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Preparation of a polar monolithic stir bar based on methacrylic acid and divinylbenzene for the sorptive extraction of polar pharmaceuticals from complex water samples

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ABSTRACT

A monolithic, hydrophilic stir bar coating based upon a copolymer of methacrylic acid and divinylbenzene [poly(MAA-*co*-DVB)] was synthesised and evaluated as a new polymeric phase for the stir bar sorptive extraction (SBSE) of polar compounds from complex environmental water samples. The experimental conditions for the extraction and liquid desorption in SBSE were optimised. Liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) was used for the determination of a group of polar pharmaceuticals in environmental water matrices. The extraction performance of the poly(MAA-*co*-DVB) stir bar was compared to the extraction performance of a commercially available polydimethylsiloxane stir bar; it was found that the former gave rise to significantly higher extraction efficiency of polar analytes (% recovery values near to 100% for most of the studied analytes) than the commercial product. The developed method was applied to determine the studied analytes at low ng L^{-1} in different complex environmental water samples.

1. Introduction

In recent years, different sampling techniques have been developed to extract organic compounds from aqueous samples. One of these techniques is stir bar sorptive extraction (SBSE), where organic compounds are enriched from aqueous samples by direct sorption onto a sorbent phase/coating which encapsulates a magnetic stir bar. Once sorption is complete, the sorbed compounds can be thermally or liquid desorbed from the sorbent. The technique has been applied successfully in environmental analyses, biomedical analyses and food analyses, *inter alia* [1–4].

The extraction mechanism of the SBSE technique is similar to solid-phase microextraction (SPME), which is an equilibrium extraction technique based on sorption. The working principle of SPME and SBSE involves the partitioning of analytes between the sample matrix and the extracting phase on the fibre or stir bar. The sorption efficiency depends primarily upon the characteristics of the selected sorbent, as well as on the type of analytes being sorbed [1,5]. Generally speaking, in terms of the sensitivity of determination of apolar analytes at trace levels in complex matrices, SBSE is recognised to be superior to SPME [4,6]. A number of different extracting phases, such as polydimethylsiloxane (PDMS), polyacrylate (PA), carboxen, carbowax-divinylbenzene (CW-DVB), poly(ethylene glycol) (PEG), amongst others, have been commercialised for SPME, however a PDMS coating is the only coating commercially available for SBSE, which sets significant limits upon the applicability of the technique. Although an excellent performance by SBSE for the sorption of apolar analytes is usually obtained [7–10], the most polar analytes present in samples are retained poorly by PDMS [11,12]. Therefore, efforts have been directed at developing new sorbents for implementation in the SBSE technique [13–19], such as monolithic materials [14–17], polyurethane foams [18] or materials based on sol-gel technology [19], in order to increase the efficiency of extraction of polar analytes.

Concerning the monolithic approach, several polar coatings for stir bars have been reported. Recently, Huang et al. prepared a series of polymeric phases for SBSE with different polarities, such as a vinylimidazole-divinylbenzene copolymer [poly(VIm-*co*-DVB)] [14], a vinylpyridine-ethylene glycol dimethacrylate copolymer [poly(VP-*co*-EGDMA)] [15] and a methacrylic acid-3-sulfopropyl ester potassium salt-divinylbenzene copolymer [poly(MASPE-*co*-DVB)] [16] for the extraction of polar and apolar compounds. The utility of the aforementioned materials was evaluated using different matrices (water, urine and honey), and gave rise to promising results. Recently, our research group disclosed methods for the preparation of a new design of stir bar based on a monolithic vinylpyrrolidone-divinylbenzene copolymer [poly(VPD-*co*-DVB)] coating, and its application towards the extraction of a group of polar and apolar analytes from complex water samples [17].

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In view of the encouraging results arising from our previous study [17], we prepared a monolithic stir bar coating incorporating a polar monomer with a ionisable functional group, since this introduces mixed-mode character into the material. The selection of the monomer was based on previous experience within our group in the use of mixed-mode sorbents in SPE [20], where a mixed-mode SPE sorbent incorporating methacrylic acid residues was applied successfully to the selectively extraction of basic analytes (the methacrylic acid residues impart weak cation-exchange character). With all of this in mind, a stir bar coated with a copolymer of methacrylic acid and divinylbenzene was prepared, and this is what is disclosed in the present paper. Post-synthesis, the sorptive capacity of the material was investigated, and the material evaluated for the SBSE of polar pharmaceuticals from complex environmental samples. The results were compared to extraction data derived from experiments with a commercially available, PDMS-coated stir bar.

2. Experimental

2.1. Reagents and standards

Methacrylic acid (MAA) (98% grade) and divinylbenzene (DVB) (80% grade) were supplied by Sigma–Aldrich (Steinheim, Germany). Stabilizers were removed from MAA and DVB by distillation under reduced pressure and by passing through a short column filled with neutral alumina (Aldrich), respectively. Cyclohexanol (99%) and 1-dodecanol (98%), both from Aldrich, were used as porogens. The 2,2′-azobisisobutyronitrile (AIBN) used as initiator (BDH, Poole, UK) was recrystallised at low temperature from acetone (Merck, Darmstadt, Germany) prior to use.

Paracetamol, caffeine, antipyrine, propranolol, carbamazepine, naproxen and diclofenac (from Aldrich) were the analytes selected to evaluate the sorptive properties of the stir bars.

Standard solutions at 1000 mg L^{-1} of each compound were prepared in MeOH. These solutions were stored at 4 °C. A standard mixture solution was prepared by diluting each individual standard solution in ultra pure water (Veolia Water Solutions & Technologies, Barcelona, Spain).

LC-grade methanol (MeOH) and acetonitrile (ACN) were supplied by SDS (Peypin, France).

Formic acid (Prolabo, Bois, France), hydrochloric acid (Probus, Barcelona, Spain), sodium hydroxide (Panreac, Barcelona, Spain) and sodium chloride (from Aldrich), were used to adjust the pH of the mobile phase or the sample prior to SBSE.

2.2. Stir bar preparation

Polymerisation conditions were selected based on previous experience from related synthetic work [17]. Purified monomers, at a ratio of 75% (w/w) DVB and 25% (w/w) MAA (50% (w/v) total monomer in feed relative to solvent), AIBN (1 mol% relative to polymerisable double bonds) and porogen (10%, w/v 1-dodecanol in cyclohexanol) were placed in a glass tube. The monomer mixture and porogen were mixed ultrasonically into a homogenous solution, then the monomer solution was purged with N_2 at 0 °C for 5 min. Subsequently, the monomer solution was poured into a glass tube of defined diameter (6.5 mm i.d.). A magnetic stir bar (12 mm × 4.5 mm o.d.) was introduced into the middle of a spring (4.5 mm i.d.) and then immersed vertically into the monomer solution. The glass tube was sealed with a septum and incubated at 60 °C for 48 h. Once the polymerisation was complete, the glass tube was cut off carefully to deliver a rigid, coherent monolith. The monolithic material which encapsulated the stir bar was then Soxhlet-extracted with MeOH for 24 h to eliminate residual monomers, porogen and initiator. The final poly(MAA-*co*-DVB) stir bar obtained had the following dimensions: length: 14 mm; polymer thickness: 1 mm, which corresponds to a polymer volume of around 350 μL.

Nitrogen sorption porosimetry measurements were performed on an ASAP 2010 Micromeritics Instrument (Norcross, GA, USA), and the specific surface areas calculated using the BET method. The carbon, hydrogen and nitrogen contents of the polymeric materials were obtained by elemental microanalysis using a Carlo-Erba EA 1106 Instrument. FTIR analyses were performed using a Perkin–Elmer Spectrum One FTIR Spectrometer (Birmingham, UK).

2.3. LC–(ESI)MS/MS analysis

The extracts were analysed on an Agilent 1200 liquid chromatograph coupled to a 6410 triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode with an ESI interface, an automatic injector, a degasser, a quaternary pump and a column oven from Agilent Technologies (Waldbronn, Germany).

The chromatographic column used for analyses was a 100 mm × 4.6 mm i.d. stainless-steel column packed with Kinetex 100 Å C_{18}, with 2.6 μm superficially porous shell particles (Phenomenex, Torrance, CA, USA). The analyses were performed at 35 °C and the injection volume was 50 μL. A binary mobile phase with a gradient elution was used. The mobile phase consisted of ultra pure water adjusted to pH 3.0 with formic acid, and ACN, and the flow-rate was set at 0.6 mL min^{-1}. The applied gradient was as follows: 10–15% ACN in 5 min then to 100% ACN in 5 min and kept constant for 5 min, and then decreased to the initial conditions in 2 min. Under the optimum conditions, the separation of the analytes was achieved in less than 13 min. The instrument operated in positive and negative modes and the ESI parameters were as follows: drying gas flow 12 L min^{-1}, desolvation temperature 350 °C, nebulising gas pressure 45.0 psi, and capillary voltage 4000 V. Nitrogen was used as collision, nebulising and drying gas. The MRM transitions, the cone voltage, the collision energy as well as the pK_a values are summarised in Table 1. Two fragmentations of $[M+H]^+$ or $[M-H]^-$ were acquired for all selected analytes. To quantify the analytes, their most intense transitions were chosen.

Table 1
ESI mode and MRM conditions used for LC–(ESI)MS/MS of target analytes.

Analyte	pK_a	Log K_{ow}	ESI ionisation mode	Cone voltage (V)	Precursor ion (m/z)	Product ions (m/z)		Collision energy (V)	
Paracetamol	9.2	0.5	+	100	152	**110**	93	15	25
Naproxen	4.8	2.9	–	50	229	**185**	170	5	30
Diclofenac	4.2	3.7	–	75	294	**250**	214	10	20
Caffeine	13.4	−0.6	+	125	195	**138**	110	15	25
Antipyrine	13.3	0.4	+	100	189	**145**	115	30	30
Propranolol	9.5	−0.2	+	125	260	**116**	183	15	15
Carbamazepine	13.7	1.9	+	150	237	**193**	179	35	35

Bold indicates the quantifier ion.

2.4. Stir bar sorptive extraction

The SBSE procedure was as follows: the stir bar was activated with 5 mL of MeOH and stirred for 5 min. After drying with lint-free tissue, the stir bar was inserted into a flask with 100 mL of sample adjusted to pH 3.0. Samples were stirred with the stir bar at 750 rpm for 4 h at room temperature (25 °C). Following the extraction, the poly(MAA-*co*-DVB) stir bar was removed magnetically from the sample solution, dipped briefly in ultra-pure water (to remove adsorbed impurities) and dried using lint-free tissue.

For the liquid desorption of analytes, the stir bar was introduced into a vial with 5 mL of MeOH and agitated at 750 rpm for 20 min. Then, the stir bar was removed magnetically and reconditioned. The extract was evaporated to dryness under nitrogen and the dry residues redissolved in 1 mL MeOH:water (20:80). The extracts were analysed by LC–(ESI)MS/MS.

The results obtained with the poly(MAA-*co*-DVB) stir bar were compared to a commercially available PDMS-coated stir bar, obtained from Gerstel (Mulheim Ruhr, Germany). From the two sizes of commercially available stir bars, the larger stir bar was chosen, since it provides high sorptive capacity for sample volumes greater than 50 mL. It consists of a 20 mm long glass-encapsulated magnetic stir bar, coated externally with a 1 mm thick layer, corresponding to a PDMS volume of 126 μL. Before their first use, the stir bars were introduced into a vial containing acetonitrile and conditioned for 24 h [21].

The poly(MAA-*co*-DVB) stir bar can be reused and the life time of a single stir bar was found to be between 30 and 40 extractions, depending on the matrix. The stir bars were reconditioned by inserting them into vials containing MeOH for a period of 20 min; then, the MeOH was refreshed and the procedure was repeated three times. Finally, the stir bars were dried using a lint-free tissue, and stored in a vial until the next analysis.

2.5. Sample collection

The river water samples were collected from the Ebre River. The effluent wastewater samples were collected from two sewage treatment plants (STPs).

All the environmental water samples were adjusted to ~pH 3 using HCl and stored at 4 °C prior to analysis. They were filtered through 0.45 μm nylon membranes (Supelco, Bellefont, PA, USA) before the stir bar extraction.

3. Results and discussion

3.1. Preparation and characterisation of polymer monolith

Different variables which can affect the polymerisation and the sorptive properties of the monolith, such as the monomer type, the crosslink density, the volume and nature of the porogen and the initiation mode in polymerisation were investigated. These parameters can influence the porous character of the monolithic material, and this is significant because it is the volume of pores which allows the analytes to penetrate for sorption processes into the polymer structure.

In previous related work within our group [17], a spring was used to stabilise/scaffold the monolithic material when in contact with the magnetic stir bar, to enhance the dimensional stability. This design was very successful and was therefore implemented in the present study as well. Since the incorporation of the polar monomer VPD into the polymer structure facilitated the extraction of polar analytes [17], we decided to increase the polarity yet further and simultaneously attempt to introduce selectivity into the extraction. Although the earlier results using a poly(VPD-*co*-DVB) sorbent were satisfactory, there was no selectivity in the extraction of the analytes, therefore we intended to make further improvements by introduction of mixed-mode character [20]. Concerning the monomer selection, MAA was identified as a suitable comonomer due to its polar and ionisable nature, and DVB was selected as a suitable crosslinking agent. Different monomer feed ratios (75/25, 50/50, 25/75 and 15/85 (w/w) of MAA/DVB) were tested. It was found that those monoliths derived from lower DVB contents in the monomer feed (25 and 50, w/w) were of low rigidity and could be damaged easily (*i.e.* they had relatively poor mechanical/dimensional stability). In contrast, when higher proportions of DVB were used in the monomer feed, the monolithic products were mechanically and dimensionally stable, and well-suited for their intended use in SBSE. To ensure that the monoliths had polar character as well as mechanical and dimensional stability, the monomer feed was fixed at 25/75 (w/w) of MAA to DVB.

We selected a mixture of cyclohexanol and 1-dodecanol as porogen, which has been demonstrated previously to be convenient in monolith material synthesis [14,15,22]. The ratio of total monomer to porogenic solvent was fixed at 50/50 (w/w); a similar ratio has been used previously in the preparation of different monolithic stir bar coatings [15,22].

FTIR spectroscopic analyses of the poly(MAA-*co*-DVB) coatings confirmed that the comonomers, DVB and MAA, had been copolymerised into the monolithic structures. The spectra showed the characteristic bands ascribed to O—H stretching (broad band at around 3450 cm^{-1}) and C=O stretching (1705 cm^{-1}) of MAA residues. The region below 1600 cm^{-1} confirmed the presence of the aromatic DVB residues (stretching of C—C bonds in the rings).

Elemental microanalysis of the poly(MAA-*co*-DVB) monolith gave the following results: carbon (80.0%), hydrogen (7.8%), nitrogen (0.1%) and oxygen (12.1%, calculated by difference). These values are in agreement with the values expected based upon statistical incorporation of the monomers from the feed, which indicates that the copolymerisation was successful.

The specific surface area of the monolith was determined to be 500 $m^2\ g^{-1}$ using Brunauer–Emmett–Teller (BET) method, thus the monolith had a well-developed pore structure which ought to facilitate the kinetics of the sorption.

3.2. Optimisation of SBSE procedure

Since the poly(MAA-*co*-DVB) monolith was expected to have mixed-mode character [20], a group of basic and acidic pharmaceuticals (pK_a detailed in Table 1) was selected to examine the potential selectivity of the monolith.

The poly(MAA-*co*-DVB) monolith can be expected to retain charged basic compounds by reversed-phase (RP) and ionic interactions arising from the presence of carboxylic acid moieties, while the remainder of the compounds are retained by RP interactions alone. Ideally, in the washing step the acidic analytes can be eliminated, while the basic analytes remain retained but can be eluted subsequently in the elution step. The parameters affecting SBSE were tested; and, several important variables affecting the extraction and desorption steps, including sample pH, ionic strength, desorption solvent, extraction and desorption time, were studied in detail to optimise the SBSE conditions.

The SBSE procedure was optimised using initial conditions to promote ionic interactions in the mixed-mode materials. The conditions were as follows: 100 mL of the sample at pH 7 (to ensure the deprotonation of the carboxylic acid and protonation of the basic compounds), agitated at 750 rpm during 1 h, and desorption with 5 mL of 2% TFA in MeOH stirred at 750 rpm for 20 min. All the experiments were performed at ambient temperature (25 °C). The % recoveries under these preliminary conditions ranged between 10 and 60%.

Before the optimisation of these parameters, preliminary tests were performed to establish the weak cation-exchange character of the coating. For this we included, supplementary to the previous conditions, a washing step (brief immersion in 1 mL of solvent) and several solvents, including MeOH, ACN, ethyl acetate, CH_2Cl_2 and 5% NH_4OH in MeOH were tested. Here, the aim was to remove acidic analytes and interferences, which were retained by RP interactions, while maintaining basic analytes retained by ionic interactions. However, we observed that all the compounds were partially or completely eliminated during the washing step, which implies that the ionic interactions were not strong enough to retain basic analytes under the conditions of study. Thus, while the poly(MAA-*co*-DVB) phase was found to be suitable for the extraction of polar analytes, its weak cation-exchange properties could not be exploited for these analytes under the specific conditions used for the sorption experiments. Thereafter, the SBSE conditions were optimised based on the RP retention behaviour on the stir bar.

Huang et al. [16] reported the preparation of a mixed-mode MASPE-DVB-based coating, where, according to the authors, the presence of sulfonic acid groups (deprotonated) in the monolithic material allowed retention of fluoroquinolones (protonated amino groups at pH 5.0), through the combination of hydrophobic and cation-exchange interactions. However, no washing step was performed in this study to demonstrate unequivocally the selectivity which can arise with mixed-mode materials.

3.2.1. Liquid desorption conditions

In methods using SBSE, thermal desorption of the analytes is usually combined with analysis by gas chromatography-mass spectrometry (GC–MS) [13]. However, due to the fact that a group of polar analytes was extracted, liquid desorption followed by LC was selected to back extract and analyse the polar pharmaceuticals under study. The influence of the liquid desorption conditions on efficiency were optimised.

To ensure the complete elution of target analytes, several different solvents were tested: MeOH and ACN, and MeOH containing different percentages of CH_3COOH or TFA. Considering the size of the stir bar and suitable vials to perform the desorption step, we used a 5 mL solvent volume to guarantee the complete immersion of the coated stir bar and ensure a proper desorption process. The results using ACN and MeOH with either CH_3COOH or TFA were marginally poorer than when using MeOH. Therefore, MeOH was selected as the desorption solvent due to the slightly higher ability to desorb polar analytes from the stir bars (~5–10% improvement in % recovery). Increasing the MeOH volume further (to 10 mL) did not improve the desorption results. Finally, 5 mL of MeOH was selected for the optimal back extraction of the target analytes.

In the next step, the desorption time of the poly(MAA-*co*-DVB) sorbent was varied from 10 to 30 min using an agitation speed of 750 rpm. The level of analytes desorbed from the stir bar increased when the desorption time was extended from 10 to 20 min, however a further increase in the desorption time did not improve the recoveries. Consequently, 20 min for the desorption was selected as optimal for the remaining investigations.

3.2.2. Extraction conditions

Once the desorption conditions were optimised, variables affecting the extraction process were examined. These included sample pH (3.0, 7.0 and 9.0), ionic strength (5, 10, 15 and 20% of NaCl w/v), stirring rate (600, 750 and 900 rpm), sample volume (50 and 100 mL) and extraction time (1–8 h).

The effect of sample pH on the extraction efficiency was examined at three representative pHs (*i.e.* 3.0, 7.0 and 9.0). The pH is an important parameter in an extraction process as it determines the protonation state of ionisable groups in the polymeric sorbent and analytes, and consequently influences their retention and

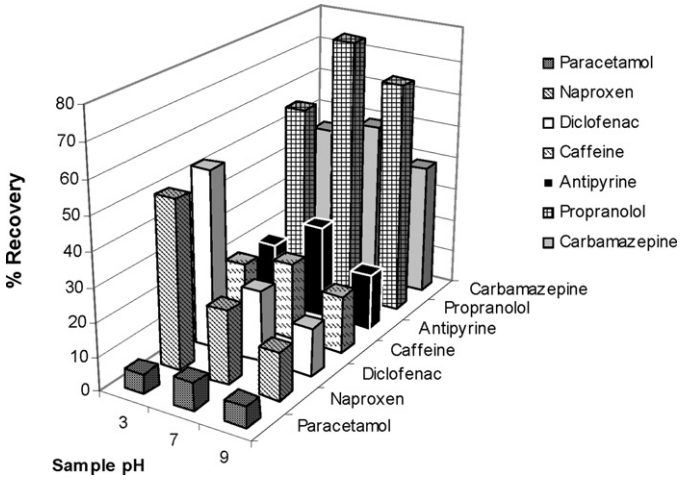

Fig. 1. The effect of sample pH on extraction recovery.

extraction efficiency. As shown in Fig. 1, the pH value significantly affected the extraction efficiency of the poly(MAA-*co*-DVB) stir bar for selected analytes. The results indicated that the extraction efficiency for acidic pharmaceuticals improved considerably when the pH value was set at 3.0 (at this pH they are in neutral form), but decreased when the pH value was higher. Only the recovery of propranolol ($pK_a \sim 9.5$) was found to be improved under higher pH conditions. In view of these results, pH 3.0 was selected for further research.

The effect of ionic strength on the extraction efficiency was also investigated. The influence of ionic strength on recoveries of the target analytes was performed by addition from 0 to 20% of NaCl (w/v) to the aqueous samples. However, the results showed that an increase in ionic strength did not enhance significantly the extraction efficiency. To simplify the extraction procedure, we therefore did not add any salt in the subsequent experiments.

It is well-known that agitation speed can affect the mass transfer of the analytes during the extraction process. Three agitation rates (600, 750 and 900 rpm) were tested to optimise the stirring conditions. The results obtained with an agitation rate of 750 rpm were better than those obtained at 600 rpm; however a further increase in the agitation rate to 900 rpm may shorten the lifetime of the monolithic coating. Since a stirring rate of 750 rpm gave rise to efficient extractions without detriment to the physical integrity of the stir bar, it was selected for further study.

When we varied the sample volume, the results were comparable when both 50 mL and 100 mL of ultra pure water samples were analysed. Therefore, 100 mL of sample was selected for further analysis.

Finally, the extraction time was varied from 1 to 8 h. The extraction efficiency increases rapidly with an increase in the extraction time from 1 to 4 h, and then changes slowly with further increases in the extraction time. Since a compromise between the extraction time and efficiency was necessary, 4 h was selected as the extraction time in the following studies.

Overall, the optimum SBSE conditions were as follows: 100 mL of sample at pH 3.0 extracted at 25 °C by agitating at 750 rpm for 4 h; liquid desorption: 5 mL of MeOH stirred at the same speed for 20 min. The recovery values (listed in Table 2) obtained in the extraction of the analytes from ultra-pure water (spiked at 100 ng L^{-1} with the analyte mixture) for most of the analytes were in the range of 60–100%, except for paracetamol (%*R* only 13%) and caffeine (%*R* 45%), which may be due to their weak hydrophobic interactions.

The bar-to-bar reproducibility of poly(MAA-*co*-DVB) monoliths was also studied by comparing the extraction efficiency of target

analytes. The bar-to-bar reproducibility %RSD ($n = 3$) was less than 11% for all analytes under study. It is also worth noting that no damage to the stir bar coatings was observed during extractions. The good reproducibility and stability indicates that poly(MAA-*co*-DVB) monoliths are eminently suitable as materials for stir bar coatings.

3.3. Comparison to the other stir bars

Taking into account the SBSE data arising from use of the poly(MAA-*co*-DVB) stir bars, we decided to compare their SBSE performance to the commercially available stir bars based on PDMS. The results obtained using a PDMS-coated stir bar are shown in Table 2. It can be noticed that when using the same SBSE conditions, the recoveries of target analytes were higher for all selected compounds on the poly(MAA-*co*-DVB) monolith. These results can be explained easily by considering the polar nature of the poly(MAA-*co*-DVB) coating, comparing to the apolar PDMS phase. The poor performance of PDMS-coated stir bars in the extraction of polar pharmaceuticals has been reported previously [17,23].

Upon comparing the new results to the results obtained in previous research work with a monolithic coating based on poly(VPD-*co*-DVB) [17], it is clear that in spite of the fact that higher sample volumes and a larger amount of extracting phase were used in the present study, the recovery values for most of the analytes were comparable, except in the case of caffeine and antipyrine where the recoveries with the poly(VPD-*co*-DVB) monolith were 20% and 42%, respectively, whereas with the new poly(MAA-*co*-DVB) stir bar the recoveries were significantly higher (45% and 61%, respectively). Thus, poly(MAA-*co*-DVB) stir bar outperforms the previously synthesised poly(VPD-*co*-DVB) stir bar.

3.4. Application to environmental water samples

Since the analytes under study are contaminants which can be found in river water and effluent wastewater from a treatment plant (WWTP), these sample matrices were selected to perform the study.

In order to improve the overall sensitivity of the method, the 5 mL extract from the liquid desorption was evaporated until dryness and reconstituted with 1 mL of MeOH/H_2O (20/80). No losses of analytes in the evaporation step were observed.

A common drawback when quantifying LC–MS with an ESI source is the ion suppression or enhancement effect arising from a number of organic and/or inorganic compounds present in the matrix sample. The resulting ion suppression or enhancement effect ranged from 0 to 17% for river water samples, and from 3 to 45% (only for some analytes) for effluent WWTP samples. The analytes most affected by matrix effects were propranolol and antipyrine. Although some authors reported that the matrix effect is less when using SBSE compared to other sorptive extraction techniques, such as SPE [24,25], in this case, due to the polarity of the sorbent material, the suppression was quite significant. Several approaches are typically applied to deal with matrix effects in quantitative analysis, such as sample dilution, improvement of the sample pre-treatment and the chromatographic separation, or the use of stable-isotopically labeled internal standards [26]. From these approaches we selected to dilute the effluent WWTP samples (1:1) with ultra-pure water. The resulting ion suppression or enhancement effect ranged between 1 and 21%.

Table 2
Recovery values (%) obtained when the poly(MAA-*co*-DVB) and PDMS-coated stir bars were applied in SBSE of 100 mL of an ultra-pure sample spiked at 100 ng L^{-1} with the analyte mixture.

Analyte	% Recovery	
	Poly(MAA-*co*-DVB)	PDMS
Paracetamol	13	–
Naproxen	107	–
Diclofenac	101	23
Caffeine	45	3
Antipyrine	61	–
Propranolol	101	–
Carbamazepine	95	1

% Relative standard deviations (%RSDs) ($n = 3$) were lower than 10% for $\%R > 15\%$. For the experimental conditions, see text.

Table 3
Recovery values (%) obtained when the poly(MAA-*co*-DVB) coated stir bar was applied in SBSE of 100 mL of river and effluent WWTP samples spiked at 100 ng L^{-1} and 200 ng L^{-1}, respectively, with the analyte mixture.

Analyte	% Recovery (%RSD, $n = 3$)	
	River	Effluent WWTP
Paracetamol	11 (3)	10 (4)
Naproxen	92 (6)	85 (10)
Diclofenac	90 (1)	62 (4)
Caffeine	35 (7)	31 (19)
Antipyrine	52 (4)	45 (6)
Propranolol	50 (7)	35 (9)
Carbamazepine	90 (2)	86 (3)

For the experimental conditions, see text.

Table 3 lists the recovery values for the different water samples. The data obtained for river water samples was good, with recoveries ranging from 50 to 100% for most analytes (except paracetamol and caffeine), and similar to the values obtained for extractions from ultra-pure water, demonstrating the satisfactory ability of this monolithic material to retain both acidic and basic pharmaceuticals even in the presence of matrix interferences. Only propranolol showed a decrease in retention ($\%R \sim 50\%$) when river water samples were analysed, possibly due to the complexity of the matrix and competition between the analyte and the other components from the sample matrix for access to those sites of the polymer where the retention takes place. In comparison to the performance of the poly(VPD-*co*-DVB) coating [17] in the SBSE of environmental samples, the recoveries of analytes were slightly better.

Thereafter, the method was validated with Ebre river water; the linear range with matrix calibration ranged from 10 to 500 ng L^{-1} for antipyrine, propranolol and diclofenac, and from 20 to 500 ng L^{-1} for the remaining analytes, except for naproxen (100–500 ng L^{-1}), with regression coefficients (r^2) greater than 0.999. The limits of detection (LODs), calculated using a signal to noise ratio of ≥ 3, were 10 ng L^{-1} for most of the compounds, with the exception of naproxen (50 ng L^{-1}). The repeatability and reproducibility of the method, expressed as the relative standard deviation (RSD) of three analyses of 100 mL of Ebre river water spiked at 100 ng L^{-1} were lower than 15% for all compounds.

We also demonstrated the applicability by analysing different environmental samples from a river and effluent WWTP using the SBSE-LC–(ESI)MS/MS method. When analysing three river water samples, analytes such as diclofenac (31–48 ng L^{-1}) and caffeine (<LOQ – 33 ng L^{-1}), were found in the samples analysed. The concentration value of carbamazepine found in river samples was below the LOD. Moreover, in one sample propranolol was found at a concentration of 19 ng L^{-1}. These values are comparable to those found in the samples from the same river [27].

Three samples of effluent from WWTPs were also analysed. Certain analytes, such as diclofenac (63–106 ng L^{-1}), caffeine (129–461 ng L^{-1}), antipyrine (76–125 ng L^{-1}), propranolol (31–48 ng L^{-1}), and carbamazepine (67–86 ng L^{-1}) were found in the effluent WWTP samples analysed. As an example, Fig. 2 shows representative MRM chromatograms from the analysis, obtained

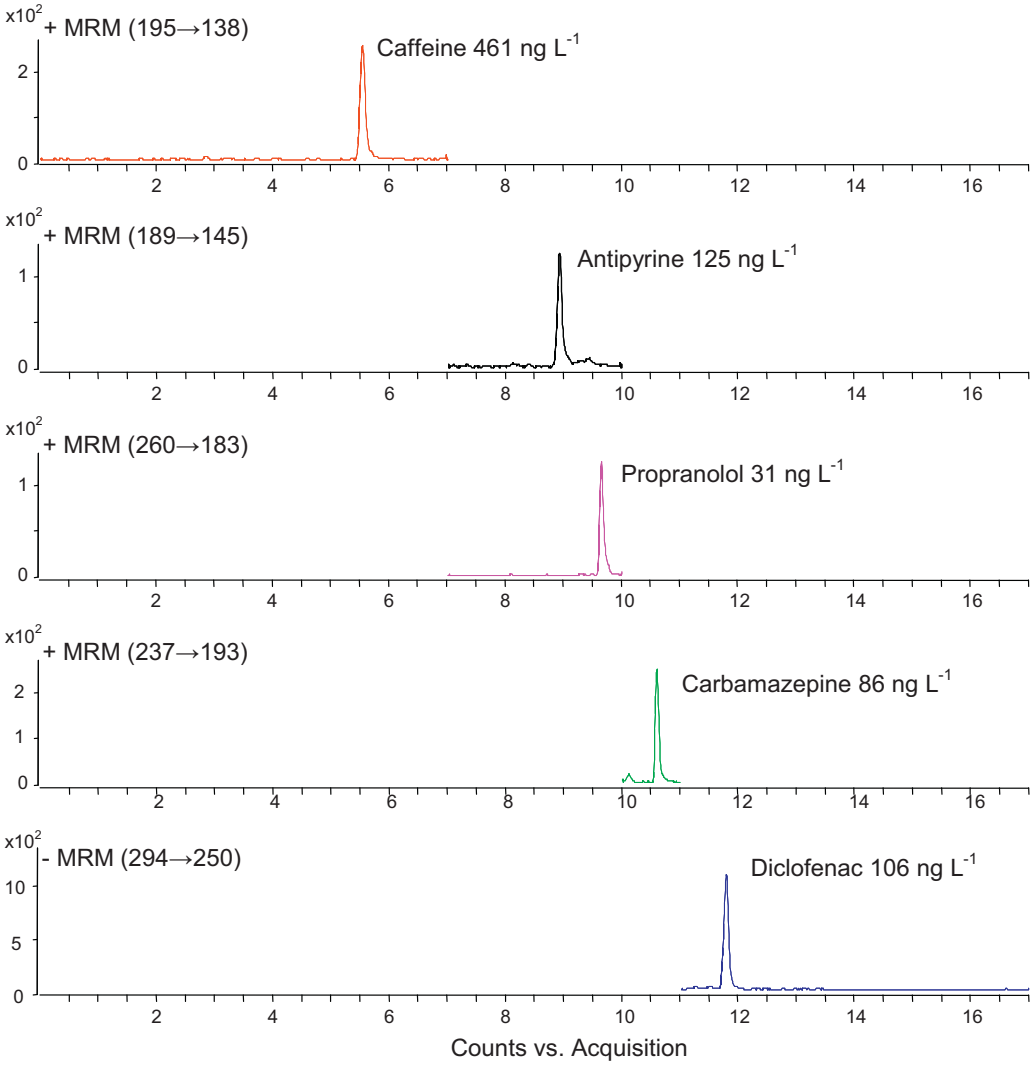

Fig. 2. MRM chromatograms of an effluent WWTP sample. For experimental conditions, see the text.

under optimum conditions from one of the effluent WWTP samples. The presence of these pharmaceuticals in similar samples was reported earlier [27–29], and the levels found here are in good agreement with previous reports.

4. Conclusions

A new poly(MAA-*co*-DVB) monolithic material was prepared as a coating for magnetic stir bar and served as an extractive polar phase in SBSE.

The poly(MAA-*co*-DVB) coated stir bar was applied successfully to the extraction of polar pharmaceuticals from complex aqueous samples; the results were superior to those obtained with a commercially available PDMS-coated stir bar.

The combination of SBSE and liquid desorption with LC-(ESI)MS/MS provided an efficient, simple and sensitive method for the determination of polar pharmaceuticals present at low levels in complex environmental samples. The optimised and validated SBSE-LC-(ESI)MS/MS method allowed the detection and quantification of the majority of the compounds studied.

Use of a poly(MAA-*co*-DVB) stir bar can be considered to be a promising alternative to conventional PDMS-coated stir bars in analytical applications.

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