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Using a novel sol–gel stir bar sorptive extraction method for the analysis of steroid hormones in water by laser diode thermal desorption/atmospheric chemical ionization tandem mass spectrometry

S. Vo Duy [a], P.B. Fayad [a], B. Barbeau [b], M. Prévost [b], S. Sauvé [a,*]

[a] Department of Chemistry, Université de Montréal, Montreal, Quebec, Canada
[b] Department of Civil, Geological and Mining Engineering, École Polytechnique de Montréal, Montreal, Quebec, Canada

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ABSTRACT

A new coating material was used for a stir bar sorptive extraction (SBSE) method coupled to a high throughput sample analysis technique. This allowed for a simple procedure for fast determinations of eight steroid hormones (estriol, estradiol, ethynylestradiol, estrone, progesterone, medroxyprogesterone, levonorgestrel, northindrone) in water. Sample pre-treatment was performed using an in-house SBSE method based on a polydimethylsiloxane/phenyltrimethylsiloxane/β-cyclodextrin sol–gel material. The analytes were desorbed by liquid extraction prior to their analysis by laser diode thermal desorption/atmospheric pressure chemical ionization coupled to tandem mass spectrometry (LDTD–APCI–MS/MS). Several parameters, including ionic strength, volume and time of extraction as well as volume and time of desorption, were investigated to maximize extraction efficiency by SBSE in aqueous solutions. The in-house stir bar showed good reproducibility and could be used for at least 50 extractions without affecting analytical performance. The recoveries of the spiked steroid hormones ranged from 55% to 96% in all water matrices studied (HPLC grade water, tap water and raw wastewater). Only one compound showed poor recovery values ($<2\%$ for estriol) in all matrices. The method detection limits (MDLs) in real matrices were within the range of 0.1–0.3 $\mu g\,L^{-1}$ except for estriol at 48 $\mu g\,L^{-1}$. The extraction performance of the in-house SBSE for the eight selected hormones was also compared with that of a commercially-available stir bar coated with polydimethylsiloxane (PDMS). This novel stir bar coating could prove to be useful method for the detection and quantification of trace levels of steroid hormones.

1. Introduction

There has been growing concerns towards the effects of emerging contaminants (ECs) in the aquatic environment and they are progressively becoming a priority for governmental and regulatory agencies as well as the general public. Among these compounds, endocrine-disrupting chemicals (EDCs), such as steroid hormones have received much attention because of their adverse effects on the reproductive physiology of wildlife populations with possible implications in human reproductive health as well [1]. To date, numerous analytical procedures have been developed to identify and quantitate steroid hormones in water matrices and often include the use of liquid chromatography (LC) coupled to tandem mass spectrometry and one purification step with solid phase extraction (SPE) [2,3] or immunosorbent assay [4,5]. An ultrafast analytical method for the quantification of eight selected steroid hormones in wastewater by laser diode thermal desorption (LDTD)/atmospheric pressure chemical ionization tandem mass spectrometry (APCI–MS/MS) has been described and this is the detection system that we will use in this work [6]. The analysis time was achieved in seconds compared to minutes for traditional LC–MS/MS method by eliminating the LC step. An SPE step was used to clean up and pre-concentrate the samples. While analysis time was significantly reduced, the commonly used solid-phase extraction sample preparation step remained tedious, time-consuming and the cartridges were not re-usable. As a simpler clean up step over SPE, stir bar sorptive extraction (SBSE) procedures have been developed to quantitate certain steroid hormones in aqueous matrixes such as drinking water, wastewater and urine prior to the analysis step [7–10]. Our approach was to demonstrate the feasibility and establish proof of concept of an in-house SBSE method for eight selected steroid hormones (estrogens as well as progestagens), many of which have not been studied with previous stir bar methods to overcome the laborious

* Corresponding author. Tel.: +1 514 343 6749; fax: +1 514 343 7586.
E-mail address: sebastien.sauve@umontreal.ca (S. Sauvé).

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SPE approach using LDTD–APCI–MS/MS. Stir bar sorptive extraction has been developed as a solventless sample preparation technique in 1999 by Baltussen et al. [11–14]. It is based on the same principles as solid phase microextraction (SPME), in that it is an equilibrium technique, but the amount of polydimethylsiloxane (PDMS) coated on the stir bar is 50–250 times larger than that on a SPME fiber, resulting in higher recovery values and extraction capacity [15]. This technique utilizes glass bars with a magnetic core, coated with a PDMS polymer as the extraction phase. In the last decade, SBSE has been successfully applied in numerous matrices (gas, liquids and solids) in environmental, food and flavor, biomedical and pharmaceutical samples [13,15–17]. Presently, a drawback of SBSE is that commercially-available stir bars are typically coated with an apolar PDMS layer, limiting their extraction efficiency towards polar and less polar compounds. Studies using PDMS coatings to analyze hormones in water, sludge and urine matrices have reported low recovery values for the majority of hormones (below 50%) with the exception of estrone with a recovery value of 80% and limits of detection (LODs) ranging from 0.5 to 1000 ng L^{-1} [7,9,18]. An additional step in the extraction procedure, in situ derivatization, has been proposed to improve extraction yields of some polar compounds, such as acidic and organic contaminants in water [19] using PDMS stir bars. This approach has improved recovery values for estrogenic hormones (above 93%) as well as LODs (0.5–2 ng L^{-1}), by applying a dual derivatization method which involved acylation of the aromatic hydroxyl group followed by the silylation of the aliphatic hydroxyl group found in estrogens [20]. However, this method requires significantly more time and reagents in the overall procedure and cannot be applied to all steroid hormones, such as progestagens, that contain different functional groups. Therefore, the need to develop novel stir bar coating materials with a higher affinity towards polar or less polar analytes, thus improving the selectivity and widening the applicability of SBSE, is of interest and has been investigated for multiple classes of compounds in the past [21–40]. Here, a sol–gel technology was chosen for the synthesis of the coating extraction phase sorbent, since it is both cost effective and simple, can be performed under mild thermal conditions and allows for strong adhesion of the coating to the substrate due to chemical bonding which increases mechanical durability as well as chemical stability [41]. In this study, a robust and reliable novel stir bar using sol–gel technology was prepared by introducing 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) or β-CD into the coating layer by the sol–gel technique for the efficient extraction of eight selected steroid hormones (Supporting information Fig. S-1) in water matrices. Analysis was performed by LDTD–APCI–MS/MS, a rapid, sensitive and high-throughput method. To the best of our knowledge, this new coating material as well as its application to these compounds in water matrices has not been reported in the literature. This method was developed and validated following the optimization of several parameters that could affect the efficiency of the SBSE process as well as being compared to a commercially available PDMS stir bar. This approach is a proof of concept study that could prove to significantly reduce analysis time, sample size and solvent consumption when compared to a previously developed SPE method [6] when applied to environmental water matrices.

2. Experimental

2.1. Reagents and standards

The stir bar coating materials, hydroxylterminated polydimethylsiloxane (OH-PDMS), poly(methylhydrosiloxane) (PMHS), phenyltrimethylsiloxane (PTMS), 3-(trimethoxysilyl)propyl methacrylate (TMSPMA), tetraethoxysilane (TEOS), β-cyclodextrin, 3-glycidoxypropyl trimethoxysilane (GPTMOS), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). All selected steroid hormones standards (purity ≥ 97%), estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2), levonorgestrel (LEVO), medroxyprogesterone (MPROG), norethindrone (NOR) and progesterone (PROG) were purchased form Sigma Aldrich. Isotopically-labeled 17α-ethinylestradiol, [$^{13}C_2$]-EE2, used as an internal standard (IS) were obtained from ACP Chemical Inc. (Montreal, QC, Canada). Individual stock solutions were prepared in methanol (MeOH) at a concentration of 1000 mg L^{-1} and kept at −20 °C for a maximum of three months. A primary mix of steroid hormone working solution was prepared daily at a concentration of 2.5 mg L^{-1} by dilution in acetonitrile (ACN) of individual stock solutions aliquots. All solvents used were of analytical grade purity from Fisher Scientific (Whitby, ON, Canada) and deionized/distilled water (dd-H_2O) was used for dilutions.

2.2. Instrumentation

Glass stir bars (7/8" × 1/4") were obtained from VWR (Montreal, QC, Canada). PDMS stir bars (Twister, coated with 10 mm in length and 0.5 mm film thickness of PDMS) were purchased from Gerstel GmbH & Co. (Mülheim and der Rühr, Germany) and used without modification. A magnetic stirrer from Labinco (Breda, Netherlands) was used for SBSE procedure and an ultrasonic bath model Branson 5200 (Bransonic^R, Danbury, CT) was used for liquid desorption. The LDTD–APCI–MS/MS consisted in an LDTD–APCI ionization source, developed and manufactured by Phytronix Technologies (Quebec, QC, Canada), mounted on a Quantum Ultra AM triple quadrupole mass spectrometer by Thermo Fisher Scientific (Waltham, MA) for analyte detection. The LDTD–APCI source was controlled by the LazSoft 4.0 software (Phytronix Inc., Quebec, QC, Canada). Resulting MS/MS peaks were integrated using the ICIS algorithm of the Xcalibur 1.2 software from Thermo Fisher Scientific.

2.3. Analytical conditions

The analytical conditions were described in more detail in previous work [6,42]. Samples were first spotted (4 μL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy with an appropriate solvent and then left to dry at room temperature. The loaded plate is then transferred to an *X*–*Y* moveable stage of the LDTD housing unit. Upon operation, a glass transfer tube is inserted into a well by an air-powered piston to avoid any sample loss. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample which is vaporized into the gas phase. The uncharged analyte molecules travel along the transfer tube by a carrier gas (medical grade purified air) to eventually reach the corona region for ionization by APCI and then transferred to the MS inlet.

The LDTD–APCI sample optimization for MS and MS/MS conditions in negative ionization mode (NI) and positive ionization mode (PI) was performed by depositing 2 μL of the standard steroid hormone of interest and the IS was at a concentration of 2 mg L^{-1} in the well plate inserts. The LDTD–APCI source parameters were set to the following values: corona discharge voltage of 5000 V in PI mode and 5500 V in NI mode, a carrier gas temperature of 50 °C, a sheath gas and auxiliary gas set at 0 for both modes and the ion transfer tube was set at 350 °C for both modes. The carrier gas flow was set at 3 L/min for all selected hormones in both PI and NI modes. The laser pattern

programming consisted of a 0.5 s ramp from 0% to 30% and held for 0.1 s at 30% before shutting off. The steroid hormones samples were analyzed in three desorption events, i.e. (i) E2, EE2, NOR and (ii) LEVO; MPROG; PROG in PI mode; (iii) E1 and E3 in NI mode. The IS was analyzed in both PI and NI modes. Two selected reaction monitoring transitions (SRM) were used as well as the relative intensities of their ratios so as to avoid false positives and confirm the presence of the detected steroid hormone. The instrument response was determined as the ratio of the analyte area to that of the isotopically-labeled IS.

2.4. Preparation of sorptive stir bars

Commercial magnetic glass stir bars were activated before coating by placing them in a 1 mol L^{-1} NaOH solution overnight to expose the maximum number of silanol groups on the surface. The treated bare glass stir bars were then subsequently cleaned with water and a 0.1 mol L^{-1} HCl solution to neutralize the excess NaOH in an ultrasonic bath for 30 min before being rinsed once again with water and dried at 100 °C for 2 h in a gas-chromatography oven. The activated stir bars were placed in a desiccator for no more than 12 h prior to the binding procedure.

The different polymerization reaction solutions and the corresponding reagents used for the coating procedure of the four different stir bars tested are listed in Table 1. The sol solutions were prepared by mixing the reagents in Table 1 according to the different stir bar protocols (1–4) and thoroughly mixing the solution on a vortex. TFA was then added to the mixture which was mixed once again and then centrifuged. The supernatant was collected and left to react at room temperature for 1 h. The resulting clear solution was used for the stir bar coating step. The treated glass bars were vertically immersed into the solution and left to react for 30 min in order for the sol–gel coating to be generated on the surface of the stir bars before being removed from the solution and left to air-dry for 10 min. The above coating procedure was repeated several times until the needed coating thickness was obtained, approximately 0.5 mm (usually between 5 and 6 coatings). The coated stir bars were placed in a desiccator at room temperature overnight to form the sol–gel polymer layer. The stir bar was thermally conditioned under nitrogen atmosphere in a gas-chromatography oven with the following temperature program: from 40 °C to 120 °C at 1 °C/min, held at 120 °C for 200 min, raised to 200 °C at 1 °C/min and kept at 200 °C for 200 min. Finally, the resulting coated stir bars were extracted with methanol (MeOH) and acetonitrile (ACN) in an ultrasonic bath for 1 h, respectively, to remove any unreacted reagents. The protocol for stir bar 4 (Table 1) was chosen as the final method of preparation once the SBSE was optimized.

In order to evaluate the performance of the stir bars, according to dimension and phase ratio, the PDMS polymer coating of a commercial Gerstel stir bar was removed from the stir bar before being recoated with the in house polymer layer (stir bar 5) as described in protocol 4 (Table 1).

Table 1
Reagent composition for the preparation of the four in-house stir bars.

Reagents	Stir bar 1	Stir bar 2	Stir bar 3	Stir bar 4
β–CD (mg)	0	0	90	90
Dimethylsulfoxide (μL)	0	0	0	100
OH-PDMS (mg)	900	900	900	900
PTMS (mg)	180	180	180	180
TEOS (mg)	0	90	90	90
TMSPMA (mg)	180	180	0	0
GPTMOS (mg)	0	0	180	180
PMHS (mg)	180	90	90	90
Dichloromethane (μL)	1350	1350	1350	1350
TFA (95% in water) (μL)	225	225	225	225

2.5. SBSE optimization procedure

Several parameters were evaluated to establish the optimum extraction conditions for the stir bar. These include sample extraction volume (20–100 mL), modifying ionic strength by the addition of NaCl (0–30%, m/v), stirring rate (200–300 rpm), time of extraction (1–2.5 h), addition of an organic modifier to enhance extraction (MeOH), pH of extraction solution, volume (0.6–1.2 mL) and nature of desorption solvent (MeOH and ACN) as well as desorption time (15–30 min). The tests were performed in a 125 mL glass vessel, spiked with 80 μL of steroid hormone working solution, where the prepared sorptive stir bar was then immersed at room temperature. After extraction, the stir bar was placed into a small glass tube containing the optimum volume of desorption solvents where IS at 100 μg/L was added before desorption of the target analytes in an ultrasonic bath at 35 °C. Following optimization, the protocol was as follows: a sample volume fixed at 50 mL, adding a NaCl solution of 30% (m/v), the stirring rate was 250 rpm, the extraction time was 2 h, the desorption solvent consisted of MeOH/ACN (50:50, v/v) and desorption time was deemed sufficient with 20 min. The organic modifier and pH did not improve extraction efficiency of the analytes. After desorption, 4 μL of the eluting solvent were spotted into the LazWell 96-well polypropylene plate cavities for analysis by LDTD–APCI–MS/MS. The stir bar was placed into 1 mL of MeOH/ACN (50:50, v/v) mixture in the same desorption conditions to prepare the stir bars for re-use. The stir bars were able to perform at least 50 extractions without affecting extraction efficiency. The method detection limits were determined as the minimum spiked concentrations that gave a signal to noise ratio between 3 and 5.

2.6. Sample preparation

The tap water samples from our laboratory and the affluent wastewater collected from the Repentigny wastewater treatment plant (WWTP) (Repentigny, QC, Canada) were previously filtered using a 0.45 μm cellulose acetate membrane filter (Watman, Florham Park, NJ) before applying the SBSE optimized procedure.

3. Results and discussion

3.1. Sol–gel coating process

Sol–gel technology is a simple and convenient pathway for the synthesis of surface coating materials. Four different protocols (Table 1) were carried out to study the effect of the functional monomers on surface coating. In all cases, OH-PDMS was introduced to the sol–gel mixture because of its ability to (i) lengthen the silica network, increasing the surface area of the coating material, (ii) uniformly distribute the stationary phase, (iii) reduce the coating layer fragility and (iv) prevent cracking of the coating layer [8]. PTMS was also added to all stir bar protocols to ameliorate the selectivity of the stir bar coating layer by improving π–π interactions between the polymer and the selected steroid hormones. As well, the PTMS could take the role of the cross-linking agent in addition to the TEOS. We also evaluated the potential advantages of the silica monomer TMSPMA as a co-precursor in the first two stir bar protocols (stir bars 1 and 2, Table 1). Due to the structure of TMSPMA, it could serve both as cross-linking agent as well as a selective stationary phase in the sol–gel coating procedure [43]. Its use in the sol–gel

solution could result in (i) the hydrolysis of the trimethoxysilyl groups in the monomer, allowing chemical bonding of TMSPMA to the sol–gel network and (ii) the polymerization reactions under high temperature during conditioning step of the vinyl substituents in the monomer [43]. Alternatively, the β-CD functional monomer co-precursor was introduced to the sol–gel network (protocols for stir bars 3 and 4, Table 1) to modify the selectivity of the stationary phase for the selected steroid hormones. β-CD is a cyclic oligosaccharide formed by seven glucosepyranose units, which shows a hydrophilic exterior and a hydrophobic cavity which increased the polarity of the SBSE stationary phase. GPTMOS was used in solution in order to bind β-CD and the sol–gel network through a ring-opening reaction [8]. β-CD is poorly soluble in sol–gel solution therefore dimethylsulfoxide (DMSO) was added to enhance the β-CD solubility in solution. Several volumes of DMSO were tested and the maximum volume used of DMSO was 100 μL. All the sol–gel solutions tested contained PMHS, used for the deactivation of the surface and coating network. PMHS does not contain any sol–gel active functionalities, but possesses active hydrogen atoms capable of derivatizing silanol groups during the high temperature conditioning step of the coated stir-bar. Also, the percentage of PMHS in the solution determines the solidification rate of sol–gel polymer. For the first stir bar, 180 mg of PMHS was used, where only 90 mg was added to the stir bars 2, 3 and 4, which reduced the solidification rate of the sol–gel allowing the formation of a more uniform surface on the stir bars with less PMHS. 90 mg of TEOS was added in the last three solutions as a complementary cross-linking agent. Dichloromethane (CH_2Cl_2) was the used solvent and TFA (95% in water) was used as catalyser for the ring-opening, hydrolysis and polycondensation reactions of the organosilanes. An acid catalyser was chosen over a basic one, since the hydrolysis reaction is favoured compared to the polycondensation reaction in less acidic medium, allowing for a more extended and less highly branched network structure [44]. This allowed for a more stable and less brittle coating layer on the stir bars. An illustration of sol–gel polymer network with the role of each reagent is presented in Fig. S-2 (supplementary).

3.2. Optimization of extraction conditions

All optimization experiments were carried out using the standard aqueous solution spiked with 0.2 μg for each steroidal hormone. Figures illustrating the influence of the different optimization parameters were made choosing two representative steroid hormones, i.e. MPROG for progestagens and E2 for estrogens, with the results for all steroid hormones shown in supplementary information (SI). The main objective for the optimization procedure was to evaluate the extraction recovery efficiencies that were determined by the area ratios of the absorbed quantity on the stir bars versus the same spiked quantity in a control solution in pure solvent. The effect of the different desorption solvents, MeOH, ACN and a mixture of MeOH and ACN on desorption efficiency was evaluated. The results showed negligible differences between the tested solvents on extraction recoveries. Therefore the MeOH/ACN (50/50, v/v) mixture was chosen as the desorption solvent, since this solvent mixture gave the optimum peak intensities (when compared to MeOH and ACN individually) when used as the deposition solvent for the analytical quantification using LDTD–APCI–MS/MS. According to the literature [14], the agitation speed may affect the SBSE extraction efficiency significantly. However, assays performed at 200, 250 and 300 rpm demonstrated that within the tested range, agitation speed had no influence on recovery value for our compounds with the prepared stir-bars. Consequently, a 250 rpm agitation speed was used in our work. The preliminary tests were performed for the four in-house stir bars (Table 1) and a commercial PDMS stir bar (Gerstel) using 20 mL of pure water spiked with the selected steroid hormones at 10 μg L^{-1}. The extraction and desorption time were 2 h and 20 min respectively. The extraction was carried out at room temperature and desorption was carried out in an ultrasonic bath at 35 °C. The desorption volume was 1 mL. As illustrated in Figs. 1 and S-3, the percent recoveries of the progestagens presented by MPROG and the estrogens presented by E2 increased in the presence of NaCl (0–10%) for all stir bars. The first two in-house stir bars showed good recoveries for the progestagens, except for norethindrone, where as the stir bars 3 and 4 more efficiently recovered both estrogens and progestagens which confirms the importance of β-CD for the retention of the estrogens when compared to OH-PDMS alone as a functional monomer for the preparation of the coating layer. The four in-house stir bars performed better than the commercial stir bar for all target hormone compounds. The pH of the extraction solution was examined to optimize the extraction efficiency. Since the sol–gel polymer was silica based, the pH range tested in solution should be between 2 and 8. Two pH values, 3 and 8, were tested in the same conditions for all stir bars but no improvement was observed (result not shown). This could be explained by the high pK_a of the target hormones (Supporting Information Table S-1) for which a pH of 3, 7 or 8 did not modify the steroidal hormone structures. The presence of an organic modifier (MeOH, 5% v/v), to enhance recoveries by limiting the absorption of the steroid hormones on

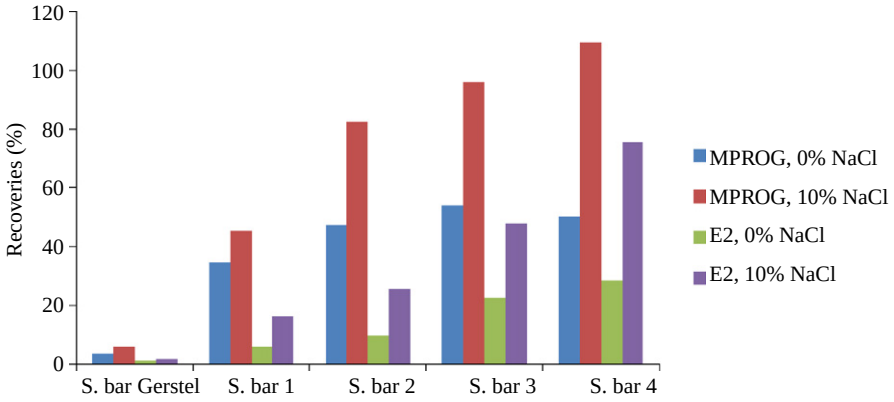

Fig. 1. Effect of NaCl addition on the recoveries of two hormones (MPROG and E2) for five stir bars at the 10 μg/L level by SBSE–LDTD/APCI–MS/MS: at 0% NaCl and 10% NaCl.

the glass vessel extraction container, in the extraction solution was not beneficial for the extraction procedure.

The last two stir bar protocols tested gave the best results for the initial extraction procedures. Therefore, all further optimization parameter discussed hereafter will focus on stir bars 3 and 4 in comparison to the commercially available one, all in pure HPLC-grade water.

3.2.1. The effect of ionic strength

The effect of salting out on the SBSE was examined with NaCl concentrations ranging from 20% to 30% (m/v), since previous results (Figs. 1 and S3) showed an improvement for recovery values from 0% to 10%. The results (Figs. 2 and S-4) indicated that the extraction efficiency for the eight steroid hormones was improved by increasing the amounts of salt. Therefore, the addition of 30% NaCl in the aqueous extraction solution was chosen for all experiments. The observed increase could be explained by the fact that water molecules form hydration spheres around the ionic salt molecules; this lowers the solubility of the steroid hormones in water, thereby driving the additional analytes into the sol–gel polymer.

3.2.2. The effect of sample volume

In order to increase the pre-concentration factor, the extraction volume was optimized. The effect of extraction volume in the range of 20–100 mL was examined while fixing the spiked quantity in solution at 2 μg for each steroid hormone. As expected (Figs. 3 and S-5), the recoveries decreased for all target compounds with increasing extraction volume. The extraction recoveries are proportional to the ratio of coated polymer volume to sample volume [13]. With a fixed volume of coated polymer on a stir bar, when the sample volume increases, this ratio will be decreased; consequently, the recoveries obtained will be reduced. The recoveries obtained from stir bar 4 for an extraction volume of 50 mL were over 64% for all steroid hormones, except for E3 at 2%, with similar results for stir bar 3, except for E2 and E3 with recoveries of 53% and 1%, respectively. The low recovery values for E3 could be attributed to its higher polarity, with the lowest log K_{ow} value (2.81, Table S-1) of all the studied hormones, and therefore reduced affinity with the coating material. A previous study [20] has shown the influence of the hydroxyl groups on estrogens, in particular for E2 and EE2, on the low recovery values which increased significantly when a derivatization method was applied. Estriol (E3) is the only hormone, of those studied, to have three hydroxyl groups in its structure. The stir bar 4 gave better results than stir bar 3 for the majority of estrogens and specifically norethindrone. The Gerstel stir bar showed low recoveries ($< 25\%$) for all steroid hormones for an extraction volume of 50 mL. A final extraction volume of 50 mL was therefore chosen although the recoveries obtained from this extraction volume were smaller than those obtained using 20 mL but it still resulted in a higher pre-concentration factor. These results were in accordance with previous work done on steroid hormones with different SBSE methods [9,10].

3.2.3. The effect of extraction time

The extraction time is an important parameter when applying SBSE. Initial experiments were performed on samples spiked with 0.2 μg steroid hormones to determine the recoveries as a function

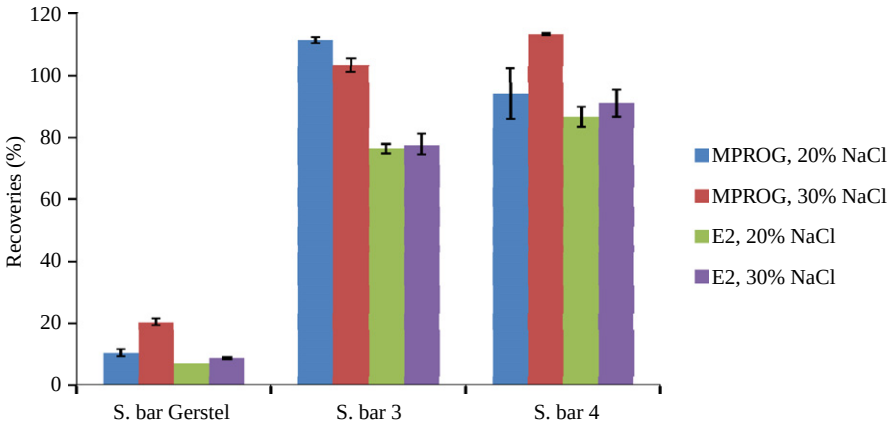

Fig. 2. Effect of NaCl addition on the recoveries of two hormones for three stir bars at the 10 μg/L level by SBSE–LDTD/APCI–MS/MS: at 20% NaCl and 30% NaCl.

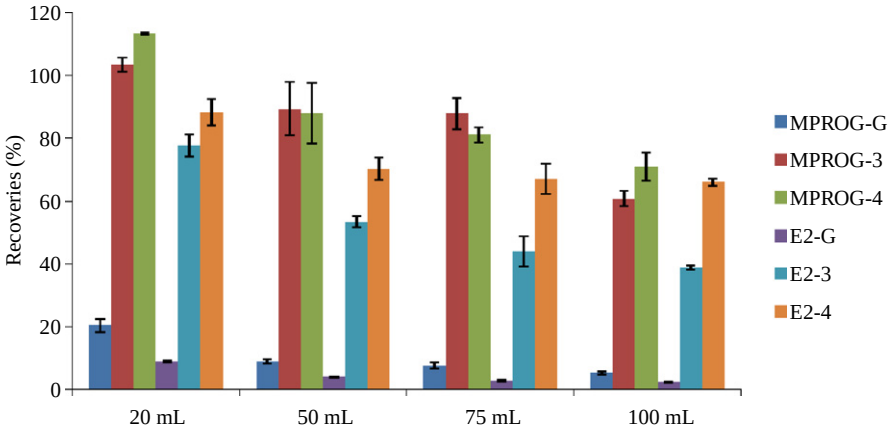

Fig. 3. Effect of the extraction volumes on the recoveries of two hormones spiked with 0.2 μg and analyzed by SBSE–LDTD/APCI–MS/MS from three stir bars. MPROG-G: MPROG from Gerstel; MPROG-3: MPROG from stir bar 3; MPROG-4: MPROG from stir bar 4; E2-G: E2 from Gerstel; E2-3: E2 from stir bar 3 and E2-4: E2 from stir bar 4.

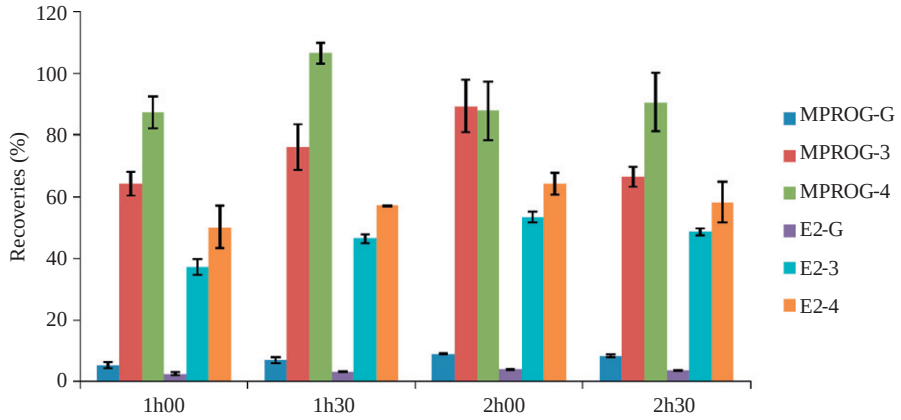

Fig. 4. Effect of the extraction time on the recoveries of two hormones spiked with 0.2 μg and analyzed by SBSE–LDTD/APCI–MS/MS from three stir bars.

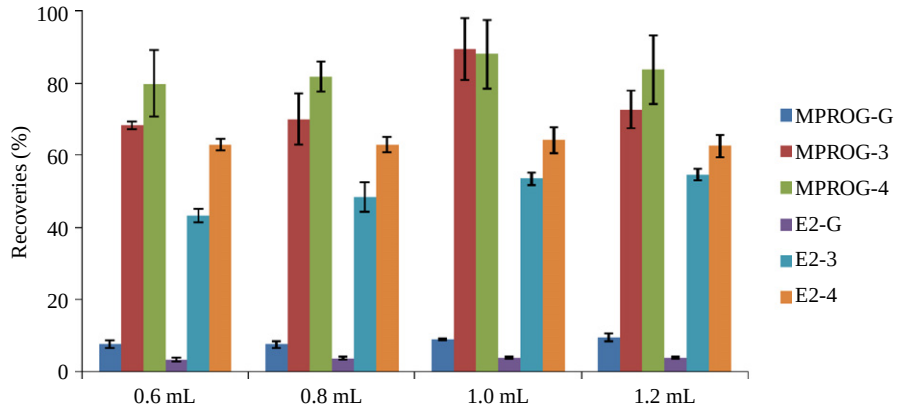

Fig. 5. Effect of the desorption volume on the recoveries of two hormones spiked with 0.2 μg by SBSE–LDTD/APCI–MS/MS from three stir bars.

of time. The extraction time recoveries were evaluated with stirring time from 1 h to 2.5 h with 30 min intervals on the 50 mL sample, as illustrated in Figs. 4 and S-6. The recovery values of the eight steroid hormones extracted by the three stir bars increased with longer extraction time between 1 h and 2 h. Longer enrichment time showed no improvement in recovery values which did not increase between 2 h and 2.5 h (Fig. 4). Therefore, an extraction time of 2 h was selected in this work.

3.2.4. *The effect of desorption volume and time*

The impact of desorption volume by MeOH:ACN (50:50 v/v) on extraction recoveries was investigated in a range from 0.6 mL to 1.2 mL. A minimum volume of 0.6 mL was used because it allowed for complete coverage of the stir bars in the glass tube, especially for the in-house stir bars, allowing for better recovery values. The results showed that the effect of desorption volume on recoveries was negligible for the commercial stir bar (Fig. 5). The same results were observed for the two in-house stir bars, except for E2 and EE2 for which the recoveries increased with a desorption volume of 1 mL (Fig. S-7b and c) although they had the lowest recovery values among all the compounds while E3 did not adsorb on any stir bar. Consequently, 1 mL was chosen as the optimum desorption volume for the SBSE procedure.

Various desorption times, between 15 min and 30 min, were tested to evaluate their influence on recovery values (Fig. 6). It was found that 20 min was enough for the desorption of all compounds from the stir bars. The fast desorption time obtained could result from the use of sonification combined with temperature (35 °C). A blank of SBSE procedures was also verified and no carry over was observed. A second desorption procedure was carried out on the stir bar following the initial desorption step and no peaks were observed when analyzed by MS/MS, which meant that the steroid hormones were fully desorbed under the optimized conditions.

3.3. *Reproducibility and lifetime of PDMS/PTMS/β-cyclodextrin coated stir bars*

The reproducibility and lifetime of PDMS/PTMS/β-CD coated stir bars were investigated using 4 μg L^{-1} of target steroid hormones in aqueous solution and applying the optimized extraction procedure. The PDMS commercial stir bar was examined in parallel under the same conditions. To evaluate reproducibility, four of each in-house stir bar (3 and 4) prepared using the polymer synthesis protocols (Table 1) as well as four different commercially available stir bars were evaluated in triplicate ($n=3$). The results (Figs. 7, S-9 and Supporting information Table S-2) show good reproducibility within batches with RSDs ranging from 0.5% to 16% for all hormones, but also between batches with RSDs between 5.3% and 16%. The only exception was E3 with poor RSDs within and between batches with values of 75% and 74%, respectively. The higher recoveries obtained for the estrogens with stir bar 4, in particular for E1, demonstrated the advantage of using DMSO to increase the solubility of β-CD to improve the coating layer selectivity by increasing its polarity, as discussed previously.

To evaluate the lifetime of both PDMS/PTMS/β-CD coated stir bars with and without DMSO and the PDMS commercial stir bar, 53 successive extractions were performed for all three stir bars from within batch C (Figs. 8 and S-10). The RSD of the mean recovery values of the target analytes after multiple extractions was evaluated to determine whether the stir bar could be reused without affecting their performance. The recoveries shown in Fig. 8 are the mean of three consecutive extractions. For the three tested stir bars,

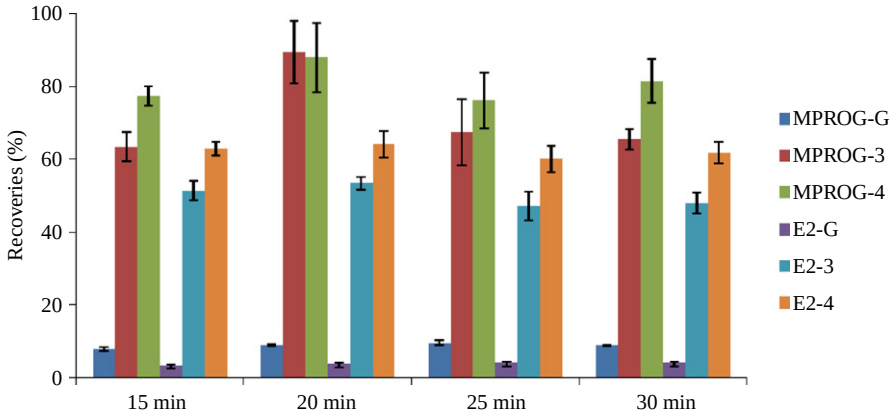

Fig. 6. Effect of the desorption time on the recoveries of two hormones spiked with 0.2 μg and analyzed by SBSE–LDTD/APCI–MS/MS from three stir bars.

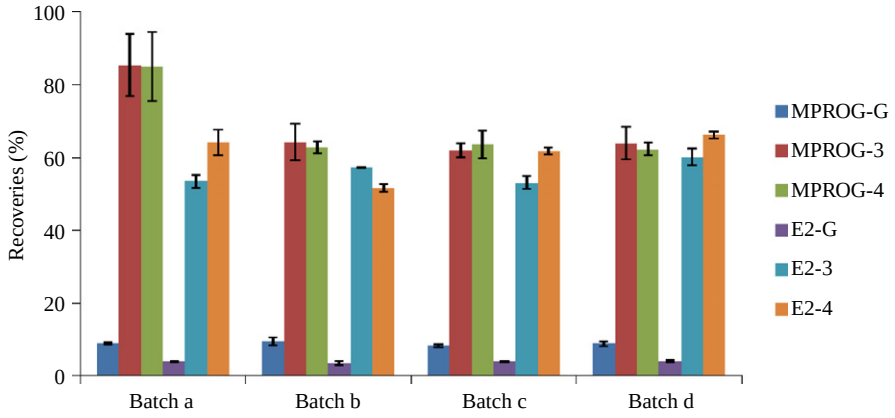

Fig. 7. Inter-batch reproducibility of three stir bars on the extraction of two hormones spiked 0.2 μg and analyzed by SBSE–LDTD/APCI–MS/MS.

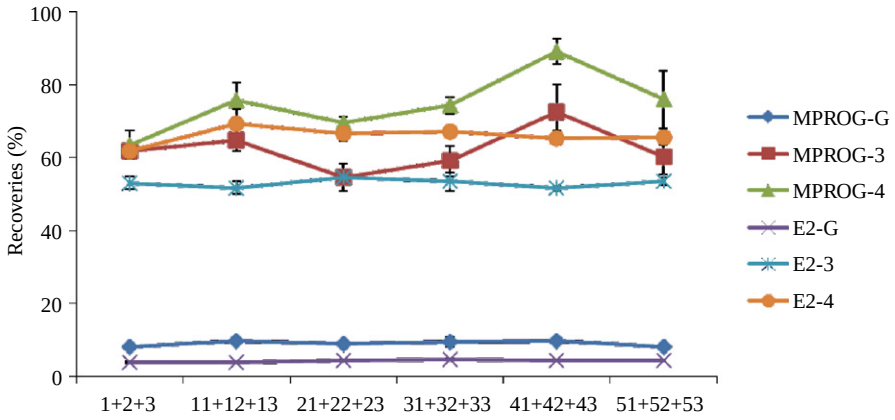

Fig. 8. Lifetime of three stir bars from batch C for 53 extractions for two hormones (each point is a mean of three consecutive extractions).

the RSDs were below 15% which was established as an acceptable response (Supporting information Table S-3). However, the RSDs of E3 were high (122%, 21% and 21% for Gerstel, 3C and 4C stir bars respectively) mostly because the stationary phase of those stir bar show a low affinity towards this relatively polar hormone. It was found that the in-house and the commercial stir bars could be used for at least 50 extractions without losing their efficiency.

3.4. *Application of PDMS/PTMS/β-cyclodextrin coated stir bars to tap water and wastewater samples*

Once optimized, the procedure was tested on real water samples, including tap water and raw sewage from a wastewater treatment plant, to demonstrate its applicability to environmentally-relevant matrices. The recovery values of the steroidal hormones (Fig. 9 and in Supporting Information Table S-4) in tap water (Fig. 9a) and in affluent wastewater extractions (Fig. 9b) were slightly lower to those obtained in pure water. This was explained by to the competition for binding sites on the stir bars between steroidal hormones and the interfering components present in the real sample matrices.

The method detection limits (MDL) of SBSE–LDTD–APCI–MS/MS for the three stir bars in the real environmental samples and for pure water are shown in Table 2. The MDLs obtained in real samples were slightly higher to the detection limits obtained in pure water because of matrix effects. The MDLs ranged from 0.1 to 0.3 $\mu g\,L^{-1}$ and from 0.5 to 4.8 $\mu g\,L^{-1}$ for the in-house and Gerstel stir bars, respectively. The MDLs for E3 were very high, with 480 $\mu g\,L^{-1}$ for the Gerstel stir bar, 80 $\mu g\,L^{-1}$ for stir bar 3 and

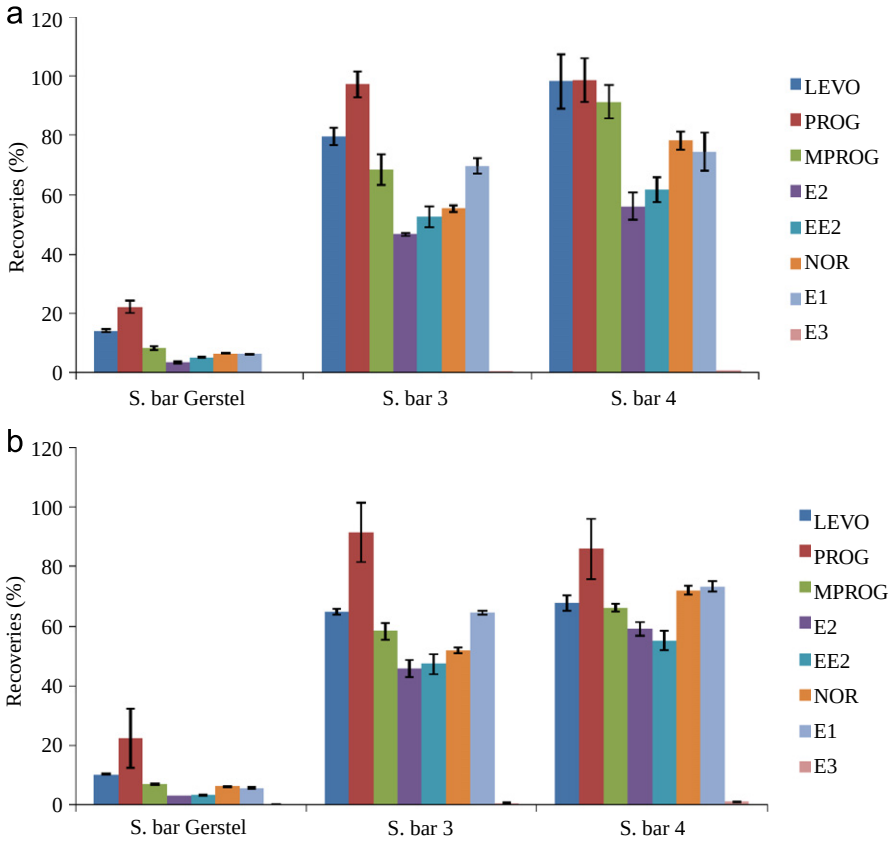

Fig. 9. Extraction recoveries (%) of eight hormones in real matrixes: (a) in tap water and (b) in wastewater.

Table 2
Method detection limits (MDL, μg/L) of SBSE–LDTD–APCI–MS/MS for three stir bars in three different matrices: Milli-Q water, tap water and affluent wastewater.

Hormones	Gerstel			Stir bar 3			Stir bar 4		
	Water mQ	Tap water	Wastewater	Water mQ	Tap water	Wastewater	Water mQ	Tap water	Wastewater
LEVO	1.6	1.6	2.1	0.2	0.3	0.3	0.2	0.2	0.3
PROG	0.5	0.5	0.5	0.1	0.1	0.1	0.1	0.1	0.1
MPROG	1.3	1.5	1.7	0.1	0.2	0.2	0.1	0.1	0.2
E2	2.6	2.9	3.2	0.2	0.2	0.2	0.2	0.2	0.2
EE2	2.8	3.1	4.8	0.2	0.3	0.3	0.2	0.3	0.3
NOR	2.8	2.8	3.0	0.3	0.3	0.3	0.2	0.2	0.3
E1	2.2	2.9	3.2	0.2	0.3	0.3	0.2	0.2	0.2
E3	160	480	480	48	80	80	25	48	48

48 μg L^{-1} for stir bar 4. Consequently, the home made stir bars can be applied to analyze samples with concentrations up to 16 folds smaller than if using the commercially available Gerstel stir bar.

3.5. *Performance of new stationary phase for hormones extraction in aqueous solutions*

In order to evaluate the performance of the new stationary phase versus the PDMS commercial phase, an in house stir bars with the same dimension as a commercial Gerstel stir bar was made. The optimized extraction protocol previously described was applied for both stir bars; the Gerstel stir bar and stir bar 5. However, as the volume of in house stir bar was greatly reduced, some extraction and desorption parameters were modified. The sample volume was adjusted to 5 mL, the hormones were spiked with 10 ug/L of the mixed hormone solution and the desorption volume was 200 μL. The results showed that stir bar 5 gave higher recoveries for the majority of studied hormones, especially for the estrogens (Fig. 10). As expected, the difference in the recoveries between the Gerstel stir bar and the in house stir bar was reduced, which demonstrates that the stir bar dimensions (surface and volume of coated polymer layer) play an important role when evaluating stir bar sorptive extraction.

4. Conclusion

This is the first application of a combination of stir bar sorptive extraction and laser diode thermal desorption coupled to atmospheric pressure chemical ionization and tandem mass spectrometry (SBSE–LDTD–APCI–MS/MS) for the quantification of eight selected steroid hormones in different environmentally-relevant water matrices.

A novel sol–gel coated polydimethylsiloxane/phenyltrimethylsiloxane/β-cyclodextrin in-house stir bar (stir bars 3 and 4) protocol has been developed to efficiently extract the selected steroid hormones. It has been shown that equilibrium time, ionic strength and extraction volume were the most important parameters affecting the stir bar recoveries for the tested hormones in aqueous solution. The

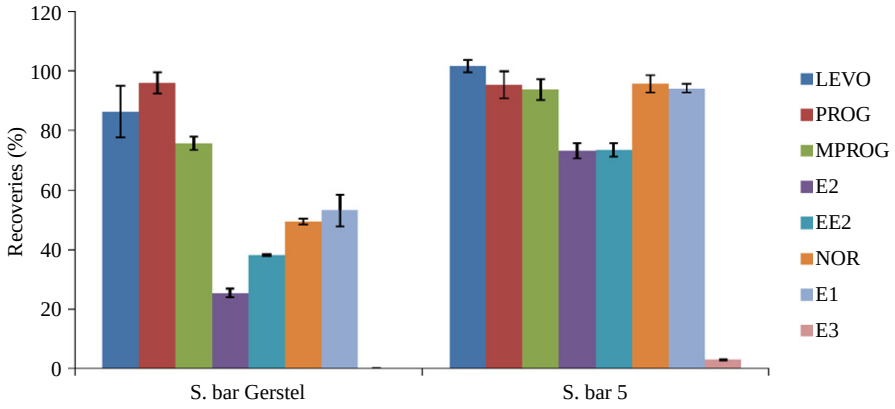

Fig. 10. Extraction recoveries (%) of eight hormones in pure water with Gerstel stir bar and stir bar 5 (modified Gerstel stir bar with in-house coating).

recoveries for all hormones in pure water for a commercially available PDMS stir bar ranged from 3.8% to 23.9%, except for E3 with values under 1%. The in-house stir bars gave recovery values from 60% to 96%, except for E3 with a recovery below 2%. The recoveries obtained in real matrixes including tap water and affluent wastewater ranged from 3.1% to 22.4% (E3 at 0%), from 46.0% to 97.3% (E3 at 0.4%), from 55.2% to 98.5% (E3 at 0.7%) for the PDMS stir bar, stir bar 3 and stir bar 4, respectively. The in-house stir bars were re-usable and gave reproducible results without affecting their performance for at least over 50 extractions before having to be replaced. SBSE–LDTD–APCI–MS/MS was shown to be a simple, rapid, reliable and sensitive method requiring a low sample volume for the analysis of steroid hormones. The MDLs obtained ranged from 0.1 to 0.3 μg/L for 7 hormones; albeit such MDLs are not sufficient for the analysis of the traces of hormones commonly found in the environment, this method would still be applicable for the analysis of contaminated samples such as raw wastewaters, sewage sludge or manure and other agricultural by-products [18,42]. This method would be especially useful for the treatment optimization studies [45,46] where a factorial setup is common and having access to an ultrafast simplified analysis is most appreciated. The time required for the SBSE extraction is approximately 2 h, but a large number of samples can be extracted simultaneously and further analysis on the MS instrument takes only seconds (in many academic settings, access time to high end MS instrumentation is limited).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.09.036.

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