microextraction (SPME)-liquid chromatography (LC) for the extraction of
122 drugs from complex sample matrices [15]; as far as we are aware, this is the
123 first time that a hypercrosslinked sorbent has been used as a weak cation-
124 exchanger for the selective extraction of basic pharmaceuticals from complex
125 environmental samples, which also contain acidic and neutral compounds.

126

127 ## 2. Experimental

128 ### *2.1 Reagents and standards*

129 The reagents used for the polymer syntheses were divinylbenzene (DVB)
130 (80% grade) supplied by Aldrich (Steinheim, Germany), methacrylic acid (MAA)
131 (98% grade) and *para*-vinylbenzyl chloride (VBC) (95% grade) supplied by
132 Fluka (Buchs, Switzerland). DVB and VBC were purified by passing through
133 short columns packed with neutral alumina. MAA was purified by vacuum
134 distillation. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied
135 by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck,

136 Darmstadt, Germany). Ferric chloride ($FeCl_3$) and anhydrous 1,2-dichloroethane
137 (DCE), from Aldrich, were used in the hypercrosslinked reactions.
138 The pharmaceutical analytes selected to evaluate the performance of the
139 sorbents in SPE were: acetaminophen, caffeine, antipyrine, propranolol,
140 carbamazepine, naproxen and diclofenac (all obtained from Sigma-Aldrich). The
141 chemical structures and pK_a values of the analytes are presented in Table 1.
142 Standard solutions at 1000 mg l^{-1} in methanol were prepared for each
143 analyte. The mixture of all the analytes was prepared by diluting the standard
144 solutions with MeOH:H_2O (1:1, v/v).
145 LC-grade acetonitrile and methanol (SDS, Peypin, France) and Milli-Q
146 water (Millipore, Bedford, MA, USA) were used to prepare the mobile phases.
147 Hydrochloric acid (Probus, Barcelona, Spain) was used to adjust the pH of the
148 mobile phase and the sample before SPE. Other reagents used in SPE
149 procedures were: ammonium hydroxide (NH_4OH) (Merck), formic acid
150 (HCOOH) (Probus) and trifluoroacetic acid (TFA) (Fluka).

151 ***2.2 Resin preparation and characterisation***

152 The micron-sized spherical particles (PP-WCX) used as swellable
153 precursors in the production of the hypercrosslinked resins (HXLPP-WCX),
154 were synthesised using an optimised precipitation polymerisation (PP) protocol
155 [16]. The monomers (10% MAA, 50% VBC and 40% DVB [% w/w]) and AIBN (2
156 mol% relative to polymerisable double bonds) were dissolved in acetonitrile
157 (200 ml) in a polypropylene bottle (250 ml) at a total monomer concentration of
158 2% w/v. The monomer solution was de-oxygenated with N_2 at 0 ^oC and the
159 bottle then placed on a low-profile roller (Stovall, Essex, UK) in a temperature-
160 controllable incubator (Stuart Scientific, Surrey, UK). The temperature was

161 ramped from ambient to 60 ^{o}C over a period of ~ 2 hours and the polymerisation
162 allowed to proceed at 60 ^{o}C for a further 46 hours. The resulting particles were
163 filtered on a 0.2 *μ*m nylon membrane filter and washed successively with
164 MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 ^{o}C.

165 The hypercrosslinked reactions of the MAA-VBC-DVB precursors were
166 carried out as described in previous study [16], using a well-established reaction
167 for the VBC-DVB precursor.

168 The hypercrosslinked resin (HXLPP-WCX) was characterised by
169 measuring specific surface area using a BET treatment of N_2 sorption isotherm
170 data generated on a Micromeritics ASAP 2000 porosimeter. The carbon (83.1%
171 w/w), hydrogen (7.2% w/w), chlorine (2.5% w/w) and oxygen (6.3% w/w,
172 calculated by difference) contents for the resin were obtained by elemental
173 microanalysis using a Carlo-Erba EA 1106 instrument. The cation-exchange
174 capacity was calculated from the microanalytical data using the theoretical
175 values. The average microsphere diameter and homogeneity in size (particle
176 size distribution) were calculated using ImageJ software from the image
177 analysis of 100 individual particles in scanning electron microscopy (SEM)
178 images, which were acquired using a JEOL 6400 Instrument. SEM image for
179 the HXLPP-WCX resin is included in Figure 1S of the Supported Information
180 Section. A schematic representation of the structure of HXLPP-WCX is depicted
181 in Figure 1. The characterisation data for all the sorbents studied is detailed in
182 Table 2.

183 ***2.3 Chromatographic equipment and conditions***

184 The chromatographic experiments were performed with an HP 1090 Liquid
185 Chromatograph equipped with an injection valve with a 20 *μ*l loop and an

186 Agilent 1200 UV spectrophotometric detector (Agilent, Waldbronn, Germany).
187 The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column
188 packed with Kromasil 100 C_{18}, 5 μm (Teknokroma, Barcelona, Spain).

189 The mobile phases were Milli-Q water adjusted to pH 3 with HCl (solvent
190 A) and acetonitrile (solvent B). The flow rate was 1 ml min^{-1} and the
191 temperature of the column oven was set at 65 $^{\circ}$C. The gradient profile was from
192 10% to 15% ACN in 5 min, then to 100% ACN in 25 min (held for 2 min), then
193 the mobile phase was returned to the initial conditions (10% ACN) in 3 min.

194 The detection wavelength for all the compounds was 210 nm.

195 ***2.4 Solid-phase extraction***

196 SPE cartridges (6 ml, polypropylene) were packed with 200 mg of the
197 HXLPP-WCX sorbent. The sorbent was retained by two frits: a 2 μm pore size
198 metal frit at the bottom, and a 20 μm pore size polyethylene frit at the top. The
199 retention capabilities of the novel sorbent was compared to commercial SPE
200 cartridges from Phenomenex (Strata-X-CW; 200 mg/6 ml) and Waters (Oasis
201 WCX; 200 mg/6 ml) (which was packed manually). A vacuum manifold
202 (Teknokroma) was used to manipulate the cartridges in the off-line SPE
203 process. One single sorbent cartridge of each type was used for the whole
204 study. The three sorbent structures are presented in Figure 1.

205 Prior to the SPE extractions, the pH of the sample was adjusted to 7 with
206 HCl or NaOH. The procedure used for all cartridges was identical: the cartridge
207 was activated with 5 ml of MeOH followed by 2 ml of Milli-Q water, and the
208 sample then loaded at a flow rate 10 ml min^{-1}. After equilibration, the cartridge
209 was washed with 2 ml 5% NH_4OH in MeOH. Finally, the compounds were
210 eluted from the cartridge using 5 ml of 2% TFA in MeOH.

211 Prior to LC analyses, the SPE eluates were evaporated to dryness and
212 then reconstituted in 1 ml of MeOH:H_2O (1:1, v/v).

213 To keep the samples under proper conditions, real water (Ebre river water
214 and effluent waste water from a treatment plant) were adjusted to ~ pH 3 with
215 HCl and kept at 4 ºC before analysis. They were filtered through 0.22 μm nylon
216 membranes (Supelco, Bellefonte, PA, USA) prior to the preconcentration step to
217 eliminate the particulate matter which is normally present in real samples.

218

219 **3. Results and discussion**

220 **3.1 Preparation of the HXLPP-WCX sorbent**

221 The novel WCX hypercrosslinked sorbent (HXLPP-WCX) was derived from
222 a swellable copolymer precursor (PP-WCX) prepared by precipitation
223 polymerisation (PP). PP is a simple, straightforward and reproducible method
224 for obtaining, in one single preparative step, spherical polymer particles with
225 average diameters in the low micron size regime which, as has been
226 demonstrated previously [14,17,18], perform well as novel sorbents in SPE
227 applications. The aim of the present work was to synthesise a hypercrosslinked
228 derivative of the terpolymer poly(MAA-co-VBC-co-DVB), and thereby access a
229 resin which combined both weak cation-exchange character (through the MAA
230 residues present) and high specific surface area derived from its high micropore
231 content (from hypercrosslinking reactions which consume the pendent
232 chloromethyl groups). During the production of the poly(MAA-co-VBC-co-DVB)
233 precursor polymer, various comonomer ratios were evaluted (data not shown);
234 the comonomer ratio reported in the present manuscript (*i.e.*, 10% MAA,

50%VBC, 40% DVB [w/w]) was found to offer the optimal balance of properties, *i.e.*, suitable ion-exchange capacity and particle size, and high specific surface area.

The resin characterisation data for HXLPP-WCX is detailed in Table 2. The specific surface area was 1125 m^2 g^{-1} and the cation-exchange capacity 0.72 meq g^{-1}. Following on from the development of a convenient synthetic route into an HXLPP-WCX resin, our aim was to evaluate the potential benefits in SPE of introducing carboxylic acid moieties into hypercrosslinked polymer microspheres, and to compare the performance of this new resin to the commercially available sorbents (more specifically, Oasis-WCX and Strata-X-CW). The characterisation data for all three sorbents is detailed in Table 2. The ion-exchange capacity is similar for all three sorbents (~ 0.75 meq g^{-1}), however the particle size of the HXLPP-WCX sorbent (~ 6 μm) is markedly lower than either of the other two materials. The lower particle size of the sorbent might provide better contact with the analytes to be extracted, and, thus, benefit in the SPE process.

3.2 SPE optimisation

Pharmaceuticals bear a variety of functional groups and they can be cationic, anionic or zwitterionic depending on the sample pH. Some pharmaceuticals contain nitrogen-containing functional groups which are basic and will therefore be readily protonated to give a cation under certain conditions [19,20]. To evaluate the cation-exchange properties of the HXLPP-WCX sorbent, and establish the scope of its sorption characteristics, we selected a group of acidic and basic pharmaceuticals with variable pK_a values (Table 1).

Besides the selection of analytes with a wide range of acidic/basic properties, to test the performance of the WCX sorbents in an accurate and reliable manner it was necessary to optimise the SPE conditions in such a way as to maximise the retention of the analytes on the sorbents. Optimal retention conditions are those for which ionic interactions between the MAA residues in the sorbent and the cationic forms of the analyte are maximised.

3.2.1 Sample loading

Since HXLPP-WCX is a cation-exchange material, the analyte retention mechanism is based on ionic interactions between carboxylic acid groups in the polymer and the pharmaceuticals. Thus, the pH of the sample during analyte extraction by the sorbent is an important parameter to be optimised.

To investigate the retention properties of the HXLPP-WCX sorbent, 100 ml volumes of two separate samples (at pH 3 and pH 7, respectively) were percolated through SPE cartridges packed with the sorbent. At pH 3 the carboxylic acid groups of the acidic compounds and the sorbent are primarily in their non-ionised form, whereas the basic compounds are fully ionised. In contrast, at pH 7 the carboxylic acid-containing acidic compounds are deprotonated and are eluted during the SPE washing step, while the carboxylic acid residues in the polymer are ionised and retain the basic pharmaceuticals (protonated) by ionic interactions. Thus, cation-exchange phenomena are expected to be more effective at pH 7 than at pH 3. When preliminary SPE experiments were performed to confirm these expectations, the recoveries of the basic compounds were found to be lower at pH 3 and very high at pH 7. For this reason, samples were adjusted to pH 7 in all the subsequent SPE experiments.

285 3.2.2 *Washing step*

286 The aim of the washing step was to eliminate interferences (including
287 acidic and neutral compounds) bound to the sorbent through reserved-phase
288 mechanisms, while retaining on the sorbent the basic compounds bound
289 through cation-exchange interactions. 1 ml volumes of various neat organic
290 solvents (such as methanol and acetonitrile) were applied in the washing step,
291 but in such cases all the analytes were eluted. Thus, we decided to use a
292 solution of NH_4OH in organic solvent as the washing solution, to maintain the
293 desired ionisation state of the analytes and the sorbent. In this regard, the
294 following solutions were evaluated: 5% NH_4OH in MeOH; 5% NH_4OH in ACN;
295 5% NH_4OH in MeOH/ACN (1/4). Of these three options, 5% NH_4OH in MeOH
296 gave higher recoveries for all analytes than the other two washing solution and
297 was thus selected as the washing solvent of choice. Thereafter, the next step
298 was to evaluate the optimum volume of the washing solvent to be used in the
299 SPE protocol. For these experiments, where the sample matrix was Milli-Q
300 water, 1 ml of 5% NH_4OH in MeOH was used initially. Although this volume of
301 washing solvent was found to be not enough to elute all the acidic compounds
302 quantitatively, 2 ml of 5% NH_4OH in MeOH was found to be effective for this
303 purpose so was established as the optimal volume of washing solvent required
304 to elute acidic compounds and interferences, whilst still allowing total retention
305 of the analytes of interest (*i.e.*, basic compounds).

306 3.2.3 *Elution of basic compounds*

307 For the elution step, in which the aim was to elute the basic compounds
308 bound to WCX sorbents through ionic interactions, various acidic solutions were
309 tested (acidification protonates the carboxylic acid residues on the sorbents,

310 break the cation-exchange interactions and leads to release of the basic
311 analytes from the sorbents thanks to the elution strength of the organic solvent
312 also present in the solution). For this purpose, 5 ml aliquots of 2% HCOOH in
313 MeOH, 2% TFA in MeOH and 2% TFA in MeOH/ACN (1/4) were investigated.
314 Since 2% TFA in MeOH delivered the best results (higher recoveries than for
315 2% HCOOH in MeOH), and did not give any significant disturbance in the LC
316 separation of the analytes, it was selected for use in the elution step. 2% TFA in
317 MeOH/ACN (1/4) delivered good results also, but required longer evaporation
318 times. 5 ml of 2% TFA in MeOH was found to be sufficient to elute completely
319 all of the basic compounds, so was set as the optimal volume of elution solvent.

320 *3.2.4 Volume of sample*

321 Once the SPE protocol had been established, the effect of varying the
322 volume of sample in the loading step (from 100 to 1000 ml) was investigated as
323 a manner to predict the extraction capacity of the sorbent. The HXLPP-WCX
324 sorbent gave rise to good recoveries of analytes even when the sample volume
325 was 1000 ml (Table 3). Typically, the recoveries of the basic analytes were
326 close to 100% for the HXLPP-WCX sorbent. Only for antipyrine did the HXLPP-
327 WCX resin gave rise to a small degree of fractionation; for example, when 1000
328 ml of sample spiked at 20 μg l^{-1} with the analyte mixture were extracted, the
329 recovery of antipyrine in the elution step was 79%, with the remainder (15%)
330 being eluted in washing step. In view of the pK_a (13.3) and chemical structure of
331 antipyrine, this behaviour may be attributable to the stronger retention of
332 antipyrine through hydrophobic interactions.

333 Therefore, we have demonstrated that the HXLPP-WCX sorbent is highly
334 effective in extracting basic analytes in a quantitive manner from high volume

335 (1000 ml) aqueous samples, after a washing step with 2 ml of 5% NH_4OH in
336 MeOH, a feature which helps greatly in the removal of interferences from the
337 sample matrix.

338 *3.3 Comparison to commercial sorbents*

339 The SPE performance of the HXLPP-WCX sorbent was compared to
340 Strata-X-CW and Oasis WCX. The former had a specific surface area of 1125
341 $m^2\ g^{-1}$ (arising from the high micropore content) whereas the commercially
342 available sorbents, which are not hypercrosslinked, have lower specific surface
343 areas (800 $m^2\ g^{-1}$). A second notable difference between the HXLPP-WCX
344 sorbent and the commercially available sorbents is the particle size; the
345 HXLPP-WCX sorbent is in the form of microspheres with average particle
346 diameter around 6 μm, whereas the average particle size of Strata X-CW and
347 Oasis WCX are both significantly larger at around 30 μm.

348 The SPE results arising from use of the three different resins are
349 presented in Table 3. It can be seen that the analyte recoveries were higher for
350 all compounds with HXLPP-WCX than either Strata-X-CW or Oasis WCX.
351 When varying sample volumes were percolated through the Strata-X-CW and
352 Oasis WCX cartridges, most of the compounds were either eluted in the
353 washing step or fractionated between the washing and the elution steps; the
354 retention of certain analytes was also low compared to HXLPP-WCX.

355 When 1000 ml of sample was percolated through Strata-X-CW, the
356 recoveries of the acidic analytes in the washing step were 9% for
357 acetaminophen, and close to 50% for naproxen and diclofenac. It was also
358 observed that all of the basic analytes were fractionated (see Table 3).

359 The Oasis WCX sorbent, which has properties similar to Strata-X-CW,
360 was found to be even less useful than Strata-X-CW for the capture of basic
361 pharmaceuticals; for Oasis WCX all the compounds retained very poorly and
362 were eluted primarily during the washing step.

363 Another interesting feature relates to the retention behavior of naproxen
364 and diclofenac, which have pK_a values of 4.8 and 4.2, respectively. These
365 compounds were eluted nearly quantitatively (%R ~100%) during the washing
366 step when percolated through the HXLPP-WCX sorbent, but they were not
367 recovered completely by the commercial sorbents, which can be attributed to
368 losses of these analytes during the loading step. This behaviour for this pair of
369 analytes may be due to the weaker reversed-phase retention mechanisms
370 operating for the commercially available sorbents. In any case, it is evident that,
371 the HXLPP-WCX sorbent gives higher recoveries for all of the target analytes
372 than the two commercial WCX sorbents, which, due to its not suitable results
373 were not further tested.

374

375 **3.3 Application to real samples**

376 Given the highly promising SPE data obtained with HXLPP-WCX when
377 the SPE protocol was applied to Milli-Q water, an analogous protocol was
378 applied to the analysis of Ebre river water and effluent waste water. As is
379 common practice, for the analysis of real water samples the sample volume
380 loaded onto the SPE cartridges is normally lower than the sample volume
381 applied when the analytes are in Milli-Q water due to the presence of
382 interferences in real samples which compete with the analytes for binding to the
383 sorbent and thereby reduce the analyte capture efficiency. To establish the

384 utility of the HXLPP-WCX sorbent for the analysis of real water samples, the
385 initial SPE experiments involved the percolation of 500 ml sample of Ebre river
386 water spiked at 1 μg l^{-1} through cartridges packed with the sorbent (thereafter,
387 the remainder of the SPE protocol was as detailed in Section 2). Table 4
388 summarises the recovery values obtained for the various analytes on the
389 HXLPP-WCX sorbent. From these results it can be observed that when 500 ml
390 of a river water sample was loaded onto the SPE cartridge the recovery values
391 for the analytes were high and similar to those obtained for Milli-Q water, with
392 the exception of antipyrine which showed a higher level of fractionation than for
393 the Milli-Q water case.

394 Fig. 2 shows the chromatograms obtained following preconcentration on
395 HXLPP-WCX of 500 ml of non-spiked (Fig. 2b, 2d) and spiked (at 1 μg l^{-1} for
396 each analyte; Fig. 2a, 2c) Ebre river water. For the river water samples, a signal
397 was detected at the retention time corresponding to caffeine (see the non-
398 spiked Ebre river water chromatogram, Fig. 2d), but further analysis by a
399 confirmatory technique such as mass spectrometry (MS) may be appropriate
400 here. Typical chromatograms for the washing step, where all the interferences
401 and acidic analytes retained on the cartridges through reversed-phase
402 mechanism are eluted from the sorbents, are shown in Fig. 2a (spiked) and Fig.
403 2b (non-spiked). Typical chromatograms for the elution step, where the target
404 analytes retained through weak cation-exchange interactions (*i.e.*, mainly the
405 basic analytes) are eluted from the sorbents, are shown in Fig. 2c (spiked) and
406 Fig. 2d (non-spiked). It is important to note the cleanliness of the
407 chromatograms, an observation which is particularly striking when one
408 considers the fact that a non-selective detector (UV) was used in these

409 analyses. Both the selectivity and sensitivity of the analyses could be improved
410 further by using more powerful detector such as mass spectrometer.

411 To demonstrate the selectivity of the HXLPP-WCX sorbent, a further set
412 of SPE experiments was performed using dirtier sample matrices, including
413 effluent water from a wastewater treatment plant (WWTP). The recovery values
414 obtained when 250 ml of effluent WWTP samples, spiked at 5 μg l^{-1}, was
415 percolated through the HXLPP-WCX sorbent, is shown in Table 4. In general,
416 the HXLPP-WCX sorbent gave good recoveries for most of the analytes
417 studied, with the exception of antipyrine and carbamazepine. The recovery of
418 carbamazepine in the elution step was 70%, the remaining 30% being eluted in
419 washing step. As regards antipyrine, its elution profile, when loaded onto the
420 HXLPP-WCX sorbent, was the reverse of that expected, *i.e.*, it was eluted in the
421 washing step. In fact, antipyrine had already presented retention problems
422 when present in other aqueous matrices, and these problems may be magnified
423 when antipyrine is present in more complex samples since natural organic
424 matter and other compounds present in wastewater matrices give rise to
425 increased competition for binding to the sorbent.

426 Fig. 3 shows the elution chromatograms obtained after percolation of 250
427 ml of an effluent WWTP sample through the HXLPP-WCX sorbent without (Fig.
428 3b) and with (Fig. 3a) the addition of the mixture of analytes at the 5 μg l^{-1} level.
429 To emphasise the importance and effectiveness of the washing step for this
430 complex sample matrix, we performed this particular analysis without a washing
431 step; after the loading of 250 ml of effluent wastewater spiked at the 5 μg l^{-1}
432 level with the mixture of analytes, all the analytes were eluted directly with 5 ml
433 of 2% TFA in MeOH without any prior washing step being used. The effect of

434 re-introducing the methanol-based washing step was then examined in an effort
435 to remove interferences. Fig. 4 shows the washing (Fig.4a, 4b) and elution
436 (Fig.4c, 4d) chromatograms obtained after the percolation of a 250 ml effluent
437 WWTP sample through the HXLPP-WCX sorbent without (Fig. 4b, 4d) and with
438 (Fig. 4a, 4c) the addition of the mixture of analytes at the 5 μg l^{-1} level. For the
439 effluent WWTP sample, peaks were observed at the retention times
440 corresponding to antipyrine, naproxen and diclofenac (Fig. 4b) and caffeine
441 (Fig. 4d), but these results should be affirmed by a more powerful detector.

442 In addition to the marked improvements in the quality of the
443 chromatograms, the new sorbent allows a more accurate quantification of
444 analytes at lower concentration levels in complex matrices without the analytes
445 being masked by interferences. The main point of all is the fact that the
446 recovery of all the basic analytes of interest is complete in these complex
447 environmental samples, on account of the WCX interactions which lead to high
448 analyte recoveries (and clean chromatograms). In addition, the cleanliness of
449 the extracts obtained after SPE with the HXLPP-WCX sorbent is an added
450 advantage in respect of the potential to reduce or avoid ion-suppression effects
451 in the case of determination by LC-MS with electrospray ionisation.

452 In validation studies using 500 ml of river water and 250 ml of effluent
453 WWTP, all the basic analytes exhibited good linearity. In river water all the
454 analytes exhibited a linear range from 0.5-50 μg l^{-1}, with determination
455 coefficients (r^2) greater than 0.992. The limits of detection (LODs), calculated on
456 the basis of a signal to noise ratio ≥ 3, were 0.1 μg l^{-1} for all the basic analytes.
457 The repeatability and reproducibility of the method, expressed as the relative
458 standard deviation (RSD) of three analyses of 500 ml of Ebre river water spiked

459 at 1 μg l^{-1}, were less than 14% for all the basic analytes. For effluent waste
460 water, all the analytes exhibited a good linear range (1-50 μg l^{-1}) with r^2 greater
461 than 0.984. The LODs were 0.5 μg l^{-1} for most of compounds, with the
462 exception of caffeine where the LOD was 1 μg l^{-1}. Although the LODs are not as
463 low as those reported for some environmental water samples [21,22], they
464 could be decreased markedly by the introduction of a more sensitive detection
465 system, such as tandem mass spectrometry. Moreover, the cleanliness of the
466 chromatograms will tend to reduce or prevent ion-enhancement/suppression
467 effects when LC-MS is used.
468
469 **4. Conclusions**
470 In this study the synthesis of hypercrosslinked polymer resin with weak cation-
471 exchange is described, and a detailed investigation carried out with respect to
472 the application of this sorbent to the SPE of basic pharmaceuticals from
473 complex environmental samples. The resin was produced *via* the
474 hypercrosslinking of swellable polymers precursors which were synthesised *via*
475 precipitation polymerisation. The WCX properties are derived from the presence
476 of carboxylic acid moieties in the polymer.
477 This is the first time that a hypercrosslinked polymer resin has been exploited
478 as weak cation-exchanger for the SPE of basic pharmaceuticals. Following
479 optimisation of the SPE protocol, it was found that the novel HXLPP-WCX
480 sorbent enabled essentially quantitative recovery, and adequate selectivity, of
481 most of the analytes tested, and performed well as a weak cation-exchanger. In
482 contrast, the commercially available sorbents Strata-X-CW and Oasis WCX
483 were unable to completely retain basic analytes *via* an ion-exchange

484 mechanism and remove acidic analytes during the washing step. The highest
485 extraction efficiency was achieved with the HXLPP-WCX sorbent. Overall, the
486 HXLPP-WCX sorbent proved to be highly effective for the preconcentration of
487 basic analytes present in complex environmental water samples.
488
489 **Acknowledgements**
490 The authors thank the *Ministry of Science and Innovation* in Spain for the
491 financial support (Projects CTQ 2008-0825 and CTM 2008-06847-CO2-01). NF
492 also thanks the Juan de la Cierva program for personal funding.
493

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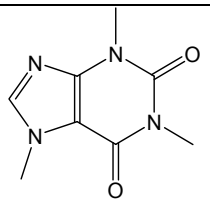
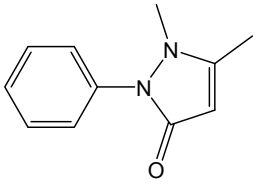
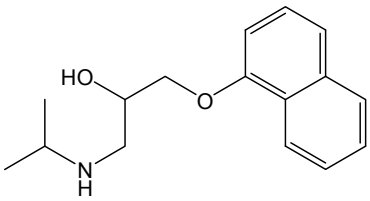
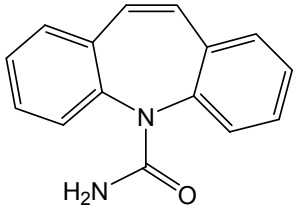
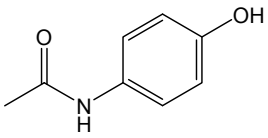
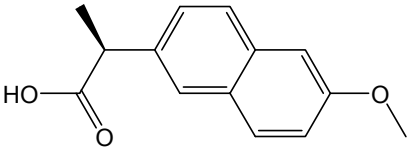
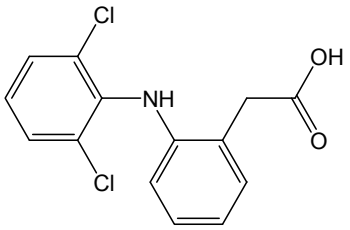
Table 1. Chemical structures and pK_a values of the selected analytes.

Compound	Compound type	Chemical Structure	pK_a [a]
Caffeine	CNS[b] stimulant		13.4
Antipyrine	Analgesic		13.3
Propranolol	β-blocker		9.5
Carbamazepine	Anti-epileptic		13.7
Acetaminophen	Analgesic		9.7
Naproxen	NSAID[c]		4.8
Diclofenac	NSAID[c]		4.2

[a] pK_a values calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs)

[b] Central nervous system

[c] Non-steroidal anti-inflammatory drug

Table 2. Characterisation data for the sorbents tested in SPE.

	HXLPP-WCX[a]	**Oasis WCX[b]**	**Strata-X-CW[b]**
	Laboratory synthesised	Waters	Phenomenex
Yield (%)[c]	85	n.d.	n.d.
I.E.C.[d] (meq g^{-1})	0.72	0.75	0.74
Specific surface area (m^2 g^{-1})	1125	800	800
Average particle diameter (μm)	6.1±1.6	30	33

n.d. no data
[a] Data measured experimentally
[b] Data provided by the supplier
[c] Relative to the mass of the corresponding (non-hypercrosslinked) precursor particles
[d] Ion-exchange capacity

Table 3. Recovery values (%) obtained when the HXLPP-WCX, Strata-X-CW and Oasis WCX sorbents were applied in SPE for the preconcentration of 1000 ml of a Milli-Q sample spiked at 20 μg l^{-1} with the analyte mixture.

Analytes	Type	HXLPP-WCX		Strata-X-CW		Oasis WCX	
		Wash	Elution	Wash	Elution	Wash	Elution
Caffeine	Basic	5	93	47	17	60	5
Antipyrine		15	79	55	11	91	0
Propranolol		0	93	48	40	73	13
Carbamazepine		4	107	31	46	90	15
Acetaminophen	Acidic	87	0	9	0	17	0
Naproxen		99	6	47	17	74	3
Diclofenac		94	13	45	22	77	11

For the experimental conditions, see text.
% Relative standard deviations (RSDs) (n=3) were lower than 12% for %R >10%.

Table 4. Recovery values (%) obtained when the HXLPP-WCX sorbent was applied in SPE for the preconcentration for different real samples spiked with the analyte mixture.

Analytes	Type	Ebre River (1 μg l^{-1})		Effluent WWTP (5 μg l^{-1})	
		500 ml		250 ml	
		Wash	Elution	Wash	Elution
Caffeine	Basic	20	90	26	82
Antipyrine		50	54	76	0
Propranolol		0	90	0	92
Carbamazepine		11	90	30	70
Acetaminophen	Acidic	113	0	100	0
Naproxen		94	0	91	0
Diclofenac		98	0	93	0

For the experimental conditions, see text.
% Relative standard deviations (RSDs) (n=3) were lower than 14% for %R >11%.

Figure captions

Fig. 1 Chemical structures of the sorbents tested: HXLPP-WCX, Strata-X-CW and Oasis WCX

Fig. 2 Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 500 ml of Ebre river water sample with (a,c) and without (b,d) addition of a 1 μg l^{-1} level of analyte mixture: washing step (a, b) and elution step (c, d).

Fig. 3 Chromatograms obtained after off-line trace enrichment with the HXLPP-WCX sorbent of 250 ml of effluent WWTP sample with (a) and without (b) the addition of a 5 μg l^{-1} level of an analyte mixture (without a washing step).

Fig. 4 Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 250 ml of effluent WWTP sample with (a,c) and without (b,d) the addition of a 5 μg l^{-1} level of analyte mixture: washing step (a,b) and elution step (c,d).

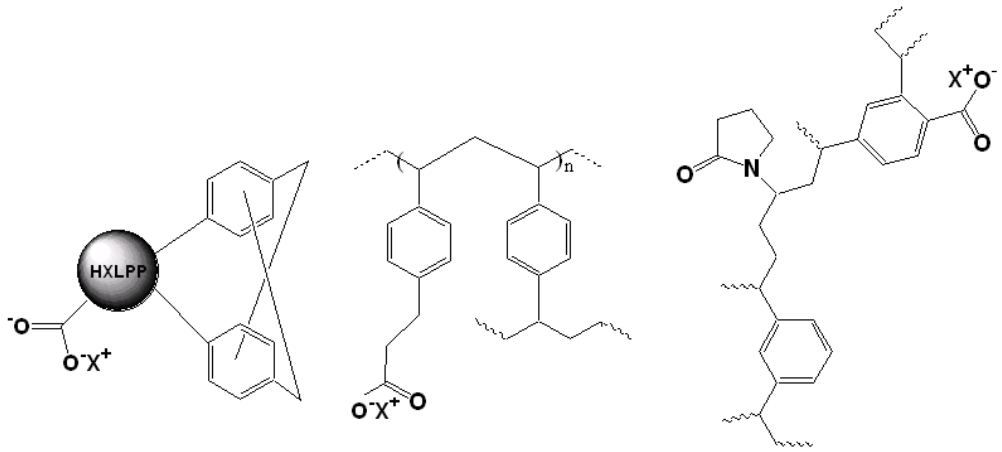
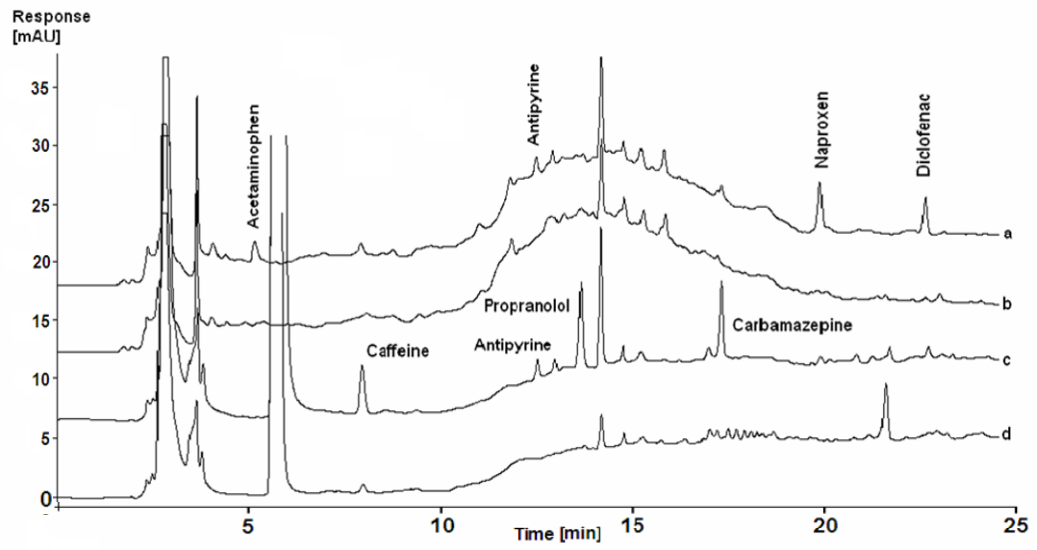


Figure 1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10
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Acetaminophen
Antipyrine
Naproxen
Diclofenac
Propranolol
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Antipyrine
Carbamazepine
a
b
c
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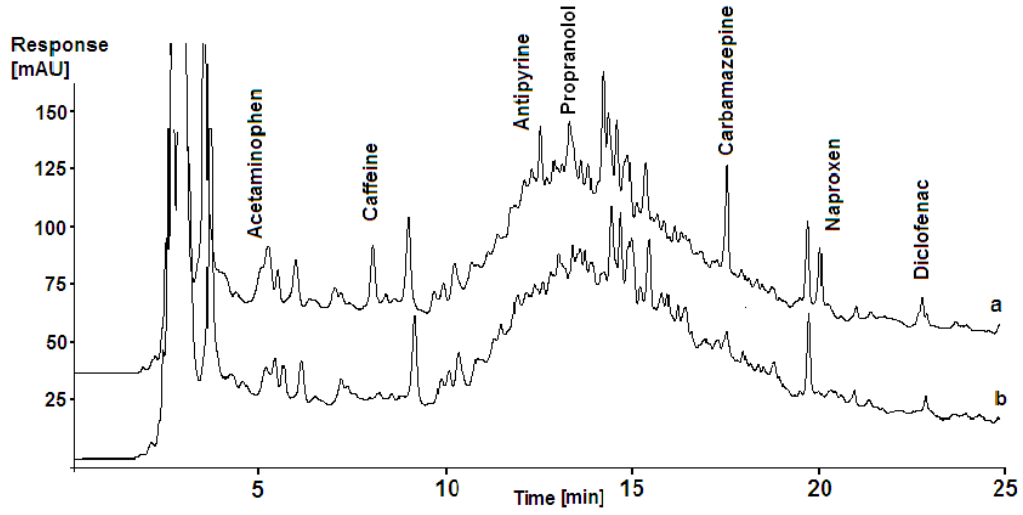
5
10
15
20
25
Time [min]

Figure 2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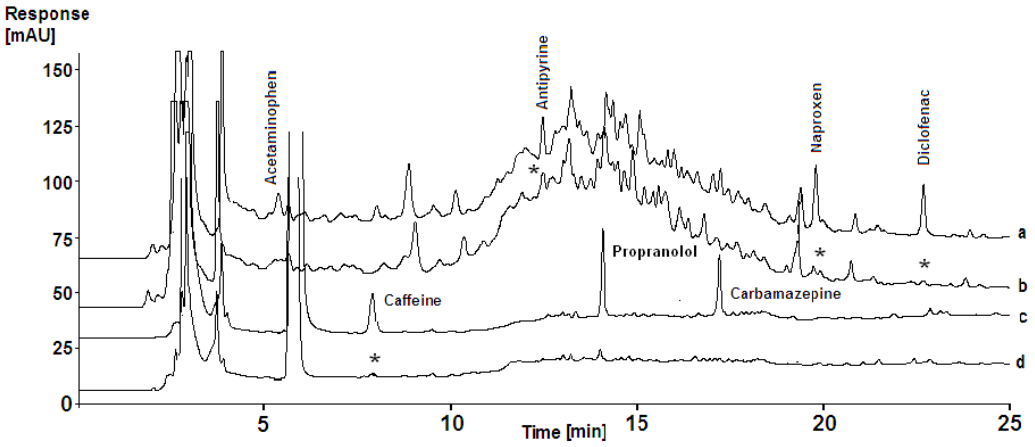
Time [min]

Figure 3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Antipyrine
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Diclofenac
Caffeine
Propranolol
Carbamazepine
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Time [min]

Figure 4