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

Alberto Moral[1], Alan Corrigan[2], Francesc Borrull[1], Peter A. G. Cormack[2]*, Núria Fontanals[1]* and Rosa Maria Marcé[1]

[1] *Department of Analytical and Organic Chemistry, Universitat Rovira i Virgili, Sescelades Campus, Marcel lí Domingo s/n, 43007 Tarragona, Spain*

[2] *WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland, UK*

*nuria.fontanals@urv.cat; Peter.Cormack@strath.ac.uk

Abstract

The application of core-shell materials as packing materials for liquid chromatography columns is common in analytical chemistry, however their use as sorbents in solid-phase extraction (SPE) is surprisingly underexplored. In the present study, core-shell polymer microspheres with strong cation-exchange character were designed and synthesized. These new materials benefit from having hypercrosslinked and relatively thin functional shells, which raises the specific surface areas and sorption capacities of the sorbents and allows for relatively shorter diffusion path lengths for analytes.

The core-shell polymer microspheres were evaluated as SPE sorbents for the extraction of basic pharmaceuticals from environmental water samples. Following optimization of the pH and volume of the loading solution, as well as optimization of the loading step, the SPE method was validated in terms of apparent and relative recoveries, matrix effect, limits of detection and quantification and precision. The method yielded very promising results in terms of apparent recoveries (>39%) and matrix effect (<±29%) and was applied successfully to the determination of basic pharmaceuticals in environmental water samples (river water, effluent wastewater and influent wastewater).

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

1. Introduction

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

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

The application of core-shell polymer microspheres to sorptive extraction techniques is particularly appealing for several reasons, not least of all because the thicknesses of the shells, their porous morphologies and the chemical functionality embedded within the shells can all be brought under synthetic control. In this way, not only can the selectivity of the material be controlled through rational selection of the functional groups, but high sorption capacities and efficient separations can be anticipated. With respect to porous morphology, hypercrosslinking chemistry has been used by us to prepare ultra-high specific surface area polymer microspheres, where specific surface areas in excess of 1,000 m^2/g arise from the presence of a large number of small pores; whilst these materials performed well in SPE, they did not have a core-shell format [6]. Since there is a positive correlation between specific surface area and sorption capacity, core-shell polymer microspheres where the shells are hypercrosslinked are particularly appealing. With respect to the installation of functional groups, this can be achieved during either the construction of the shells, through copolymerization strategies, or through post-polymerization chemical modification strategies. For example, sulfonic acid groups can be installed into some polymers using sulfonation reactions [7], yielding products with strong cation-exchange (SCX) character [8] that can be applied to the extraction of basic compounds through ion-exchange interactions, in a fashion similar to SPE reports exploiting commercial [9,10] and non-commercial SCX sorbents [11–13].

In the present study, core-shell polymer microspheres with SCX character in the shells have been prepared using a versatile synthetic strategy which exploits free radical polymerization chemistry for the production of the cores and the shells. More specifically, the precipitation polymerization of divinylbenzene-55 (DVB-55) under dilute monomer conditions yields high-quality, non-porous cores that can be shelled in a second precipitation polymerization, where divinylbenzene-80 (DVB-80) and 4-vinylbenzyl chloride (VBC) are used as comonomers. The presence of VBC residues in the crosslinked shells enables the shells to be hypercrosslinked to increase their specific

surface area, and this is followed by the installation of SCX character using sulfonation chemistry. In this paper, we describe the design and preparation of the core-shell polymer microspheres and their exploitation as porous, functional sorbents in the SPE of basic pharmaceuticals from river water, effluent wastewater and influent wastewater samples.

2. Experimental

2.1. Reagents and standards

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

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

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

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

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

The pharmaceuticals venlafaxine (VEN), propranolol (PRO), metoprolol (MTO), atenolol (ATE) and trimethoprim (TRI) were acquired as pure standards (purity >96%) from Sigma-Aldrich. 1000 µg/L solutions of the standards were prepared in MeOH and stored at -20 ºC in brown bottles. Working solutions of mixtures of analytes were prepared weekly in ultrapure water and MeOH (90/10, v/v) and stored at 4 ºC in brown bottles.

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

2.2. Synthesis of core-shell polymer microspheres

Figure 1 outlines the synthetic procedure used to prepare the core-shell polymer microspheres.

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

°C, the product was isolated as a free-flowing white powder (4.93 g, 27%). The characterization data for this product can be found in the Supplementary Material.

(ii) Synthesis of poly(DVB-80-*co*-VBC) shells on polyDVB-55 cores: PolyDVB-55 cores (2 g), DVB-80 (3.60 g, 27.65 mmol), VBC (2.40 g, 15.73 mmol), AIBN (0.216 g, 1.32 mmol, 2 mol% relative to the total number of polymerizable double bonds) and ACN (100 mL) were charged to a 500 mL Nalgene® bottle. Thereafter, the procedure was similar to that used for the synthesis of polyDVB-55 cores, although the reaction was held at 60 ºC for 46 h rather than 22 h. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing white powder (3.60 g, 27%). The characterization data for this product can be found in the Supplementary Material.

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

(iv) Sulfonation of shells: For the sulfonation reactions, lauroyl sulfate was prepared freshly immediately prior to the sulfonation reactions. In a typical sulfonation reaction, lauric acid (0.335 g, 1.67 mmol) was dissolved in cyclohexane (12 mL). Chlorosulfonic

acid (0.11 mL, 0.193 g, 1.656 mmol) was added and the mixture stirred for one hour at ambient temperature under an inert atmosphere. Whilst the lauroyl sulfate was being prepared, hypercrosslinked core-shell polymer microspheres (1.25 g) and DCE (30 mL) were charged to a three-necked, round-bottomed flask fitted with an overhead stirrer and reflux condenser. The mixture was then stirred at ambient temperature for one hour under N_2 to wet the microspheres. The lauroyl sulfate solution was then injected into the three-necked flask *via* syringe. The temperature was raised to 50 °C and the mixture stirred for 24 h at 80 rpm, after which time the reaction mixture was red/brown in colour. After cooling, the particles were isolated by vacuum filtration on a 0.45 μm nylon membrane filter and washed on the filter with successive volumes (50 mL) of petroleum ether 60-80 and petroleum ether 30-40. After drying overnight *in vacuo* (0.01 bar) at 40 °C, the product was isolated as a free-flowing, orange powder (1.27 g). FT-IR spectrum ($\bar{\nu}$/cm^{-1}): 3022 (aromatic C-H str.), 2916 (aliphatic C-H str.), 1163 (S=O symmetric str.), 825 (1,4-disubstituted aromatic out-of-plane C-H def.), 792 and 707 (1,3-disubstituted aromatic out-of-plane C-H def.). Sulfonic acid loading level = 3.7 mmol/g. SEM microscopy: mean particle diameter = 3.1 μm, C_v = 14%. Langmuir specific surface area = 550 m^2/g; specific pore volume = 0.30 cm^3/g; mean pore diameter = 3.1 nm.

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

2.3. SPE procedures

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

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

2.4. Chromatographic conditions

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

The analysis of water samples was performed by LC-HRMS using an Accela 1250 UHPLC system from Thermo Scientific (Bremen, Germany) equipped with an automatic injector (Accela Autosampler) and a quaternary pump. The LC system was coupled to

an Exactive Orbitrap (Thermo Scientific) with a heated electrospray ionization (HESI) source and a high-energy collision dissociation cell (HCD) to fragment the analytes for confirmation purposes. Solvent A was changed to water with 0.1% of HCOOH, however the remainder of chromatographic conditions were the same as used for LC-DAD.

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

For data acquisition, the mass range selected was 50 – 450 m/z, and two alternative scan events were employed; the first consisted of a full scan at 50,000 FWHM (Full Width Half Maximum) with an injection time of 250 ms, whereas the second consisted of a fragmentation scan at 10,000 FWHM with an injection time of 50 ms and a collision voltage of 20 eV. The protonated ion was measured with a mass accuracy window of 5 ppm. Table S1 includes information on the pK_a values, protonated ions, and the fragment ions and the ratios between them, for the analytes acquired for quantification and confirmation purposes. Instrumental calibration curves (R^2 > 0.995) were obtained through weighted calibration regression for the compounds: the lower limit was 0.05 µg/L for TRI, MTO and PRO, and 0.1 µg/L for ATE and VEN. The upper limit was 500 µg/L in all cases.

3. Results and Discussion

3.1 Synthesis of the sorbent

Core-shell polymer microspheres with hypercrosslinked shells and SCX character were synthesized using a four-step methodology, as follows: (i) Precipitation polymerization of DVB-55 to give non-porous polyDVB-55 cores; (ii) Precipitation polymerization of DVB-80 and VBC in the presence of polyDVB-55 cores to give core-shell polymer microspheres; (iii) Hypercrosslinking of the shells in a solvent-expanded state, using

241 $FeCl_3$ as a Lewis acid; (iv) Sulfonation of the shells using lauroyl sulfate. Precipitation
242 polymerization was the polymerization method of choice because it is a surfactant- and
243 stabilizer-free method of free radical polymerization which delivers good yields of high-
244 quality polymer microspheres with low mean particle diameters and narrow particle size
245 distributions. Particle diameters, porous morphology and chemical functionality can all
246 be controlled through rational selection of reagents and the polymerization conditions.

247 *3.2. Characterization of the sorbent*

248 The physical format, porous morphology and chemical constitution of the sorbents were
249 characterized using scanning electron microscopy (SEM), Fourier Transform infrared
250 (FT-IR) spectroscopy and nitrogen sorption analysis. The ion-exchange capacity (IEC)
251 was determined by titration, and batch-to-batch reproducibility evaluated. In respect of
252 batch-to-batch reproducibility, three different batches of polymer were synthesized and
253 the precision between batches evaluated through SPE of the basic analytes from
254 ultrapure water samples. The RSD% (n=3) was lower than 18% for all analytes.

255 Figure 2 shows SEM images of the core microspheres (Figure 2a) and the core-shell
256 polymer microspheres (Figure 2b). The mean diameter of the core microspheres was
257 2.75 µm and the mean diameter of the hypercrosslinked core-shell polymer
258 microspheres with SCX character was 3.1 µm. This gives a mean shell thickness of 0.18
259 µm. Furthermore, it can be seen from the SEM images that both sets of microspheres
260 are relatively uniform. Whilst not monodisperse, ImageJ analysis of the core-shell
261 polymer microspheres reveals that the particles are quasi-monodisperse (size dispersity
262 = 14%).

263 Figure S1 presents the FT-IR spectra acquired for the core polymer microspheres
264 (Figure S1a), the core-shell polymer microspheres before hypercrosslinking (Figure
265 S1b), the hypercrosslinked core-shell polymer microspheres (Figure S1c) and the
266 hypercrosslinked core-shell polymer microspheres with SCX character (Figure S1d). All

four spectra are consistent with the proposed structures. In these spectra, the C-Cl band at 1265 cm^{-1}, which is assignable to VBC residues, is particularly diagnostic. A signal at around 1265 cm^{-1} appears in the FT-IR spectrum of the poly(DVB-80-*co*-VBC) shells on polyDVB-55 cores (Figure S1b), and diminishes in relative intensity for the hypercrosslinked derivative (Figure S1c) because the hypercrosslinking reaction consumes chloromethyl groups. Thus, the signal at 1265 cm^{-1} verifies the incorporation of VBC into the microspheres through copolymerization and is a very convenient way to track the progress and success of the hypercrosslinking reaction. In addition, in Figure S1d there is a band at 1163 cm^{-1} which can be assigned to sulfonic acid groups.

Nitrogen sorption analysis was used to measure the specific surface areas of the core-shell materials. Compared to the core microspheres, which are non-porous in the dry state (specific surface area < 5 m^2/g), the hypercrosslinked core-shell polymer microspheres with SCX character have a very well-developed porous morphology (specific surface area = 550 m^2/g). When one takes into account the fact that the cores are non-porous, one can estimate that the specific surface of the shells must be considerably in excess of 1,000 m^2/g (possibly even approaching 2,000 m^2/g), undoubtedly because the shell contains a large number of small pores (micropores and mesopores). This characteristic, combined with the fact that we are dealing with a core-shell format and that the ion-exchange capacity is high (3.7 mmol/g of sulfonic groups, as measured by titration), suggests that the final material is an attractive SPE sorbent candidate. The specific surface area (estimated) and IEC calculations are presented in the Supplementary Material.

3.3. Optimization of the SPE protocol

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

294 the ratio of the measured concentration after SPE to the theoretical concentration when
295 working with ultrapure water. All the quantifications carried out during the optimization of
296 the SPE protocol were done through LC-DAD.

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

305 The use of a washing step involving an organic solvent can be included in an SPE
306 protocol to improve the selectivity of the extraction and reduce the matrix effects. In the
307 present study, the use of 1 and 5 mL of MeOH as washing solvent was evaluated; the
308 $\%R_{SPE}$ values are presented in Figure 4. As can be observed, there is a slight decrease
309 in the $\%R_{SPE}$ values (less than 5%) for all compounds, except TRI, when the volume of
310 washing solvent is increased to 5 mL. This decrease in recovery was considered to be
311 largely insignificant, so 5 mL of MeOH was selected as the washing step. Previous
312 studies report the use of MeOH as washing solvent at different volumes (3 – 10 mL)
313 when working with SCX sorbents [13,14,16]. Regarding the elution step, 5 mL of 5%
314 NH_4OH in MeOH gave good results and was consistent with other studies in which
315 NH_4OH solutions at various concentrations were used with other SCX sorbents [14–17].

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

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

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

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

345 clogging/blocking of the cartridge – and it will be interesting to evaluate the sorbent in
346 on-line SPE in a future study.

347 *3.5. Method validation*

348 The optimized method was validated in terms of apparent recovery (%R_{app}) at two
349 concentration levels, matrix effect (%ME), accuracy, detection and quantification limits,
350 linear range, and precision for the determination of the basic pharmaceuticals in river
351 water, effluent wastewater and influent wastewater samples. Bearing in mind our
352 previous experiences, it was decided to reduce the loading volume to 50 mL for effluent
353 wastewater samples, and to 25 mL for influent wastewater samples. It should be noted
354 that for method validation and sample analysis the determination was carried out through
355 LC-HRMS.

356 The apparent recoveries were determined by dividing the measured concentration of the
357 spiked samples (with the blank concentration subtracted) by the theoretical
358 concentration. The %R_{app} were obtained by spiking the water samples at two
359 concentration levels: 0.5 and 5 µg/L for river water samples, 1 and 10 µg/L for effluent
360 wastewater samples and 2 and 20 µg/L for influent wastewater samples. Table 1 shows
361 that the %R_{app} for all compounds were higher than 39%. It can be observed that PRO
362 provided very high recoveries with river water samples (84 and 91%) and that the values
363 decrease significantly when the complexity of the sample is increased (67 and 58% for
364 effluent, 45 and 50% for influent). This trend was not observed for any of the other
365 compounds, moreover the recoveries of ATE, TRI and MTO for influent samples were
366 slightly higher than for effluent samples. This can be explained by the fact that the loading
367 volume for effluent wastewater samples was 50 mL whereas the loading volume for
368 influent wastewater samples was 25 mL. Gilart *et al.* [14] reported recoveries ranging
369 from 39 to 97% for effluent samples and from 24 to 97% for influent samples when
370 determining basic pharmaceuticals using a non-commercial polymeric SCX sorbent.
371 Krizman *et al.* [18] extracted opioids from river water, effluent and influent wastewater

samples by applying an Oasis MCX sorbent in the SPE. The recoveries obtained were 73 – 109% for river water, 78 – 116% for effluent wastewater and 44 – 95% for influent wastewater samples.

The matrix effect was calculated by comparing the concentration obtained upon spiking (0.5 µg/L for river water samples, 1 µg/L for effluent samples and 2 µg/L for influent samples) a non-spiked sample after SPE, subtracting the blank signal from the theoretical concentration. A negative value indicates signal suppression while a positive value indicates signal enhancement. It can be observed in Table 1 that, in most cases, there was signal suppression, and that low matrix effects were observed considering that values in the range of ±20% are considered acceptable. For river water samples, only PRO presented values higher than ±20% (+24%), for effluent samples TRI and VEN had values of -29 and -24%, respectively, and for influent samples VEN and PRO had values of -24 and +25%, respectively. Despite these values being higher than ±20%, they are still quite low. Prosen *et al.* [15] obtained %ME values ranging from -3 to -41% for river water samples, from -46 to +1% for effluent and from -49 to +16% for influent samples when determining drugs of abuse with Oasis MCX; even then, it should be noted that in the case of influent samples, a 1/5 dilution was required to reduce the matrix effect. When using Oasis HLB for the extraction of pharmaceuticals from wastewater, Mostafa *et al.* [19] observed matrix effects higher than ±20% for most of their compounds. When working with sorbents like Oasis HLB, it is possible to retain a wide range of compounds, however it is not possible to introduce an exhaustive washing protocol, and this leads to significantly high matrix effects.

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

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

408 The precision was evaluated as the repeatability (intra-day precision) and reproducibility
409 between days (inter-day precision) (n=4 in both cases). The precision was excellent (the
410 %RSD values obtained were always lower than 13%).

411 *3.6. Analysis of samples*

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

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

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

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

Influent samples presented the highest occurrences of the analytes, reaching levels higher than 5,000 ng/L for TRI, MTO and VEN. On the other hand, one of the samples presented concentrations for all the compounds lower than 400 ng/L. The occurrences found agree with data reported by Gilart *et al.* [14] when determining ATE, TRI and MTO in similar samples with concentrations ranging from 116 to 3,293 ng/L for these three

compounds. Moreover, the occurrences reported by Vergeynst *et al.* [26] for TRI and VEN in influent samples from Belgium were close to the lower limits of the present study, reporting concentrations from 158 to 228 ng/L for TRI and from 119 to 480 ng/L for VEN. The concentrations found by Li *et al.* [27] in samples from Scotland were in the range from 155 to 2,100 ng/L for TRI, showing similar levels to the present study, however PRO was one of the target compounds and was not detected.

4. Conclusions

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

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Figure caption

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

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

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

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

Tables

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

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

Table 2. Method detection and method quantification limits.

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

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

	River water	Effluent wastewater	Influent wastewater
ATE	<MDL – 178 ± 19	1090 ± 190 – 5453 ±640	332 ± 62 – 4030 ± 490
TRI	<MDL – 279 ± 33	282 ± 32 – 6730 ± 800	257 ± 32 – 8560 ± 710
MTO	<MDL – 165 ± 11	65 ± 9 – 6661 ± 770	272 ± 32 – 8000 ± 990
VEN	<MQL – 320 ± 34	357 ± 42 – 4530 ± 550	387 ± 43 – 7370 ± 890
PRO	<MQL – 268 ± 33	68 ± 8 – 3760 ± 320	139 ± 16 – 4280 ± 480