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

D. Bratkowska [a], R.M. Marcé [a], P.A.G. Cormack [b], D.C. Sherrington [b], F. Borrull [a], N. Fontanals [a,*]

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Campus Sescelades, Marcel.lí Domingo s/n, 43007 Tarragona, Spain
[b] WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, Scotland, United Kingdom

ARTICLE INFO

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 μm, 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 $\mu g\,l^{-1}$ levels of basic pharmaceuticals present in 500 ml of river water and 250 ml of effluent waste water.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Solid-phase extraction (SPE) is a powerful analytical tool used widely for preconcentration, 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 preconcentration 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 sam-

ple 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), $\sim$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 anion-exchange (WAX) character, and the application of these novel sorbents to the SPE of acidic pharmaceuticals from aqueous samples [14].

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

2. Experimental

2.1. Reagents and standards

The reagents used for the polymer syntheses were divinylbenzene (DVB) (80% grade) supplied by Aldrich (Steinheim, Germany), methacrylic acid (MAA) (98% grade) and *para*-vinylbenzyl chloride (VBC) (95% grade) supplied by Fluka (Buchs, Switzerland). DVB and VBC were purified by passing through short columns packed with neutral alumina. MAA was purified by vacuum distillation. The 2,2′-azobisisobutyronitrile (AIBN) used as initiator was supplied by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck, Darmstadt, Germany). Ferric chloride (FeCl$_3$) and anhydrous 1,2-dichloroethane (DCE), from Aldrich, were used in the hypercrosslinked reactions.

The pharmaceutical analytes selected to evaluate the performance of the sorbents in SPE were: acetaminophen, caffeine, antipyrine, propranolol, carbamazepine, naproxen and diclofenac (all obtained from Sigma–Aldrich). The chemical structures and pK_a values of the analytes are presented in Table 1.

Standard solutions at 1000 mg l^{-1} in methanol were prepared for each analyte. The mixture of all the analytes was prepared by diluting the standard solutions with MeOH:H$_2$O (1:1, v/v).

LC-grade acetonitrile and methanol (SDS, Peypin, France) and Milli-Q water (Millipore, Bedford, MA, USA) were used to prepare the mobile phases. Hydrochloric acid (Probus, Barcelona, Spain) was used to adjust the pH of the mobile phase and the sample before SPE. Other reagents used in SPE procedures were: ammonium hydroxide (NH$_4$OH) (Merck), formic acid (HCOOH) (Probus) and trifluoroacetic acid (TFA) (Fluka).

2.2. Resin preparation and characterisation

The micron-sized spherical particles (PP-WCX) used as swellable precursors in the production of the hypercrosslinked resins (HXLPP-WCX), were synthesised using an optimised precipitation polymerisation (PP) protocol [16]. The monomers (10% MAA, 50% VBC and 40% DVB [w/w%]) and AIBN (2 mol% relative to polymerisable double bonds) were dissolved in acetonitrile (200 ml) in a polypropylene bottle (250 ml) at a total monomer concentration of 2 w/v%. The monomer solution was de-oxygenated with N$_2$ at 0 °C and the bottle then placed on a low-profile roller (Stovall, Essex, UK) in a temperature-controllable incubator (Stuart Scientific, Surrey, UK). The temperature was ramped from ambient to 60 °C over a period of $\sim$2 h and the polymerisation allowed to proceed at 60 °C for a further 46 h. The resulting particles were filtered on a 0.2 μm nylon membrane filter and washed successively with MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 °C.

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

The hypercrosslinked resin (HXLPP-WCX) was characterised by measuring specific surface area using a BET treatment of N$_2$ sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. The carbon (83.1 w/w%), hydrogen (7.2 w/w%), chlorine (2.5 w/w%) and oxygen (6.3 w/w%, calculated by difference) contents for the resin were obtained by elemental microanalysis using a Carlo-Erba EA 1106 Instrument. The cation-exchange capacity was calculated from the microanalytical data using the theoretical values. The average microsphere diameter and homogeneity in size (particle size distribution) were calculated using ImageJ software from the image analysis of 100 individual particles in scanning electron microscopy (SEM) images, which were acquired using a JEOL 6400 Instrument. SEM image for the HXLPP-WCX resin is included in Figure 1S of the Supported Information Section. A schematic representation of the structure of HXLPP-WCX is depicted in Fig. 1. The characterisation data for all the sorbents studied is detailed in Table 2.

2.3. Chromatographic equipment and conditions

The chromatographic experiments were performed with an HP 1090 Liquid Chromatograph equipped with an injection valve with a 20 μl loop and an Agilent 1200 UV spectrophotometric detector (Agilent, Waldbronn, Germany). The analytical column was a 250 mm × 4.6 mm I.D. stainless-steel column packed with Kromasil 100 C$_{18}$, 5 μm (Teknokroma, Barcelona, Spain).
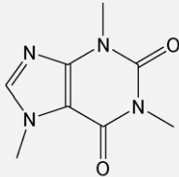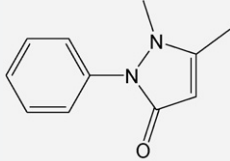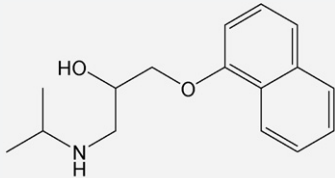
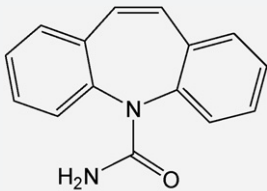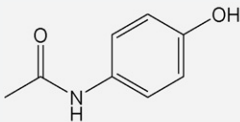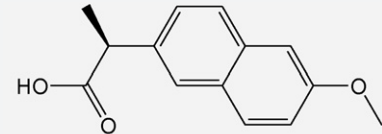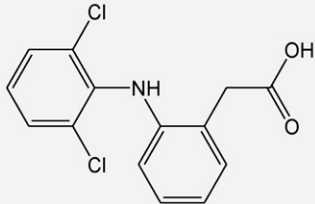The mobile phases were Milli-Q water adjusted to pH 3 with HCl (solvent A) and acetonitrile (solvent B). The flow rate was 1 ml min^{-1} and the temperature of the column oven was set at 65 °C. The gradient profile was from 10 to 15% ACN in 5 min, then to 100% ACN in 25 min (held for 2 min), then the mobile phase was returned to the initial conditions (10% ACN) in 3 min.

The detection wavelength for all the compounds was 210 nm.

2.4. Solid-phase extraction

SPE cartridges (6 ml, polypropylene) were packed with 200 mg of the HXLPP-WCX sorbent. The sorbent was retained by two frits: a 2 μm pore size metal frit at the bottom, and a 20 μm pore size polyethylene frit at the top. The retention capabilities of the novel sorbent was compared to commercial SPE cartridges from Phenomenex (Strata-X-CW; 200 mg/6 ml) and Waters (Oasis WCX;

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.

200 mg/6 ml) (which was packed manually). A vacuum manifold (Teknokroma) was used to manipulate the cartridges in the off-line SPE process. One single sorbent cartridge of each type was used for the whole study. The three sorbent structures are presented in Fig. 1.

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

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

To keep the samples under proper conditions, real water (Ebre river water and effluent waste water from a treatment plant) were adjusted to ∼pH 3 with HCl and kept at 4 °C before analysis. They were filtered through 0.22 μm nylon membranes (Supelco, Belle-fonte, PA, USA) prior to the preconcentration step to eliminate the particulate matter which is normally present in real samples.
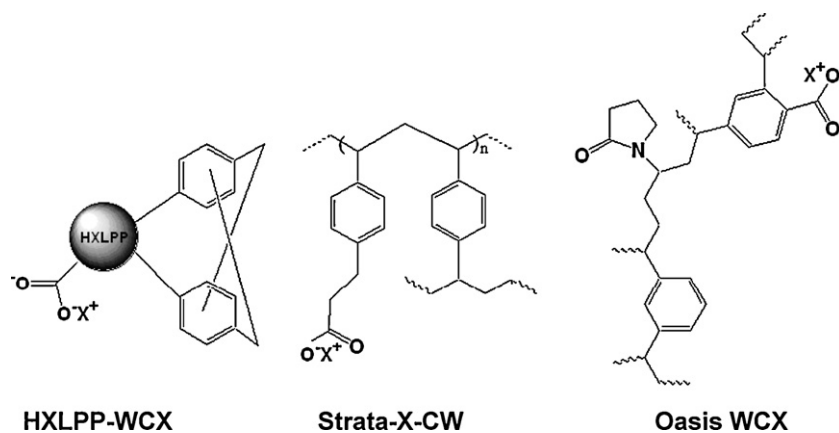
Fig. 1. Chemical structures of the sorbents tested: HXLPP-WCX, Strata-X-CW and Oasis WCX.

3. Results and discussion

3.1. Preparation of the HXLPP-WCX sorbent

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

Table 2
Characterisation data for the sorbents tested in SPE.

	HXLPP-WCX[a] Laboratory synthesised	Oasis WCX[b] Waters	Strata-X-CW[b] Phenomenex
Yield (%)[c]	85	n.d.	n.d.
I.E.C.[d] (mequiv. 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%.

tonated 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.

3.2.2. Washing step

The aim of the washing step was to eliminate interferences (including acidic and neutral compounds) bound to the sorbent through reserved-phase mechanisms, while retaining on the sorbent the basic compounds bound through cation-exchange interactions. 1 ml volumes of various neat organic solvents (such as methanol and acetonitrile) were applied in the washing step, but in such cases all the analytes were eluted. Thus, we decided to use a solution of NH$_4$OH in organic solvent as the washing solution, to maintain the desired ionisation state of the analytes and the sorbent. In this regard, the following solutions were evaluated: 5% NH$_4$OH in MeOH; 5% NH$_4$OH in ACN; 5% NH$_4$OH in MeOH/ACN (1/4). Of these three options, 5% NH$_4$OH in MeOH gave higher recoveries for all analytes than the other two washing solution and was thus selected as the washing solvent of choice. Thereafter, the next step was to evaluate the optimum volume of the washing solvent to be used in the SPE protocol. For these experiments, where the sample matrix was Milli-Q water, 1 ml of 5% NH$_4$OH in MeOH was used initially. Although this volume of washing solvent was found to be not enough to elute all the acidic compounds quantitatively, 2 ml of 5% NH$_4$OH in MeOH was found to be effective for this purpose so was established as the optimal volume of washing solvent required to elute acidic compounds and interferences, whilst still allowing total retention of the analytes of interest (*i.e.*, basic compounds).

3.2.3. Elution of basic compounds

For the elution step, in which the aim was to elute the basic compounds bound to WCX sorbents through ionic interactions, various acidic solutions were tested (acidification protonates the carboxylic acid residues on the sorbents, breaks the cation-exchange interactions and leads to release of the basic analytes from the sorbents thanks to the elution strength of the organic solvent also present in the solution). For this purpose, 5 ml aliquots of 2% HCOOH in MeOH, 2% TFA in MeOH and 2% TFA in MeOH/ACN (1/4) were investigated. Since 2% TFA in MeOH delivered the best results (higher recoveries than for 2% HCOOH in MeOH), and did not give any significant disturbance in the LC separation of the analytes, it was selected for use in the elution step. 2% TFA in MeOH/ACN (1/4) delivered good results also, but required longer evaporation times. 5 ml of 2% TFA in MeOH was found to be sufficient to elute completely all of

the basic compounds, so was set as the optimal volume of elution solvent.

3.2.4. Volume of sample

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

Therefore, we have demonstrated that the HXLPP-WCX sorbent is highly effective in extracting basic analytes in a quantitive manner from high volume (1000 ml) aqueous samples, after a washing step with 2 ml of 5% NH$_4$OH in MeOH, a feature which helps greatly in the removal of interferences from the sample matrix.

3.3. Comparison to commercial sorbents

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

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

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

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})	500 ml	Effluent WWTP (5 μg l^{-1})	250 ml
		Wash	Elution	Wash	Elution
Caffeine		20	90	26	82
Antipyrine	Basic	50	54	76	0
Propranolol		0	90	0	92
Carbamazepine		11	90	30	70
Acetaminophen		113	0	100	0
Naproxen	Acidic	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 >10%.

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

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

3.4. Application to real samples

Given the highly promising SPE data obtained with HXLPP-WCX when the SPE protocol was applied to Milli-Q water, an analogous protocol was applied to the analysis of Ebre river water and effluent waste water. As is common practice, for the analysis of real water samples the sample volume loaded onto the SPE cartridges is normally lower than the sample volume applied when the analytes are in Milli-Q water due to the presence of interferences in real samples which compete with the analytes for binding to the sorbent and thereby reduce the analyte capture efficiency. To establish the utility of the HXLPP-WCX sorbent for the analysis of real water samples, the initial SPE experiments involved the percolation of 500 ml sample of Ebre river water spiked at 1 μg l^{-1} through cartridges packed with the sorbent (thereafter, the remainder of the SPE protocol was as detailed in Section 2). Table 4 summarises the recovery values obtained for the various analytes on the HXLPP-WCX sorbent. From these results it can be observed that when 500 ml of a river water sample was loaded onto the SPE cartridge the recovery values for the analytes were high and similar to those obtained for Milli-Q water, with the exception of antipyrine which showed a higher level of fractionation than for the Milli-Q water case.

Fig. 2 shows the chromatograms obtained following preconcentration on HXLPP-WCX of 500 ml of non-spiked (Fig. 2b and d) and spiked (at 1 μg l^{-1} for each analyte; Fig. 2a and c) Ebre river water. For the river water samples, a signal was detected at the retention time corresponding to caffeine (see the non-spiked Ebre river water chromatogram, Fig. 2d), but further analysis by a confirmatory technique such as mass spectrometry (MS) may be appropriate here. Typical chromatograms for the washing step, where all the interferences and acidic analytes retained on the cartridges through reversed-phase mechanism are eluted from the sorbents, are shown in Fig. 2a (spiked) and Fig. 2b (non-spiked). Typical chromatograms for the elution step, where the target analytes retained through weak cation-exchange interactions (i.e., mainly the basic analytes) are eluted from the sorbents, are shown in Fig. 2c (spiked) and Fig. 2d (non-spiked). It is important to note the cleanliness of the chromatograms, an observation which is particularly striking when one considers the fact that a non-selective detector (UV) was used in these analyses. Both the selectivity and
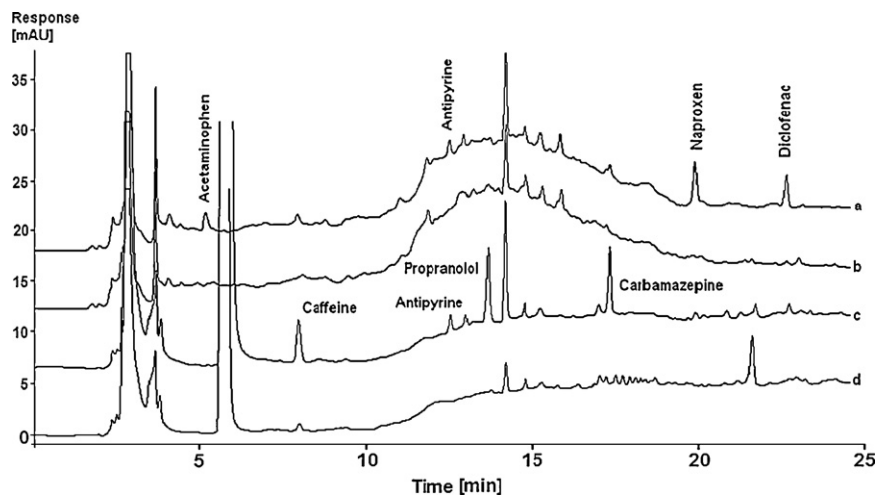
Fig. 2. Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 500 ml of Ebre river water sample with (a and c) and without (b and d) addition of a 1 μg l^{-1} level of analyte mixture: washing step (a and b) and elution step (c and d).
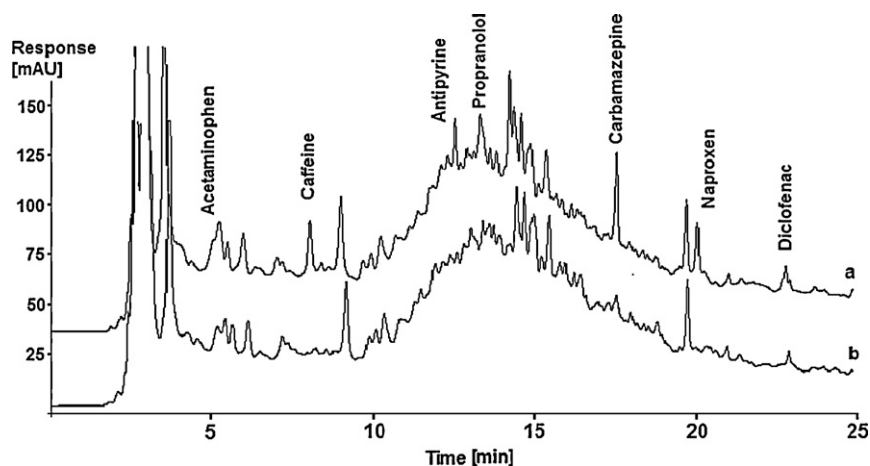
Fig. 3. Chromatograms obtained after off-line trace enrichment with the HXLPP-WCX sorbent of 250 ml of effluent WWTP sample with (a) and without (b) the addition of a 5 µg l^{-1} level of an analyte mixture (without a washing step).

sensitivity of the analyses could be improved further by using more powerful detector such as mass spectrometer.

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

Fig. 3 shows the elution chromatograms obtained after percolation of 250 ml of an effluent WWTP sample through the HXLPP-WCX sorbent without (Fig. 3b) and with (Fig. 3a) the addition of the mixture of analytes at the 5 µg l^{-1} level. To emphasise the importance and effectiveness of the washing step for this complex sample matrix, we performed this particular analysis without a washing step; after the loading of 250 ml of effluent wastewater spiked at the 5 µg l^{-1} level with the mixture of analytes, all the

analytes were eluted directly with 5 ml of 2% TFA in MeOH without any prior washing step being used. The effect of re-introducing the methanol-based washing step was then examined in an effort to remove interferences. Fig. 4 shows the washing (Fig. 4a and b) and elution (Fig. 4c and d) chromatograms obtained after the percolation of a 250 ml effluent WWTP sample through the HXLPP-WCX sorbent without (Fig. 4b and d) and with (Fig. 4a and c) the addition of the mixture of analytes at the 5 µg l^{-1} level. For the effluent WWTP sample, peaks were observed at the retention times corresponding to antipyrine, naproxen and diclofenac (Fig. 4b) and caffeine (Fig. 4d), but these results should be confirmed by a more powerful detector.

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

In validation studies using 500 ml of river water and 250 ml of effluent WWTP, all the basic analytes exhibited good linearity. In river water all the analytes exhibited a linear range from 0.5 to
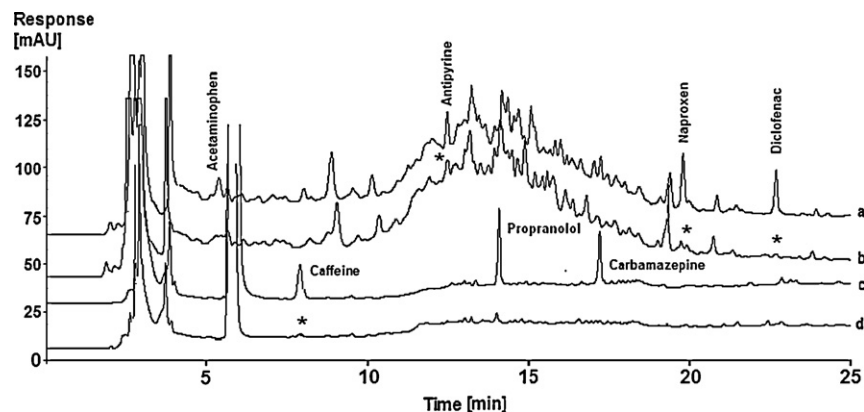

Fig. 4. Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 250 ml of effluent WWTP sample with (a and c) and without (b and d) the addition of a 5 µg l^{-1} level of analyte mixture: washing step (a and b) and elution step (c and d).*Peaks at the same time of studied analytes.

$50 \,\mu g \, l^{-1}$, with determination coefficients (r^2) greater than 0.992. The limits of detection (LODs), calculated on the basis of a signal to noise ratio ≥ 3, were $0.1 \,\mu g \, l^{-1}$ for all the basic analytes. The repeatability and reproducibility of the method, expressed as the relative standard deviation (RSD) of three analyses of 500 ml of Ebre river water spiked at $1 \,\mu g \, l^{-1}$, were less than 14% for all the basic analytes. For effluent waste water, all the analytes exhibited a good linear range ($1–50 \,\mu g \, l^{-1}$) with r^2 greater than 0.984. The LODs were $0.5 \,\mu g \, l^{-1}$ for most of compounds, with the exception of caffeine where the LOD was $1 \,\mu g \, l^{-1}$. Although the LODs are not as low as those reported for some environmental water samples [21,22], they could be decreased markedly by the introduction of a more sensitive detection system, such as tandem mass spectrometry. Moreover, the cleanliness of the chromatograms will tend to reduce or prevent ion-enhancement/suppression effects when LC–MS is used.

4. Conclusions

In this study the synthesis of hypercrosslinked polymer resin with weak cation-exchange is described, and a detailed investigation carried out with respect to the application of this sorbent to the SPE of basic pharmaceuticals from complex environmental samples. The resin was produced *via* the hypercrosslinking of swellable polymers precursors which were synthesised *via* precipitation polymerisation. The WCX properties are derived from the presence of carboxylic acid moieties in the polymer.

This is the first time that a hypercrosslinked polymer resin has been exploited as weak cation-exchanger for the SPE of basic pharmaceuticals. Following optimisation of the SPE protocol, it was found that the novel HXLPP-WCX sorbent enabled essentially quantitative recovery, and adequate selectivity, of most of the analytes tested, and performed well as a weak cation-exchanger. In contrast, the commercially available sorbents Strata-X-CW and Oasis WCX were unable to completely retain basic analytes *via* an ion-exchange mechanism and remove acidic analytes during the washing step. The highest extraction efficiency was achieved with the HXLPP-WCX sorbent. Overall, the HXLPP-WCX sorbent proved to be highly effective for the preconcentration of basic analytes present in complex environmental water samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.037.

References

[1] N. Fontanals, R.M. Marcé, F. Borrull, Trends Anal. Chem. 24 (2005) 394.
[2] C.F. Poole, Trends Anal. Chem. 22 (2003) 362.
[3] S.D. Richardson, T.A. Ternes, Anal. Chem. 77 (2005) 3807.
[4] H. Kataoka, Trends Anal. Chem. 22 (2003) 232.
[5] H. Mai-Ling, J. Ming, W. Peng, M. Su-Rong, L. Yan-Fei, H. Xiao-Zhong, S. Yun, L. Bin, D. Kang, Anal. Bioanal. Chem. 387 (2007) 1007.
[6] P. Puig, F. Borrull, M. Calull, C. Aguilar, Anal. Chim. Acta 616 (2008) 1.
[7] T. Heberer, Toxicol. Lett. 131 (2002) 5.
[8] F. Ahmadi, A.A. Shahsavari, M. Rahimi-Nasrabadi, J. Chromatogr. A 1193 (2008) 26.
[9] B. Rezaei, S. Mallakpour, N. Majidi, Talanta 78 (2009) 418.
[10] A. Beltran, E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 597 (2007) 6.
[11] M.S. Landis, J. Pharm. Biomed. Anal. 44 (2007) 1029.
[12] N. Fontanals, R.M. Marcé, F. Borrull, J. Chromatogr. A 1152 (2007) 14.
[13] V. Davankov, M. Tsyurupa, M. Ilyin, L. Pavlova, J. Chromatogr. A 965 (2002) 65.
[14] N. Fontanals, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. A 1215 (2008) 21.
[15] M. Zhang, F. Wei, Y.-F. Zhang, J. Nie, Y.-Q. Feng, J. Chromatogr. A 1102 (2006) 294.
[16] N. Fontanals, P. Manesiotis, D.C. Sherrington, P.A.G. Cormack, Adv. Mater. 20 (2008) 1298.
[17] N. Fontanals, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, J. Chromatogr. A 1191 (2008) 118.
[18] D. Bratkowska, N. Fontanals, F. Borrull, P.A.G. Cormack, D.C. Sherrington, R.M. Marcé, J. Chromatogr. A, in press, doi:10.1016/ j.chroma.2009.08.091.
[19] S. Mitra, Sample Preparation Techniques in Analytical Chemistry, Wiley, New York, 2003.
[20] O. Lorphensria, J. Intravijita, D.A. Sabatinib, T.C.G. Kibbeyb, K. Osathaphanc, C. Saiwand, Water Res. 40 (2006) 1481.
[21] L. Tong, P. Li, Y. Wang, K. Zhu, Chemosphere 74 (2009) 1090.
[22] A. Togola, H. Budzinski, Anal. Bioanal. Chem. 388 (2007) 627.