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Adsorptive micro-extraction techniques—Novel analytical tools for trace levels of polar solutes in aqueous media

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ABSTRACT

A novel enrichment technique, adsorptive μ-extraction (AμE), is proposed for trace analysis of polar solutes in aqueous media. The preparation, stability tests and development of the analytical devices using two geometrical configurations, *i.e.* bar adsorptive μ-extraction (BAμE) and multi-spheres adsorptive μ-extraction (MSAμE) is fully discussed. From the several sorbent materials tested, activated carbons and polystyrene divinylbenzene phases demonstrated the best stability, robustness and to be the most suitable for analytical purposes. The application of both BAμE and MSAμE devices proved remarkable performance for the determination of trace levels of polar solutes and metabolites (*e.g.* pesticides, disinfection by-products, drugs of abuse and pharmaceuticals) in water matrices and biological fluids. By comparing AμE techniques with stir bar sorptive extraction based on polydimethylsiloxane phase, great effectiveness is attained overcoming the limitations of the latter enrichment approach regarding the more polar solutes. Furthermore, convenient sensitivity and selectivity is reached through AμE techniques, since the great advantage of this new analytical technology is the possibility to choose the most suitable sorbent to each particular type of application. The enrichment techniques proposed are cost-effective, easy to prepare and work-up, demonstrating robustness and to be a remarkable analytical tool for trace analysis of priority solutes in areas of recognized importance such as environment, forensic and other related life sciences.

1. Introduction

In the last years, modern sample preparation approaches in analytical chemistry are characterized by simplification, miniaturization and high-throughput to enhance selectivity and sensitivity in particular for trace analysis [1]. Nowadays, sorptive extraction techniques have been extensively developed and applied to monitor many classes of organic solutes in several types of matrices [2,3]. For instance, solid phase extraction, solid phase micro-extraction (SPME) and more recently, stir bar sorptive extraction (SBSE) are some of the most widely used sorptive enrichment approaches prior to chromatographic analysis [4–6]. The latter one in particular, was introduced as a novel sample preparation method based on the same principles of SPME, which has been successfully applied to screen traces of priority organic pollutants in water and many other matrices [7–11]. In SBSE, the stir bars are typically coated with 24–126 μL of polydimethylsiloxane (PDMS), a nonpolar polymeric phase, presenting a substantial higher amount than on a SPME fiber usually with a maximum volume of 0.5 μL (100 μm film thickness). The lower phase ratio between the extraction medium and the sample provides an increasing capacity, and much higher recoveries can be reached by SBSE especially for nonpolar solutes. Consequently, this recent approach enables to increase the sensitivity by a factor within 50 and 250, decreasing the detection limits at the ultra-trace level. In the SBSE theory [7], the efficiency of the analyte partitioning between the polymeric phase of the stir bar and the water sample present a similar behavior as the distribution described by the octanol–water partition coefficients ($K_{PDMS/W} \approx K_{O/W}$) during the static equilibrium. Therefore, the $K_{O/W}$ and the phase ratio β ($=V_W/V_{SBSE}$, in which V_W is the volume of the water sample and V_{SBSE} is the PDMS volume), are important parameters to predict the theoretical recovery. In general, SBSE has been currently applied in association with gas and liquid chromatography as well as hyphenated techniques, using both thermal and liquid desorption modes [8,9]. In the meantime, if we focus our attention just on solutes with polar characteristics, *i.e.* $\log K_{O/W} < 3$, some of the most used sorptive extraction techniques present, in many cases, great limitations on the enrichment efficiency even by using convenient derivatization steps or polymeric phases (*e.g.* polyurethanes), as recently demonstrated [12–15].

So far, it is well known that polar solutes are easily adsorbed on specific solid materials having porous structure with suitable active sites, where the electrostatic and/or dispersive phenomena ("adsorption–desorption" properties) take place [16]. In powder

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form, the large specific areas exhibited (≈1000 $m^2 g^{-1}$) by these materials (*e.g.* activated carbons, ACs) present a remarkable adsorptive capacity (≈100–500 $\mu g\, mg^{-1}$), just depending on pH_{pzc} (pH at the point of zero charge) and texture [17]. For trace analysis in particular, we are definitely below the isothermal plateau (saturation of the sorbent) and therefore, the *Langmuir* and *Freundlich* theoretical considerations are not applicable [18]. Meanwhile, from the experimental point of view, it is very difficult to manipulate solids in powder form after the enrichment process, once we are dealing with strongly divided materials composed by microscopic particles (<30 μm). However, if these materials could be fixed to convenient substrates without losing the textural and surface chemical properties, would be a breakthrough of great importance for trace analysis. Recently, our group has been involved on the development of novel adsorptive micro-extraction (AμE) techniques, which could represent a great alternative to monitor a wide range of polar solutes in real matrices. The new AμE approaches can be applied through small analytical devices presenting appropriate geometry, where specific sorbents are easily supported through "sticking-based technologies". Since most of the polar solutes are non-volatile and some of them present thermolabile properties, liquid desorption (LD) followed by high performance liquid chromatography are definitely the subsequent combination of choice for analytical purposes.

In this contribution, we propose for the first time, AμE techniques as novel analytical tools for the enrichment of trace levels of polar solutes in aqueous media, using suitable sorbent phases. The description of the preparation, tests, development and the assessment of several applications will be discussed in detail using two geometrical configurations, *i.e.* bar adsorptive micro-extraction (BAμE) and multi-spheres adsorptive micro-extraction (MSAμE). The analytical performance and advantages of these approaches will be evaluated as new enrichment techniques to monitor trace levels of several classes of polar solutes and metabolites in matrices from areas of recognized importance such as environment, forensic and other related life sciences. The comparison of the sensitivity and selectivity obtained by the proposed analytical approaches with stir bar sorptive extraction based on polydimethylsiloxane polymeric phase (SBSE(PDMS)) is also addressed.

2. Experimental

2.1. Chemicals and samples

All reagents and solvents were of analytical grade and used without further purification. HPLC-grade methanol (MeOH, 99.9%) and acetonitrile (ACN, 99.9%) were purchased from Merck (Germany). Ultra-pure water was obtained from Milli-Q water purification systems (Millipore, USA). High-grade acetone (99.5%) dichloromethane (DCM, 99.8%) and ethyl acetate (99.5%) were obtained from Panreac (Spain). Hydrochloric acid (HCl, 37%), *n*-pentane (99%), ethanol (99.8%) and acetic acid (99.8%) were obtained from Riedel-de Haën (Germany). *n*-Hexane (99.5%) was purchased from Fluka (Germany). Isopropanol (99.9%) was obtained from Fisher Scientific (UK). Formic acid (99%) was purchased from Merck (Germany). Sodium hydroxide (NaOH, 98.0%) was obtained from AnalaR BDH Chemicals (UK). Acetaminophen (98.0%), caffeine (99.0%), D-(−)-norgestrel (99.0%), estriol (97.0%), progesterone (98.0%), estrone (99.0%), 17β-estradiol, trimethoprim (98.0%), sulphamethoxazole (99.0%) and sulphathiazole (98.0%) were supplied from Sigma–Aldrich (Steinheim, Germany). Sulphadimethoxine (99.0%) and enrofloxacin (≥98.0%) were provided from Fluka (Buchs, Switzerland). Diethylstilbestrol (99.5%) and 19-norethisterone (98.5%) were supplied from Riedel-de Haën (Seelze, Germany). Simazine (99.9%) and atrazine (99.2%) were purchased from Supelco (USA). Ibuprofen was synthesized by Shasun Chemicals and Drugs Ltd. (lot IBU0307598, India). Morphine and codeine in methanolic solutions were supplied by "Instituto de Desporto de Portugal" (Lisbon, Portugal). Propanal (97%), *trans*-2-hexenal (98%) and pentafluorophenyl hydrazine (PFPH, 96%) were purchased from Alfa Aesar (Germany). Formaldehyde (37%), acetaldehyde (99%) and butanone (99.5%) were purchased from Merck (Germany). The sorbents tested were commercial AC powders (Riedel-de Haën and Salmon & Cia), octadecylsilane and octylsilane (Supelco), zeolites (Aldrich), laponite (Laporte), ionic exchange resins (Biorad) and titanium dioxide (Degussa). Magnesium silicate, polystyrene divinylbenzene co-polymer (PS-DVB), silica, alumina, SCX (strong cation exchange), granular AC, zinc oxide and copper oxide were supplied from Merck. The OASIS HLB, MAX (mixed mode anion exchange and reverse phase), MCX (mixed mode cation exchange and reverse phase), WAX (weak anion exchange) and WCX (weak cation exchange) sorbents were provided by Waters. The ACs from cork were home-made prepared [19]. Surface water samples were obtained from the metropolitan area of Lisbon (Portugal). Urine samples were collected in the morning from a healthy 27 years old woman and 29 years old man. One of the samples was collected after consumption of Brufen® 600 (two doses, one per night) and the other sample was obtained without consumption of any kind of pharmaceuticals by the individual for control purposes. All samples were previously filtered (No. 1 filters, Whatman) and stored refrigerated at 4 °C until their analysis.

2.2. Experimental set-up

2.2.1. Preparation of the μ-extraction devices

The μ-extraction devices were prepared "in-house" through "sticking technology" using adhesive and thermal fixation of the sorbents. For the bar configuration, the μ-extraction devices were prepared by coating polyethylene hollow cylindrical tubes (15 mm length and 3 mm diameter) with adhesive films, followed by covering it with powdered sorbents inside a flask through manual shaking. For the multi-spheres configuration, the μ-extraction devices were prepared by coating 5–10 polystyrene spheres, attached in a thread, with powdered sorbents followed by thermal treatment in a muffle furnace (Raypa—drying oven: 160 °C) for 2 h, where final spheres with 2 mm diameter in average were obtained. The amount of material fixed depends on the sorbent and the configuration involved, generally in between 1.0 and 5.0 mg. Before use, both μ-extraction devices were previously cleaned through sonification treatment using appropriate organic solvents or ultra-pure water depending on the sorbent selected. The detailed description of both devices manufacturing can already be consulted [20,21].

2.2.2. Robustness and stability tests

To evaluate the robustness and stability of both μ-extraction devices, several tests using different solvents, temperature and pH values were previously assayed before use. The solvent tests were performed by immerging the μ-extraction devices in the most common solvents used for back-extraction, such as MeOH, ACN, mixtures of both (1:1), ethanol, isopropanol, acetone, ethyl acetate, *n*-pentane, *n*-hexane, DCM, formic and acetic acid for 60 min under sonification treatment (Brandson 3510). For the temperature tests, experiments were carried out as before by using ultra-pure water to submerge the μ-extraction devices under sonification for 3 h with temperatures ranging from 20 to 50 °C (seven points with 5 °C of variation). The pH tests (Metrohm 744 pH meter) were carried out making 3 h of extractions in ultra-pure water with the desired value (1 < pH < 14, 14 points) and adjusting by adding 5% HCl or 0.1 M NaOH. After each extraction process, the μ-extraction devices were transferred to a vial filled with ultra-

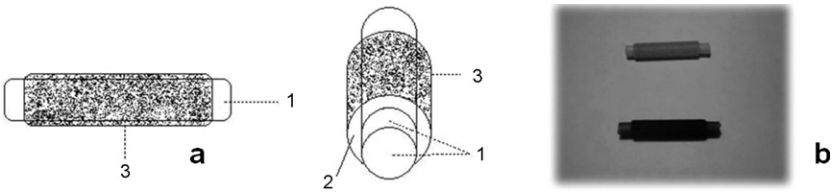

Fig. 1. Schematic representation (a) and images (b) of the BAμE device proposed in the present work [20]. 1: Polypropylene supporting bar; 2: adhesive film; 3: sorbent phase.

pure water for clean-up and taken for sonification treatment during 1 h.

2.2.3. Recovery, application and comparison assays

In a typical assay, 30 mL of ultra-pure water spiked with working standards at the desired concentration, a μ-extraction device and a conventional Teflon magnetic bar were introduced into glass flasks (30 mL, Germany). For the optimization purposes assays were performed in a multi-point agitation plate (Variomag H+P Labortechnik AG Multipoint 15, Germany) at room temperature (25 °C). Parameters such as extraction time, agitation speed, pH, organic modifier and ionic strength were systematically studied in triplicate. For back-extraction, the μ-extraction devices were removed with clean tweezers and placed into 2 mL vials containing the stripping solvent, ensuring their total immersion prior to sonification treatment at constant temperature (25 °C). To evaluate the best LD conditions, assays were performed in triplicate by using several solvents under different desorption periods. After back-extraction, the μ-extraction devices were removed with clean tweezers, the stripping solvent was evaporated to dryness under a gentle stream of nitrogen (>99.5%) followed by reconstitution with 200 μL of a suitable eluent. The vials were then sealed and placed on the auto-sampler for high performance liquid chromatography–diode array detection (HPLC–DAD) analysis. For biological assays, 1 mL of urine was diluted to 30 mL with ultra-pure water, being performed under optimized conditions. Blank assays were also carried out using the procedure above described without spiking. For comparison purposes with other sorptive extraction techniques, assays under similar optimized conditions were performed with commercial stir bars (Twister, Gerstel, Germany) coated with PDMS (20 mm length and 0.5 mm film thickness, 47 μL and 20 mm length and 1.0 mm film thickness, 126 μL). Before use, the stir bars were cleaned by treatment with ACN.

2.3. HPLC–DAD settings

The analyses were carried out on an Agilent 1100 Series LC system (Agilent Technologies, Germany), constituted by the following modules: vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostated column compartment (G1316A) and diode array detector (G1315B). The data acquisition and instrumental control were performed by the software LC3D ChemStation (version Rev.A.10.02[1757], Agilent Technologies). Analyses were performed on a Tracer excel 120 ODS-A column, 150 mm × 4.0 mm, 5 μm particle size or Mediterranean Sea 18 column, 150 mm × 2.1 mm, 5 μm particle size (Teknokroma, Spain). The mobile phase consists on several eluent compositions (MeOH, ACN, water with 0.1% H_3PO_4 or formic acid) operating in isocratic and gradient conditions depending on the type of application with a flow of 1.0 mL min^{-1}. The injection volume was 20 μL with a draw speed of 200 μL min^{-1}. Several wavelengths were selected: 220 nm (morphine, codeine and ibuprofen); 205 nm (caffeine and acetaminophen), 225 and 260 nm (antibiotics); 220, 226 and 252 nm (disinfection by-products); 205 and 240 nm (hormones). For identification purposes, standard addition was used by spiking the samples with pure standards, as well as by comparing the retention parameters and peak purity with the UV–vis spectral reference database. For recovery calculations, peak areas obtained from each assay were compared with the peak areas of standard controls used for spiking. For quantification purposes on real matrices, calibration plots using the standard addition methodology (SAM) were also performed.

2.4. SEM analysis

The morphologic characterization of the μ-extraction devices was performed by field emission scanning electron microscopy (FE-SEM; JEOL, Model JSM-7001F). Samples were previously coated with gold.

3. Results and discussion

3.1. Preparation and test of the μ-extraction devices

Since the very beginning, our intention was the development of new analytical tools that could overcome the main limitations of SBSE(PDMS) concerning the μ-extraction of the more polar solutes from aqueous media. Therefore, AμE techniques were developed with this purpose, as novel environmental friendly sample preparation technologies mainly devoted for the enrichment of trace levels of polar compounds prior to the combination with convenient analytical separation techniques (*e.g.* HPLC).

In a first approach, the μ-extraction devices were prepared using sticking-based technologies and suitable powder sorbents, where two geometrical configurations were designed at this stage, namely, through bar (BAμE) and multi-sphere (MSAμE) substrates. Figs. 1 and 2 depict schematic representations of both BAμE and MSAμE devices and the corresponding images, exemplifying final μ-collectors supported with AC and PS-DVB sorbent phases. In bar μ-extraction devices, the sorbent phases were fixed with adhesive films on polypropylene hollow cylindrical substrates (adhesive supporting), whereas in multi-sphere μ-extraction, the devices cover initially the polystyrene spherical substrates followed by fixation through thermal treatment (thermal supporting). Fig. 2c depicts a SEM micrograph of a MSAμE device coated with AC powder, where it can be observed, in a qualitative way, some surface characteristics of the sorbent phase after thermal treatment, in which the grains are fixed to the substrate device in a very homogeneous way.

During the preparation of both devices, several different sorbent phases were tested, including ACs, PS-DVB, silanes, alumina, silica, metal oxides, zeolites, magnesium silicate, ionic exchange resins, among other polymers and solids (Table 1), which are known to present strong sorptive properties. However, for the thermal supporting in particular, only ACs and PS-DVB sorbent phases were tested to prepare the multi-sphere μ-extraction devices.

Before being applied to particular samples, the μ-extraction devices must be evaluated in terms of stability and robustness of the fixation involved through appropriate physico-chemical tests.

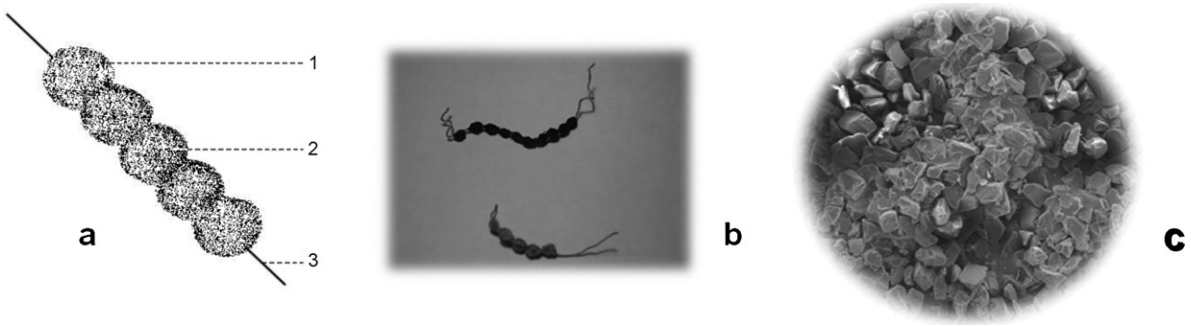

Fig. 2. Schematic representation (a), images (b) and a FE-SEM micrograph (c, 35×) of the MSAμE device proposed in the present work [21]. 1: Polystyrene supporting spheres; 2: sorbent phase; 3: thread.

Thus, the supported μ-collectors were submitted to several organic solvents having different polarity characteristics (*n*-pentane, *n*-hexane, DCM, acetone, ethyl acetate, MeOH, ethanol, isopropanol, ACN, formic and acetic acids), pHs, temperatures, mechanical treatments as well as regeneration tests. As expected and unlike BAμE devices, initial assays showed that MSAμE devices presented a much better stability behavior regarding the fixation of the different powdered materials, once the thermal supporting treatment is much more effective.

Preliminary stability tests were performed to evaluate the behavior of the adhesive supporting films on the μ-extraction bar devices, regarding the interaction with different organic solvents and therefore, the occurrence of possible desegregation phenomena of the sorbent phases. From the assessment made, the μ-extraction bars supported with different powdered materials presented, in general, good stability in almost all the solvents studied, with the exception of DCM, acetone, ethyl acetate, formic and acetic acids. On the other hand, the μ-extraction multi-spheres supported with ACs, presented remarkable stability in almost of the solvents studied, with the exception of DCM, acetone, ethyl acetate and ACN. Nevertheless, when supported with PS-DVB phases, much better stability in MeOH, ethanol, isopropanol, formic and acetic acids was achieved. Regarding the temperature tests performed on the μ-extraction bar devices, the sorbent phases stayed fixed and stable in the substrates until 40 °C, whereas above this value, they start to desegregate from the polypropylene adhesive supporting films. For the μ-extraction multi-sphere devices in particular, the desegregation phenomena promoted by higher temperatures do not occur, once the sorbent phases become much better fixed when thermal supporting treatment was adopted. Concerning the pH tests, the data obtained showed excellent stability for values ranging from 2 to 12. However, for extreme acidic and basic media (pH < 2 and pH > 12), the sorbent phases fixed in the μ-extraction bars showed a tendency to desegregate from the polypropylene adhesive supporting films. These observations can probably be explained because at extreme pH or temperature, the adhesive tends to degrade and therefore, the sorbent phases cannot hold to the polypropylene supporting films anymore. In opposition, the μ-extraction multi-sphere devices present much better stability for all pH range studied since in this case the thermal supporting promotes much higher robustness from the fixation point of view. Mechanical tests were also performed under sonification and stirring treatment in particular for the μ-extraction bar devices. From the data observed in the sonification tests, it should be pointed out that after more than 3 h the sorbent phases start to desegregate from the polypropylene adhesive supporting films. This observation seems to be explained mainly because the raise of temperature generated by this treatment, promotes the desegregation phenomena as stated before. Concerning the stirring tests, good stability was observed for assays performed for several hours. Subsequently, re-utilization tests were also assessed, where it was observed that both μ-extraction devices can be re-used several times depending

Table 1
Summary of the preliminary average recovery yields and physico-chemical stability tests performed on the BAμE devices with different sorbent phases.

Sorbents	Average recovery yields (%)[a]	Chemical and mechanical stability tests on BAμE			
		Solvents	Temperature	pH	Sonification
Commercial AC powder	80	• Sorbents supported are stable in MeOH, ACN and mixtures (1:1), ethanol, isopropanol, *n*-pentane and *n*-hexane • Sorbents supported disperses in DCM, acetone, ethyl acetate, formic and acetic acids	• Stable in between 20 and 40 °C • Disperses above 40 °C	• Stable in between 2 and 12 • For pH < 2 or pH > 12, the adhesive dissolves and the sorbents disperse in aqueous media	• Stable until 3 h • After 3 h the sorbents disperse in the solvent
PS-DVB	78				
OASIS HLB	75				
Granular AC	50				
Octadecylsilane	37				
Octylsilane	30				
Silica	20				
Alumina	20				
Magnesium silicate	7				
SCX	5				
MAX	5				
MCX	5				
WAX	–				
WCX	–				
Zeolites	–				
Laponite	–				
Ionic exchange resins	–				
Copper oxide	–				
Titanium dioxide	–				
Zinc oxide	–				

[a] Model system: target: atrazine; extraction conditions: 25 mL of spiked (10 μg L^{-1}) ultra-pure water for 3 h (1000 rpm); back-extraction conditions: MeOH or ACN (60 min).

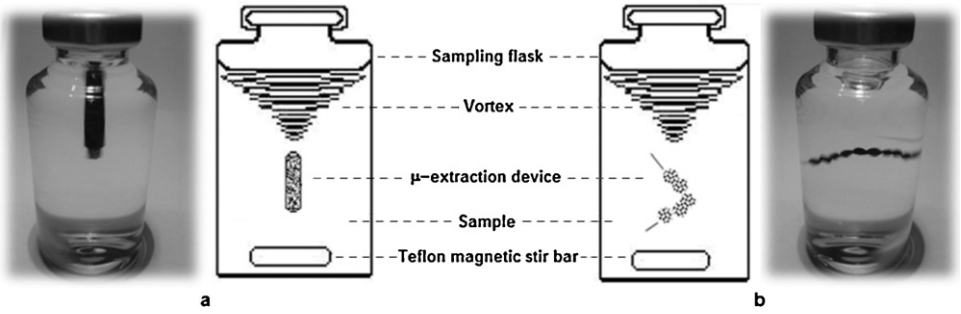

Fig. 3. Schematic representation and images of BAμE (a) and MSAμE (b) during the μ-extraction process [20,21].

on the type of sample matrix, solutes and experimental conditions, as well as on the sorbent phases involved. In general, it was clearly observed that if the sorbent phases are based on ACs, the μ-extraction devices can be used more times because this type of materials presents a much stronger fixation stability and mechanical robustness when compared with other solids or sorbent powdered materials. Table 1 summarizes preliminary average recovery assays and physico-chemical stability tests performed for several sorbent phases supported on the BAμE devices.

3.2. Analytical performance of AμE techniques

After evaluating the stability and robustness of both μ-extraction devices, we started to focus our attention on the analytical performance of AμE techniques. Since these approaches deal with a static equilibrium during the enrichment process, both BAμE and MSAμE devices coated with convenient powdered materials were introduced into the sampling flaks under agitation with a conventional teflon magnetic stirring bar, to promote the rotational motion of the liquid matrix and simultaneously, the μ-extraction of the solutes towards the sorbent phase. On the other hand, these μ-collectors are constituted with substrates lighter than water and therefore, during the enrichment process they will be standing just below the vortex formed by the agitation motion. Fig. 3 depicts schematic representations and images exemplifying the behavior of both BAμE and MSAμE devices during the μ-extraction process. Although presenting a bit different enrichment philosophy when compared with other sorptive extraction techniques (*e.g.* SPME and SBSE), the optimization of the experimental conditions for both BAμE and MSAμE is quite similar and a must to each specific type of application. Beyond the sorbent materials type and amounts involved, the recovery yields are greatly influenced by parameters such as extraction time, agitation speed and matrix characteristics, *i.e.* pH, polarity and ionic strength, in order to maximize the efficiency of both AμE techniques. Since the preparation of BAμE and MSAμE devices is very easy to manipulate and independent on the sorbent phase involved, the great advantage of these novel analytical tools over other sorptive extraction techniques is the ability to choose the most convenient specific sorbent as well as the corresponding amount for a particular solute or class of compounds. For BAμE, the amount of sorbent phase involved is limited to the available area of adhesive supporting film (±4 mg), whereas for MSAμE the number of multi-spheres can be chosen, according to the expected content level for particular target compounds. Meanwhile, the subsequent back-extraction conditions prior to instrumental analysis, *i.e.* the solutes desorption from the sorbent materials by an appropriate solvent also needs to be evaluated for each particular application. Therefore, back-extraction parameters such as desorption solvent type and time with or without sonification treatment must be criteriously optimized. Apart of the selection of the best experimental conditions, the overall optimization must always play an important role to establish the most convenient validation for any particular application.

During this stage, preliminary recovery assays were performed in order to assess the extraction efficiency of twenty different sorbent phases through BAμE, using water samples spiked with atrazine ($\log K_{O/W} = 2.82$) [13,14,19] as model system, under standard experimental conditions. From the data obtained, and summarized in Table 1, ACs and PS-DVB showed the best efficiency performance (>75%) for BAμE, giving a very good indication as potential sorbent phases to retain trace levels of polar metabolites in aqueous media. Apart of the better stability demonstrated in the previous section as sorbent phases either by BAμE or MSAμE, ACs present interesting features such as convenient textures (*e.g.* mesopores) conjugated with suitable strong adsorptive properties, which are much more indicated to retain polar solutes. On the other hand, PS-DVB sorbents present appropriate functionality since they can join reverse phase with ionic exchange properties. However, it must be emphasized that the enrichment performance of the sorbent phases for a specific solute depends on a case by case assessment, *i.e.* the material should be criteriously selected for each particular type of application. In general, AμE techniques must be understood as advantageous analytical tools comparatively to SBSE since we have the possibility to choose and easily prepare the right sorbent material for a particular polar solute, whereas for the latter just PDMS is commercially available, which is definitely indicated for nonpolar compounds.

3.3. Application of BAμE and MSAμE to real matrices

After establishing the best preparation procedures and preliminary tests, it was our intention to apply these novel enrichment tools (BAμE and MSAμE) to monitor polar solutes and metabolites in aqueous media. In a first approach, assays were performed to evaluate the behavior of these analytical devices to monitor priority solutes, among others, in several types of water matrices. Fig. 4 exemplifies some chromatographic profiles obtained by the application of BAμE (a and b) and MSAμE (c and d) using AC and PS-DVB as sorbent phases followed by LD/HPLC–DAD analysis, under optimized experimental conditions. The data presented show the successful application of both approaches to water samples spiked with drugs of abuse (Fig. 4a), disinfection by-products (Fig. 4b) and antibiotics (Fig. 4c and d). For the μ-extraction of these target metabolites, specific materials such as ACs in particular, were criteriously selected to achieve suitable selectivity and sensitivity at the trace level. Throughout this work, we also prepared and characterized novel AC materials in particular from cork waste, which showed a remarkable performance when compared with other ones commercially available [19]. Furthermore, Fig. 5 exemplifies chromatogram profiles obtained from the application of MSAμE (a) using commercial ACs and BAμE (b) with ACs from cork waste as sorbent phase followed by LD/HPLC-DAD analysis for

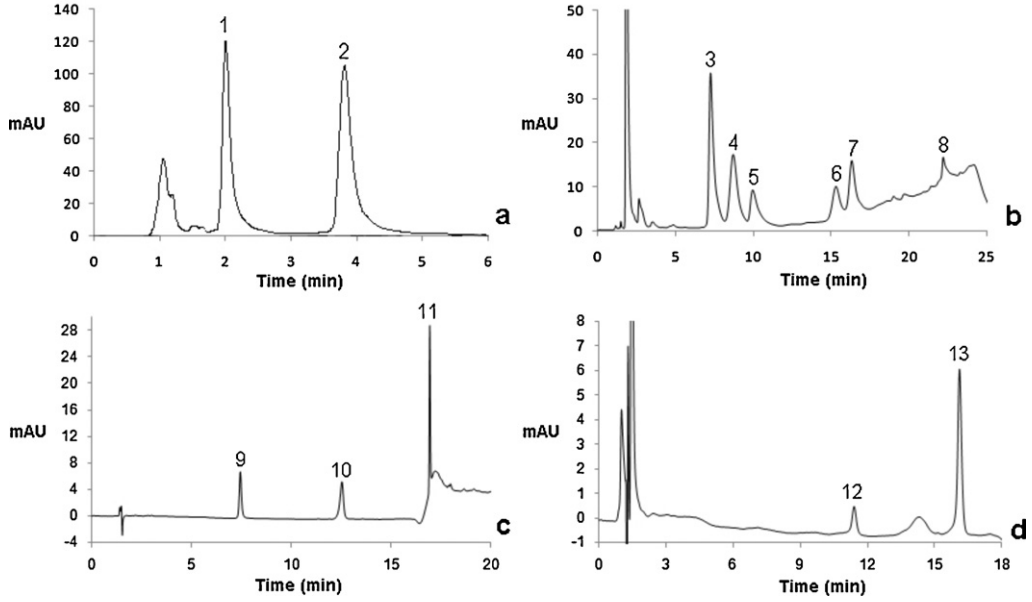

Fig. 4. Chromatogram profiles of drugs of abuse (a) and disinfection by-products (b) obtained by BAμE(AC) and antibiotics (c and d) through MSAμE(PS-DVB) from spiked water samples followed LD/HPLC–DAD analysis, under optimized experimental conditions. 1: Morphine; 2: codeine; 3: formaldehyde–PFPH; 4: acetaldehyde–PFPH; 5: acetone–PFPH; 6: propanal–PFPH; 7: butanone–PFPH; 8: 2-hexenal–PFPH; 9: sulphathiazole; 10: sulphamethoxazole, 11: sulphadimethoxine, 12: trimethoprim; 13: enrofloxacin.

the determination of acetaminophen and caffeine in spiked surface water sample and the occurrence of contents of ibuprofen in urine samples from different patients, respectively. From the data obtained, the novel analytical technologies besides being very sensitive also demonstrated high selectivity in complex matrices such as biological fluids, presenting negligible analytical interferences. As stated before, the great advantage of these novel analytical tools is the possibility to prepare or use commercially available sorbent materials more indicated for a specific application. Moreover, it must be emphasized that the proposed novel analytical technologies present a remarkable performance for the matrices assessed when compared with other dedicated sample enrichment methods.

3.4. Comparison of BAμE, MSAμE and SBSE techniques

Taking into consideration that BAμE and MSAμE methodologies were mainly developed with the purpose of overcoming the limitations of SBSE with respect to the more polar metabolites, several assays were performed to compare the analytical performance in between these approaches, as can be observed in Figs. 6 and 7. Fig. 6a depicts the data obtained by the application of BAμE on the analysis of drugs of abuse (morphine and codeine) in aqueous media, using suitable AC phases. As it can be observed, the recovery yields through BAμE(AC) are around 40%, while SBSE(PDMS) is totally unsuitable for the direct analysis of these metabolites, under similar experimental conditions. Fig. 6b displays the efficiency obtained for priority pesticides (simazine and atrazine) by BAμE(AC), allowing recoveries of around 100%, whereas by SBSE(PU), it is shown once again that even by using a coating phase more appropriate to μ-extract triazinic compounds, as previously reported [13,14], the maximum recovery yields were around 20% (atrazine), under similar experimental conditions. Fig. 6c depicts the assays carried out on a pharmaceutical and personal care compound (ibuprofen), which is an active ingredient prescribed in medicines. Since this drug is being widely used, the corresponding detection occurs with increasing frequency in aquatic systems, particularly in drinking water sources. The data depicted (Fig. 6c) for this particular compound through BAμE(AC), using AC prepared from cork waste, surpass the performance of SBSE(PDMS) with recovery yields around 100%. We also studied disinfection by-products (carbonyl type) that can occur in drinking

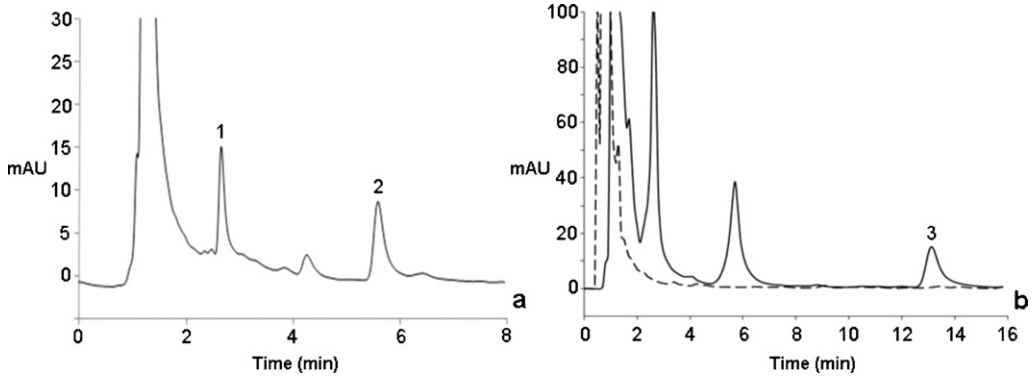

Fig. 5. Chromatogram profiles of acetaminophen and caffeine obtained by MSAμE(AC) from spiked surface water samples (a) and ibuprofen through BAμE(PS-DVB) in urine samples (b) followed LD/HPLC–DAD analysis, under optimized experimental conditions. 1: Acetaminophen; 2: caffeine; 3: ibuprofen in urine samples with (—) and without (- - -) consumption of ibuprofen.

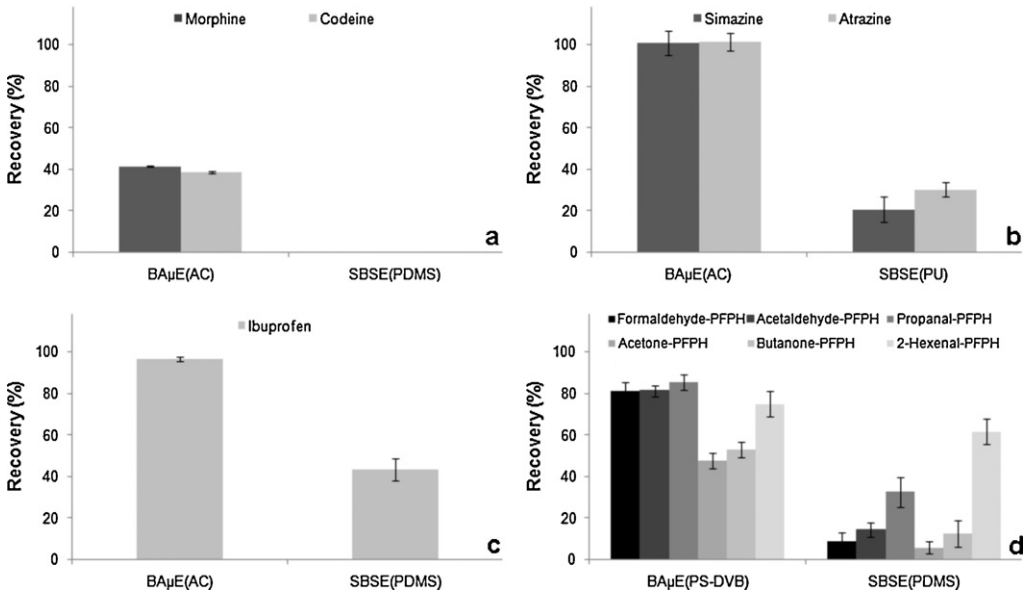

Fig. 6. Comparison of the recovery yields obtained in between BAμE coated with different sorbent phases (ACs and PS-DVB) and SBSE(PDMS) for drugs of abuse in urine (a), herbicides in surface water (b), pharmaceuticals in water (c) and disinfection by-products in drinking water (d). The conditions are as following: (a) 30 mL of spiked (30 μg L^{-1}) water sample, extraction: 2.5 h (1000 rpm) at pH 7.0, back-extraction: MeOH/ACN (1:1) for 30 min; (b) 30 mL of spiked (25 μg L^{-1}) water, extraction: 16 h (1000 rpm), pH 2.0 and NaCl 10% (w/v), back-extraction: MeOH/ACN (1:1) for 45 min; (c) 10 mL of spiked (10 μg L^{-1}) water sample, extraction: 16 h (1000 rpm), pH 5.0 and NaCl 15% (w/v), back-extraction: ACN for 45 min; (d) 30 mL of spiked (25 μg L^{-1}) water sample, extraction: 4 h (1250 rpm), pH 5.5 and NaCl 10% (w/v), back-extraction: MeOH for 30 min.

water samples by BAμE(PS-DVB) following LD/HPLC–DAD analysis, where PS-DVB proved to be the best sorbent phase. In this study, *in situ* derivatization was introduced for detection purposes using pentafluorohydrazine (PFPH) as derivatization agent. As shown in Fig. 6d, the recovery yields attained are in between 50 and 80%, whereas for SBSE(PDMS) it ranges from 10 to 30%; only the most nonpolar compound reached about 60%. The data depicted in Fig. 7 (a, b, and c) relate assays carried out with compounds belonging solely to the class of pharmaceutical and personal care products. Fig. 7a exemplifies the remarkable performance obtained either by BAμE(AC) or MSAμE(AC) from the analysis of caffeine and acetaminophen in water samples. As it can be observed, the recovery yields are about 80% for both methodologies, which means that neither the different sticking-based

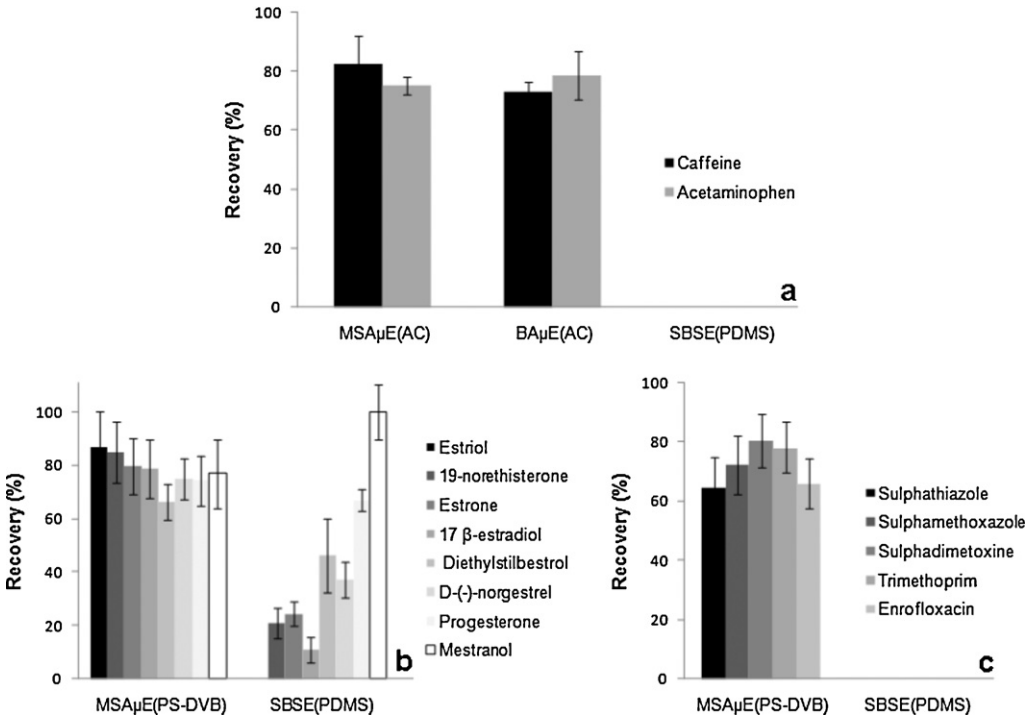

Fig. 7. Comparison of the recovery yields obtained in between MSAμE and BAμE coated with different sorbent phases (ACs and PS-DVB) and SBSE(PDMS) for pharmaceuticals in surface water (a), sexual steroid hormones in water (b) and antibiotics in water (c). The conditions are as following: (a) 30 mL of spiked (10 μg L^{-1}) water sample, extraction: 17 h (1000 rpm) at pH 6.5, back-extraction: formic acid for 30 min; (b) 25 mL of spiked (10 μg L^{-1}) water sample, extraction: 6 h (1000 rpm) at pH 7.0, back-extraction: MeOH for 15 min; (c) 25 mL of spiked (10 μg L^{-1}) water sample, extraction: 17 h (1000 rpm) at pH 7.0, back-extraction: MeOH for 45 min (for sulphathiazole, sulphamethoxazole and sulphadimetoxine) and extraction: 6 h (1000 rpm) at pH 4.0, back-extraction: formic acid for 30 min (for trimethoprim and enrofloxacin).

technologies nor the μ-extraction devices shape affects the properties of the AC involved for this particular application. By comparing the data obtained from these two approaches with SBSE(PDMS), the latter proved to be totally unsuitable for direct analysis of these compounds. Moreover, Fig. 7b depicts the efficiency for eight sexual steroid hormones (estriol, 19-norethisterone, estrone, 17β-estradiol, diethylstilbestrol, D-(−)-norgestrel, progesterone and mestranol), where MSAμE(PS-DVB) shows excellent performance, allowing recoveries ranging from 66 to 87%. However, by SBSE(PDMS), the efficiencies are up to 67% and only mestranol is 100% recovered, the most nonpolar compound of the steroid hormones studied. Additionally, Fig. 7c relates with the analysis of antibiotics (sulphathiazole, sulphamethoxazole, sulphadimetoxine, trimethoprim and enrofloxacin) in water samples through MSAμE(PS-DVB), presenting yields ranging from 60 to 80% and demonstrating once again the remarkable performance showed before in comparison with SBSE(PDMS) methodology, where the latter proved to be completely inefficient to μ-extract this type of compounds.

In short, the applications presented here clearly demonstrate that both BAμE and MSAμE methodologies containing suitable sorbent phases and under optimized experimental conditions, have undoubtedly great effectiveness and remarkable precision to μ-extract priority solutes and metabolites with polar characteristics at trace level from real matrices, unlike SBSE with PDMS phase, among others, more dedicated to nonpolar compounds. The detailing data regarding these and other applications by AμE techniques concerning the validation requirements and other particular issues will be published soon.

4. Conclusions

A novel μ-extraction technique for trace analysis of polar compounds in aqueous media is described. The analytical devices are easy to prepare using both bar (BAμE) or multi-sphere (MSAμE) geometrical configurations. From several sorbents tested, activated carbons and polystyrene divinylbenzene phases demonstrated the best stability, robustness and good μ-extraction efficiency. Additionally, this new analytical approach presents also the advantage to tune the most suitable sorbent to each specific type of application.

The application of both BAμE and MSAμE showed a remarkable performance to monitor polar solutes and metabolites (*e.g.* pesticides, disinfection by-products, drugs of abuse and pharmaceuticals) in water matrices and biological fluids at the trace level. By comparing AμE techniques with stir bar sorptive extraction based on polydimethylsiloxane phase (SBSE(PDMS)), the former showed to overcome the limitation of the latter concerning the recovery yields of the more polar solutes. Furthermore, the AμE techniques are cost-effective, easy to work-up, demonstrating to be a remarkable analytical tool for trace analysis of priority solutes in areas of recognized importance such as environment, forensic and other related life sciences.

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