Solid phase microextraction devices prepared on plastic support as potential single-use samplers for bioanalytical applications

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Abstract

This study presents new thin-film solid phase microextraction (SPME) devices prepared on plastic as potential single-use samplers for bioanalysis. Polybutylene terephthalate (PBT) was selected as a support due to its well-known chemical resistance, low cost, and suitability as a material for different medical grade components. The herein proposed samplers were prepared by applying a hydrophilic-lipophilic balanced (HLB) - polyacrylonitrile (PAN) coating on rounded and flat PBT pieces previously sanded with regular sandpaper. SPME devices prepared on PBT were evaluated in terms of robustness, chemical stability, and possible interferences upon exposure to different solvents and matrices. Rewarding results were found when these samplers were employed for the quantitative analysis of multiple doping substances in common biological matrices such as urine, plasma, and whole blood. Finally, the proposed thin-film SPME devices made on a PBT were evaluated by conducting multiple extractions from whole blood

and plasma using the Concept 96 system. Results showed that more than 20 extractions from plasma and whole blood can be performed without observed decreases in coating performance or peeling of the extraction phase from the plastic surface. These findings demonstrate the robustness of PAN-based coatings applied on such polymeric substrate, and open up the possibility of introducing new alternatives and cost-effective materials as support to manufacture SPME biocompatible devices for a wide range of applications, particularly in the clinical field.

Key words: thin-film, solid phase microextraction, high throughput, disposable sampler, sample preparation, polybutylene terephthalate, PBT

Introduction

Solid phase microextraction (SPME) has demonstrated unquestionable advantages as an alternative sample preparation approach over traditional methods for several applications (e.g. liquid-liquid extraction and protein precipitation). Minimum solvent use, integration of sampling and sample preparation steps, simple operation, and suitability for on-site and *in vivo* determinations are just some of the important benefits of using SPME. Indeed, the introduction of biocompatible coatings suitable for direct extraction of diverse compounds from complex matrices has led to an expansion in SPME applications, especially in the field of bioanalysis.[1–6] These coatings, which consist of a biocompatible binder used to immobilize solid phase extraction (SPE) particles and other sorbents, have demonstrated great selectivity towards small molecules, minimum or negligible protein fouling, and sufficient stability even for long-term reusability (140 extractions from plasma).[7,8]

Biocompatible SPME coatings are available in two main configurations: fibers and thin-films. The term “thin-film” is used to designate another geometry of SPME, in which devices consisting of a large coated surface area and a thin coating thickness (large extraction phase surface area-to-volume ratio) provide better sensitivity than traditional SPME fibers without sacrificing analysis time (short equilibration times).[9] Both, fiber and thin-film SPME devices, have been subjected to automation for high throughput analysis in the 96-well plate format; however, the enhanced sensitivity offered by thin-film geometry is preferred in several cases (*e.g. in vitro* biofluids analysis). Various studies reporting on the suitability of high-throughput thin-film SPME for analytical determinations in different matrices have been published recently.[10–12] In the area of bioanalysis, some recently reported applications of thin-film SPME include for instance the determination of tranexamic acid and rocuronium bromide in plasma, and the analysis of multiple doping substances in both urine and plasma samples.[13–15] Although, as already emphasized, the robustness of these biocompatible devices allows for reusability even in complex matrices, in some cases (e.g. doping analysis), it is certainly preferable, if not mandatory, to use disposable or single-use samplers. In this sense, exploring alternative materials for the construction of SPME devices, as well as simplifying the manufacturing process itself, are key factors to facilitate the widespread acceptance of this sample preparation method.

The introduction of novel materials in the fabrication of SPME samplers has been mainly directed towards finding alternative and cost-effective coating types.[16–18] However, broadening the current list of substrate materials used to immobilize different SPME extraction phases can also provide unique opportunities. While self-supported SPME samplers (*e.g.*

polydimethylsiloxane sheet pieces (PDMS)) are available for selected applications,[9,19] a solid substrate is typically required as base for application of the SPME coating, as well as to define the device geometry (*e.g.* wire or monofilament type of substrate for traditional SPME fibers, flat supports for thin-films and blade spray devices, and a metallic mesh for SPME-transmission mode).[20–22] Fused silica, StableFlex™, and metal non-ferrous alloys such as nitinol have been all used as fiber cores for gas chromatography (GC)-amenable SPME coatings (Table S1), while stainless steel and nitinol are currently employed as substrate materials of choice to immobilize biocompatible extraction phases.[16–18] Despite the satisfactory performance of biocompatible SPME samplers manufactured on such supports, certain applications will definitely benefit from the introduction of biocompatible substrates that are cheaper to manufacture, in addition to being more flexible, and easier to mold. For example, while the original *in vivo* SPME approach involves the use of traditional fibers by exposing them directly to the bloodstream or by inserting them in different types of tissue, [23–26] the development of devices for less invasive *in vivo* applications, such as saliva and mucosa analysis, might require alternative support materials.

Various requirements should be fulfilled when looking for substitute materials to manufacture SPME devices for bioanalysis. First of all, the material should be biocompatible, suitable for sterilization, and able to handle temperatures normally employed to cure the polymeric binders used for SPME coatings. Once applied, the coating should be sufficiently stable on the substrate, and ideally, the coated material should not release any type of interferences, neither in the sample nor in the desorption medium. Substitute support materials for SPME should also offer good chemical resistance, low moisture absorption, affordability, and be easily accessible.

Several materials, including metals and polymers, are used to manufacture various commercially available medical devices.[27,28] Among this list of medical grade materials, thermoplastics represent an important group of polymers that are widely used for the production of healthcare components.[27,28] Given that these plastics display many of the features required for the preparation of SPME samplers, in this work, polybutylene terephthalate (PBT) was tested as a potential support for application of SPME biocompatible extraction phases. For this purpose, coating preparation conditions were modified according to the new support material characteristics, and thin-film SPME devices were prepared using two different PBT geometries: rounded and flat. In this study, an SPME coating made of hydrophilic-lipophilic balanced particles (HLB) and polyacrylonitrile (PAN) was selected due to its suitability to extract a wide range of compounds.[15] A thorough evaluation of the newly prepared samplers in terms of stability, reproducibility, and performance in the extraction of seventeen doping substances from various biological matrices (urine, plasma, and whole blood) is herein presented. The model compounds chosen for this work covered logarithmic octanol-water partition coefficients (log P) values ranging from 0.33 to 6.56, and included drugs bearing on their structure different moieties. Figures of merit corresponding to determinations of total concentrations of the selected analytes spiked in different biofluids are also reported. Lastly, this work presents a discussion regarding the impact of sorbent type on coating wettability; a comparison of water contact angles measured on HLB-PAN, C18-PAN, and only-PAN coatings provided remarkable evidence on the advantages of using HLB-PAN as SPME coating for the analysis of aqueous matrices.

Experimental section

Materials and supplies

The following compounds were selected as model analytes to evaluate the SPME-HLB-PBT devices: amphetamine, 17-α-trenbolone, benzoylecgonine, bisoprolol, clenbuterol, codeine, exemestane, GW501516, methamphetamine, metoprolol, morphine, nikethamide, propanolol, salbutamol, stanozolol, strychnine, and toremifene. Codeine-d_3, oxycodone-d_3, cannabidiol-d_3, methadone-d_3, (±)11-nor-9-carboxy-Δ^9-THC-d_3 (THCCOOH-d_3), testosterone-d_3 and salbutamol-d_3 were used as internal standards. Further details about compounds suppliers and properties, as well as information about other materials and supplies, are provided in the Supporting Information (Table S2).

Preparing thin-film SPME devices on PBT support

Rounded PBT pieces were trimmed to a 53 mm length. In the case SPME-HLB-PBT devices on flat support, rectangular PBT pieces with a 0.5 mm thickness (10 x 8 cm) were cut using a conventional paper trimmer. Prior to the application of the coating, the area to be coated was uniformly sanded using commercial sandpaper (first using a sandpaper of a medium macro grit (P150) and then using one of ultra-fine micro grit (1500)). All PBT pieces were cleaned with methanol and acetonitrile (20 min in each solvent under sonication), and then left to dry at room temperature.

For the coating procedure, a slurry was prepared by mixing approximately 0.7 g of HLB particles, 3 mL of DMF, and 10 mL of 7 % (w/w) PAN/DMF solution (prepared by mixing PAN with DMF,

and heating the obtained solution at 90 ˚C for 1 h). A 2 cm length coating was applied by spraying uniform layers of slurry and curing each layer at 125 ˚C for 2.5 min (14 cycles in total) (Figures 1A, 1B and 1C). From the coated rectangular pieces, SPME-HLB-PBT samplers with a 2.3 mm width were cut using a paper trimmer as well. Six of these devices were over-coated with PAN by dipping them in 7 % PAN solution, as reported in the literature.[29] Finally, twelve SPME-HLB-PBT devices on flat support (6 of them over-coated with PAN) were arranged to ensure compatibility with the Concept 96 system, as shown in Figure 1D. All the SPME devices were cleaned in a solution of 2:1:1 v/v methanol:acetonitrile:isopropanol for 120 min.

To characterize the prepared devices, scanning electron microscopy (SEM) images were taken using a LEO 1530 field emission (Carl Zeiss NTS GmbH, Germany), and microscope pictures were taken using an Olympus SZX10 stereomicroscope system equipped with a SC30 digital camera (Olympus, Japan).

The previously described coating procedure was also followed to apply HLB-PAN, C18-PAN and only-PAN (without sorbent particles) coatings on flat PBT pieces (2 x 10 cm). These coated PBT pieces were subsequently used to investigate the wettability of these extraction phases. The wettability of these three coatings was assessed by measuring the contact angle of 30 µL water drops according to the sessile drop method. Images were acquired every 2 s, and contact angle measurements were collected using Axisymmetric Drop Shape Analysis (ADSA).

Sample preparation for LC-MS/MS analysis

An initial assessment of PBT as a substrate for the manufacture of thin-film SPME devices was carried out with the rounded pieces (Figures 1A and 1B). For this purpose, PBS and three

different biological matrices (urine, plasma, and whole blood) were used to test the plastic devices. Aliquots (1080 µL) of each matrix were spiked with methanolic stock solutions containing the model compounds. The organic solvent content was kept below 1 % in all cases. Once spiked, sample pre-incubation was allowed for 1 h under constant vortex agitation conditions to ensure proper equilibration between the model compounds and the different tested matrices. Table 1 summarizes sample preparation parameters selected for urine, plasma, and blood analysis. These experimental conditions were chosen based on previous work done in our group.[14,15] As can be seen, the same parameters used for plasma analysis were employed for extraction from whole blood, except for an additional washing step that was introduced after pre-conditioning, and three 5 s wash steps conducted after each extraction. In addition, a higher concentration of internal standard was used for blood and plasma samples compared to urine samples with the aim of compensating for low recoveries due to matrix binding effects. Since the number of SPME-HLB-PBT samplers on rounded PBT support was limited, matrix matched calibration curves were constructed for each biological fluid using one device per calibration level (0.1, 0.25, 0.5, 1, 5, 10, 25, 50, 75, and 100 ng mL^{-1}). Accuracy and precision were evaluated at 1.6, 15, 35, and 70 ng mL^{-1}, using three devices per concentration level. Limits of quantification (LOQ) were determined as the lowest concentration points at which both deviations from nominal concentration values and relative standard deviations were below 20 % for each individual compound. All steps of the SPME method were carried out at room temperature, and uniform stirring was conducted by placing the vials in a multi-tube vortex agitator. SPME-HLB-PBT devices on flat PBT support were employed to evaluate the robustness of the coating immobilized on the selected substrate. For this purpose, consecutive extractions

from plasma and whole blood spiked with six of the model compounds were performed using a set of 12 films arranged in the Concept 96 system (Figure 1 D). For this part of the study, experimental conditions were kept as already described for whole blood analysis, except for extraction time and desorption volume, which were set at 60 min and 1200 µL, respectively. All extracts were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Full details concerning the LC gradient and monitored transitions are provided in the Supporting Information.

Table 1. Summary of experimental conditions selected for SPME.

Parameter	Urine	Plasma	Whole blood
Sample volume	1080 µl		
Phosphate buffer (pH = 7)	120 µL (2 M buffer)	120 µL (1 M buffer)	
Internal standard	Spiked in buffer at 200 ng mL^{-1}	15 µL of 8 µg mL^{-1} solution	
Preconditioning	30 min in 1500 µL of 1:1 methanol:water		30 min in 1500 µL of 1:1 methanol:water + 10 s wash in nanopure water
Extraction	90 min		
Washing	10 s in 1500 µL of nanopure water (manually performed using vortex agitation)		3 times each in 1500 µL of nanopure water for 5 s
Desorption	20 min in 600 µL of 4:1 methanol: acetonitrile acidified with 0.1% formic acid		

Results and Discussion

Characterization of PAN-HLB coating prepared on plastic and evaluation of PBT as a support for SPME

The optimized procedure for preparing thin-film PAN-based SPME coatings on a stainless steel support has been already reported.[8] In that study, spraying over a previously etched surface and curing at 180 ˚C for 2 min were found to be optimum conditions. Due to the characteristics of the material selected as a new support for SPME devices, modifications to the already reported protocol were necessary. First of all, since etching with concentrated acid was not feasible, the plastic surface was only sanded with regular sandpaper. This step was taken with the aim of improving the adherence of the biocompatible coating on PBT. Secondly, considering that the maximum temperature for long term use of PBT is 125 ˚C, the coating curing procedure was conducted at 125 ˚C for 2.5 min.

As can be seen in both the microscope picture and SEM image presented in Figures 1B and 1C, a uniformly coated surface was obtained. The robustness of the coated plastic devices was tested by exposing them to water, methanol, and acetonitrile for a total period of 20 h under constant vortex agitation. No noticeable changes in the coating structure were observed, and no detachment of the coating from the plastic surface occurred. These findings demonstrated the stability of the HLB-PAN layer immobilized on the newly proposed substrate and validated the selected conditions for the coating procedure. Provided that no etching with concentrated hydrochloric acid is required, a simplified and greener coating application approach can be established.

After verifying the stability of the SPME coating on PBT, inter-device reproducibility was also assessed. Satisfactory results were obtained for 20 rounded devices employed to extract from spiked PBS, showing RSD values below 13 % for all the tested compounds (Figure S1, Supporting Information). However, it is worth noting that the rounded shape of the plastic support may have hindered the uniform application of the SPME coating by spraying. Although 13 % was an acceptable RSD value, better results were obtained when a flat support was used.

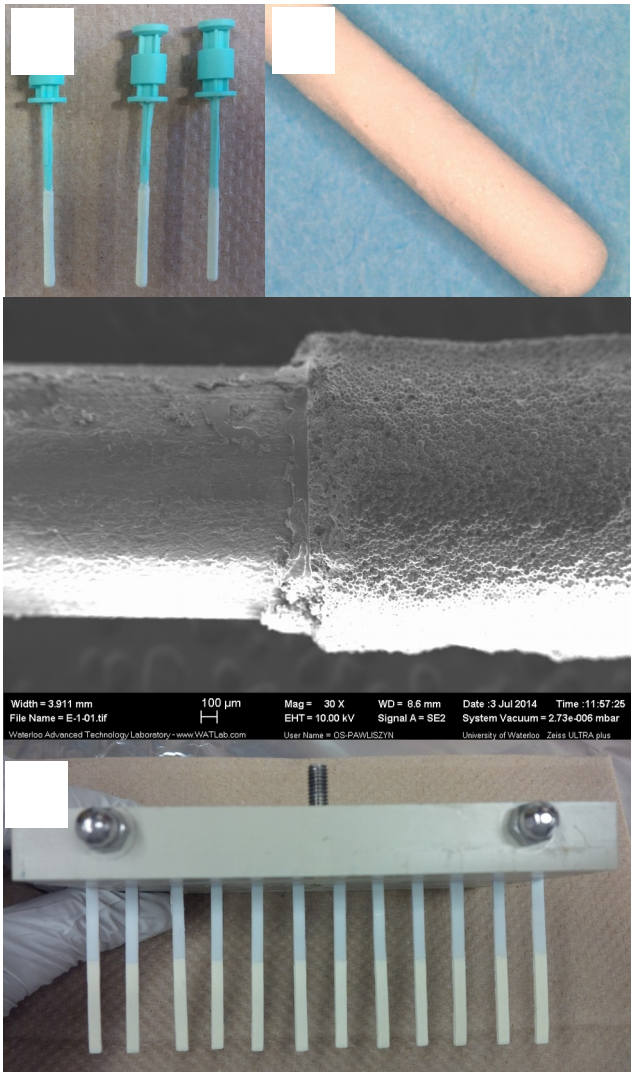

Figure 1. Rounded thin-film SPME devices prepared as described (A), microscope pictures of the HLB coating applied on the rounded plastic pieces (B), SEM image of the morphology of the

coating on PBT support using 30x magnification (C) and SPME-HLB-PBT devices (on flat PBT support) in an arrangement compatible with the Concept 96 system (D).

Following the initial positive results obtained for the new coated material, an evaluation was conducted regarding possible interferences arising from the substrate that may be introduced into the sample or desorption media. For this purpose, an initial assessment of the background provided by the SPME coating prepared on the PBT support was carried out. Various new SPME-HLB-PBT devices were desorbed in 4:1 methanol:acetonitrile (v/v) acidified with 0.1 % formic acid for 20 min. Prior to blank desorption, a cleaning step was performed by exposing the plastic devices to a mixture of organic solvents (2:1:1 v/v methanol :acetonitrile:isopropanol) under vortex agitation conditions for 120 min. Extracts were then run in full scan mode and compared with solvent blanks and extracts from the new HLB-PAN coatings prepared on a regular stainless steel. As can be seen in Figure S2 (Supporting Information), no significant differences were observed among the background signals obtained for all blanks. As a second means of verifying the absence of interferences coming from the proposed devices, absolute matrix effects were estimated according to the procedure proposed by Matuszewski et al.[30] Extracts obtained from the desorption of blank SPME-HLB-PBT samplers were spiked with the target compounds at 50 ng mL^{-1}, and their response (peak area) was compared with the one from standards prepared in neat solvent at the same concentration level. Likewise, extracts from urine and plasma blanks obtained with SPME-HLB-PBT devices (n = 6) were post-spiked and compared with neat standards as well. As shown in Table S4 (Supporting Information), no absolute matrix effects coming from the devices prepared on the PBT support were found for all the studied compounds. In addition, no significant matrix effects (80 – 112 %) were observed for the

compounds spiked in urine and plasma blank extracts. These results demonstrated the stability of the SPME coatings on the polymeric support, while proving the absence of possible interferences that might be released upon contact between the coated PBT and the sample medium or desorption solvent.

Using SPME devices on polymeric support for the analysis of doping substances in urine, plasma, and blood

Urine and plasma analysis

The proposed rounded PBT devices were used for the analysis of urine and plasma samples according to the experimental conditions provided in Table 1. As can be seen in Table 2, rewarding figures of merit were obtained when using SPME-HLB-PBT devices in both matrices. Satisfactory results were observed for most analyzed drugs, with good linearity (R^2 > 0.99), accuracy (80 – 120 %), and precision (RSD < 7%) measures. As expected, less polar compounds such as toremifene (logP 6.56) and GW501516 (logP 6.46) exhibited higher LOQ values in plasma than in urine due to protein binding effects. On the other hand, polar compounds such as salbutamol (log P 0.64) and morphine (log P 0.89), which are not significantly affected by protein binding interactions, showed the same LOQ values in both matrices. Indeed, the LOQ value for a given analyte is directly correlated to the absolute SPME recovery, which, in turn, depends on several parameters, namely, the extraction time, the convection conditions, the coating volume, and the affinity of the compound for the matrix components and for the SPME coating. Although in SPME a small amount of analyte proportional to the analyte concentration in the sample is extracted, depending on the previously listed factors and on the proportion

between coating and sample volumes, exhaustive, or almost exhaustive extraction can take place. For some of the model compounds, this can be reflected in the higher enrichment factors (EF, analyte concentration in the extract/analyte concentration in the sample) estimated in urine compared to those EFs calculated in plasma or in whole blood (Table S5). In regards to urine results, it is also worth emphasizing that the LOQs determined complied with or were lower than the minimum required performance levels (MRPL) stipulated by WADA. For instance, the LOQ value determined for clenbuterol, the compound with the lowest MRPL (0.2 ng mL^{-1}), not only fulfills WADA requirements but also allows for a quantification that is two times lower than the drug MRPL. Indeed, the observed differences between the LOQ values presented in this work and those already reported by Boyacl et al.[14] and Reyes- Garcés et al.[15] can be attributed to a reduction in the desorption solvent volume (600 µL instead of 1200 µL), a distinct SPME coating volume (coated stainless steel blades vs. coated rounded devices) and, in the case of urine, the use of HLB particles instead of C18. Figure S3 shows representative chromatograms corresponding to extracts obtained from the different biofluids spiked at LOQ levels.

Table 2. Figures of merit for urine and plasma analysis using rounded SPME-HLB-PBT devices.

Compound (logP)[a]	Urine						Plasma					
	LOQ, ng mL^{-1}	R^2	Accuracy %, (RSD, n=3)				LOQ, ng mL^{-1}	R^2	Accuracy %, (RSD, n=3)			
			1.6 ng mL^{-1}	15 ng mL^{-1}	35 ng mL^{-1}	70 ng mL^{-1}			1.6 ng mL^{-1}	15 ng mL^{-1}	35 ng mL^{-1}	70 ng mL^{-1}
Morphine (0.89)	0.1	0.9984	100 (0.1)	89 (1.0)	102 (1.4)	102 (0.4)	0.1	0.9958	116 (0.1)	107 (1.1)	92 (3.8)	110 (2.0)
Salbutamol (0.64)	0.1	0.9952	103 (0.1)	87 (0.7)	97 (0.9)	98 (1.7)	0.1	0.9936	109 (0.1)	104 (0.5)	103 (2.7)	108 (3.7)
Nikethamide (0.33)	0.5	0.9895	103 (0.1)	81 (1.5)	90 (1.7)	85 (1.7)	0.5	0.9986	100 (0.1)	102 (1.2)	100 (3.2)	104 (5.3)
Codeine (1.19)	0.1	0.9977	101 (0.1)	87 (0.5)	103 (1.1)	101 (1.0)	0.1	0.994	109 (0.1)	104 (0.4)	105 (2.7)	109 (3.9)
Benzoylecgonine (2.71)	0.5	0.9978	99 (0.1)	86 (1.8)	114 (0.8)	107 (3.0)	1	0.9993	87 (0.3)	110 (2.5)	107 (3.9)	113 (3.2)
Amphetamine (1.76)	0.25	0.9935	95 (0.1)	82 (1.4)	88 (1.6)	94 (3.8)	0.25	0.9919	105 (0.2)	104 (0.7)	112 (3.1)	99 (2.5)
Methamphetamine (2.07)	0.1	0.9928	94 (0.1)	84 (2.1)	89 (1.4)	94 (3.4)	0.25	0.9909	105 (0.2)	103 (0.9)	115 (2.5)	98 (4.2)

Strychnine (1.93)	0.5	0.9959	94 (0.1)	85 (1.9)	103 (1.6)	99 (4.6)	5	0.9935	-	121 (0.7)	94 (3.8)	116 (5.5)
Exemestane (3.11)	1	0.9975[b]	107 (0.1)	87 (0.8)	88 (1.1)	108 (3.2)	1	0.9922	118 (0.2)	106 (1.0)	112 (2.2)	111 (4.7)
Trenbolone (2.27)	1	0.9958[b]	104 (0.2)	74 (0.6)	81 (0.5)	98 (7.8)	1	0.9922	107 (0.1)	105 (1.0)	105 (4.4)	105 (4.8)
Metoprolol (1.88)	0.25	0.9940	102 (0.1)	84 (0.6)	102 (1.9)	88 (2.3)	0.25	0.9982	107 (0.1)	107 (0.4)	112 (3.1)	109 (4.6)
Stanozolol (4.42)	0.1	0.9997[b]	94 (0.1)	81 (0.5)	87 (1.0)	105 (2.7)	5	0.9843	-	95 (0.4)	102 (3.2)	93 (4.4)
Clenbuterol (2.61)	0.1	0.9906	102 (0.1)	86 (1.0)	106 (1.7)	95 (1.6)	1	0.9968	79 (1.3)	123 (1.5)	105 (2.9)	118 (5.7)
Bisoprolol (1.89)	0.1	0.9953	93 (0.1)	82 (1.1)	103 (2.2)	86 (3.5)	0.5	0.9951	79 (1.0)	116 (0.7)	118 (2.7)	112 (5.7)
GW501516 (6.46)	0.1	0.9963	96 (0.4)	80 (1.8)	80 (4.9)	96 (3.9)	5	0.9940	-	98 (0.5)	98 (3.2)	103 (1.7)
Propranolol (3.48)	0.1	0.9913	91 (0.1)	74 (2.0)	93 (1.7)	86 (3.1)	5	0.9952	-	110 (0.9)	101 (3.4)	112 (2.1)
Toremifene (6.56)	1	0.9959	105 (0.2)	75 (1.3)	72 (4.8)	96 (6.5)	5	0.9976	-	85 (2.0)	101 (3.4)	118 (5.3)

Regression coefficients were calculated using LOQ values and 100 ng mL^{-1} as the lowest and highest calibration points, respectively.

[a] log P values were taken from Chemspider[31]

[b] Highest calibration point was 75 ng mL^{-1}

Whole blood analysis

Due to the high content of proteins and the presence of red blood cells, using whole blood as a matrix is more challenging and subsequently less common than analyzing serum and plasma. Based on the already discussed results corresponding to urine and plasma, the suitability of SPME-HLB-PBT devices for whole blood analysis was also investigated.

A previous study published by Mirnaghi and Pawliszyn in 2012 reported irreversible attachment of red blood cells to C18-PAN thin-film SPME devices when they undergo direct immersion in whole blood using the Concept 96 workstation system.[29] As a feasible solution to this problem, and looking toward coating reusability in an automated fashion, a modified extraction phase consisting of an external layer of PAN over a C18-PAN coating was proposed.[29] This approach considerably improved the coating compatibility with blood and enabled its reusability for multiple extractions in such a complex matrix. However, it is worth emphasizing that the presence of the extra PAN layer leads to a decrease in extraction kinetics, and therefore affects

method sensitivity, especially at pre-equilibrium conditions. Figure S4 (Supporting Information) shows a comparison of the absolute recoveries obtained for six of the model compounds, using SPME-HLB-PBT devices prepared on flat support with and without a PAN over-coating (applied by dipping). As can be seen, a decrease of more than 70 % for some of the analytes was found due to the over-coating PAN layer. Given that in this case, potential application of the proposed devices as single use samplers is being considered, a different approach to test the rounded PBT coated pieces in whole blood analysis was taken.

The typical SPME workflow was modified by introducing three additional washing steps, as described in Table 1. A washing step was implemented after preconditioning in order to remove any excess organic solvent remaining on the coating. This prevented possible protein precipitation or cell disruption from occurring on the extraction phase when the wet SPME coating got in contact with the whole blood matrix. Three wash steps of 5 s were also conducted after direct blood extraction (each wash step was performed in a new vial with clean water). It is worth highlighting that an evaluation of four different washing approaches (10 s static, 10 s with vortex agitation, two washing steps of 5 s with vortex agitation and three washing steps of 5 s with vortex agitation) showed that three consecutive washing steps did not cause any significant losses for any of the studied compounds for SPME devices coated with HLB particles (see Figure S5, Supporting Information). Indeed, the washing step should be carefully optimized according to the SPME coating selected and the analytes of interest. For example, in the case of the HLB-PAN, it is expected to observe high affinity toward compounds bearing on their structures lone electron pairs and able to display π-π interactions with the divinylbenzene moiety.[32,33] The almost exhaustive recoveries provided by HLB-PAN coating when used for the extraction of basic

(e.g. clenbuterol, bisoprolol, and propranolol) and steroidal analytes (e.g. 17-α-trenbolone and stanozolol) from PBS (Figure S5) are in agreement with other reports where the properties of HLB as SPE sorbent were investigated.[34,35] It is also worth to emphasize that, among the model compounds, salbutamol presented the lowest absolute recovery. Albeit for this compound a lower distribution coefficient is predictable due to its high hydrophilicity and degree of ionization, reproducible recoveries, even after several washing steps, were still attained.

By following this modified SPME procedure, analytical figures of merit in whole blood were investigated. As can be seen in Table 3, satisfactory results in terms of linearity, LOQ, accuracy, and precision were obtained for all prohibited drugs under analysis. These findings also reflected the absence of irreversible fouling which, as already reported by several authors, can affect the kinetics of extraction with SPME.[36–38] SEM images and microscope pictures of the HLB coating after exposure to blood for 90 min (Figure S6, Supporting Information) confirmed that irreversible protein attachment on the coating surface did not take place. In this regard, it is important to note that the feasibility of using the proposed devices for direct immersion in whole blood without any PAN over-coating layer may be related to the agitation conditions, the composition of the slurry employed to prepare the devices (lower SPE particles/PAN solution ratio compared to previous reports[8]), as well as the dimensions and rounded shape of the PBT support. In point of fact, shear stress imparted by blood flow conditions has been reported as a critical factor in determining whether a device is blood compatible.[39–42] The vigorous agitation provided by the multi-tube vortex system, together with the rounded shape and the specific dimensions of the coated devices (2.0 mm diameter), might have led to different shear conditions from those normally encountered when using the Concept 96 under its typical

operational configuration. Another aspect that should be also taken into account is that the evaluation of plastic devices was carried out in vials. Whole blood, when exposed to an open environment for a substantial period of time, as it occurs when using the Concept 96 system, is more prone to undergo alterations compared to blood that remains capped in a vial during the extraction process.[41] For this reason, additional considerations should be taken into account when expecting an antifouling SPME extraction phase at such conditions (e.g. using SPME coatings with an extra layer of PAN). Overall, the results demonstrated high blood compatibility for both the coating and support employed at the selected experimental condition for the proposed devices.

The presence of an absolute matrix effect after extracting from whole blood was assessed in the same manner reported for urine and plasma (Table S6, Supporting Information). As presented in Table S6, no significant ion suppression/enhancement was found for any of the evaluated compounds at the selected experimental conditions. Indeed, using vortex stirring for the washing steps provided very effective cleaning/removal of possible interferences due to the strong agitation provided by the vortex. As a second approach to verify the performance of the plastic SPME devices in terms of analytical specificity at the tested conditions, transition ratios were also calculated for all the evaluated biofluids. In quantitative analysis using mass spectrometry, the ratio between qualifier and quantifier transition signals should be the same for both the standards and biological samples.[43] Results presented in Table S7 (Supporting Information) evidenced an overall satisfactory performance of the proposed methodology for the majority of the model compounds. Only trenbolone extracted from blood showed a

significant deviation from the ratio calculated in standard; however, this may be overcome by improving the separation conditions.

Table 3. Figures of merit for whole blood analysis using rounded SPME-HLB-PBT devices.

Compound (log P)[a]	LOQ, ng mL^{-1}	R^2	Accuracy %, (RSD, n=3)			
			1.6 ng mL^{-1}	15 ng mL^{-1}	35 ng mL^{-1}	70 ng mL^{-1}
Morphine (0.89)	0.1	0.9984	107 (0.1)	102 (1.0)	99 (1.4)	108 (6.0)
Salbutamol (0.64)	0.1	0.9992	102 (0.1)	100 (0.3)	95 (0.7)	105 (5.0)
Nikethamide (0.33)	0.5	0.9993	110 (0.1)	101 (1.3)	91 (1.2)	106 (2.3)
Codeine (1.19)	0.1	0.9992	101 (0.1)	98 (0.3)	95 (0.5)	105 (5.5)
Benzoylecgonine (2.71)	0.5	0.9992	122 (0.2)	109 (2.0)	102 (1.7)	117 (10.6)
Amphetamine (1.76)	1	0.9994	86 (0.1)	101 (0.7)	94 (1.9)	106 (4.6)
Methamphetamine (2.07)	1	0.9987	83 (0.1)	104 (1.1)	95 (1.6)	108 (2.9)
Strychnine (1.93)	5	0.9980	-	103 (0.3)	97 (1.0)	103 (5.4)
Exemestane (3.11)	1	0.9988	87 (0.2)	94 (0.8)	95 (0.1)	104 (5.6)
Trenbolone (2.27)	1	0.9994	88 (0.1)	94 (0.5)	90 (0.9)	100 (6.8)
Metoprolol (1.88)	0.25	0.9970	97 (0.1)	99 (0.3)	94 (1.2)	100 (6.0)
Stanozolol (4.42)	5	0.9944	-	105 (2.2)	99 (3.5)	110 (5.4)
Clenbuterol (2.61)	1	0.9982	80 (0.1)	102 (0.3)	95 (0.8)	102 (5.7)
Bisoprolol (1.89)	0.25	0.9964	94 (0.1)	98 (0.5)	92 (0.5)	99 (6.4)
GW501516 (6.46)	5	0.9945	-	95 (0.5)	91 (2.6)	98 (5.2)
Propranolol (3.48)	5	0.9958	-	106 (0.9)	94 (0.4)	102 (7.5)
Toremifene (6.56)	5	0.9956	-	102 (1.8)	88 (3.1)	107 (10.0)

Regression coefficients were calculated using the LOQ value and 100 ng mL^{-1} as the lowest and highest calibration points, respectively.

[a] log P values were taken from Chemspider[31]

Assessment of HLB-PAN coating wettability

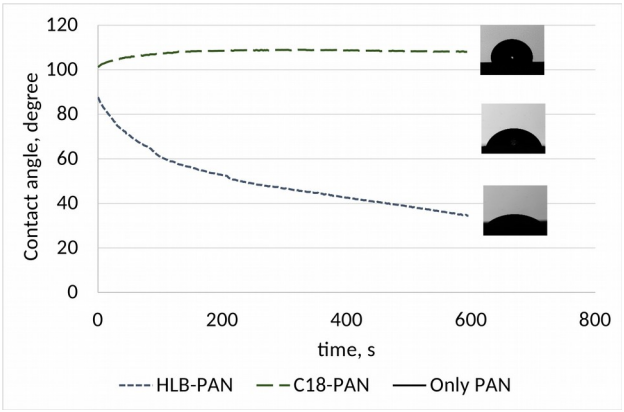

Figure 2. Water contact angles for HLB-PAN, C18-PAN and only-PAN coatings. Images were acquired every 2 s over a 10 min period.

Surface wettability has been traditionally conceived as an essential parameter in the design of materials with efficient resistance to protein fouling.[44,45] However, a direct relationship between low water contact angles (increasing wettability) and negligible protein adsorption or material biocompatibility has not been found in all the studied cases.[39,45–47] Despite this, determining the degree of wettability of PAN-based SPME coatings may provide important information considering the aqueous nature of biofluids. Indeed, easily wettable extraction phases are expected to facilitate the sample-coating interaction during the extraction process. Figure 2 shows the water contact angle variation over time found for HLB-PAN, C18-PAN and only-PAN coatings. Prior to the contact angle measurements, the HLB-PAN and C18-PAN surfaces were conditioned in a 1:1 methanol:water solution for 10 min, wiped with Kimwipes, and allowed to dry for approximately 10 min. As can be seen in Figure 2, although PAN was used as a binder in all the tested surfaces, HLB particles imparted a high degree of wettability to the coating. On the other hand, C18 conferred significant hydrophobicity to the extraction phase, yielding estimated

water contact angles above 100˚ over the entire measurement time. In relation to SPME extraction phases, these results suggest that using HLB combined with PAN, or even using other wettable materials, may provide improved performance compared to other sorbents, such as C18, when dealing with biological samples. As such, the development of SPME devices intended for spot sampling or even for *in vivo* sampling, where convection is certainly restricted, may need to include the determination of coating wettability as an important criterion.

Evaluation of the robustness and reusability of SPME thin-film devices prepared on plastic substrate using high-throughput configuration.

After verifying the suitability of PBT as a substrate for SPME devices, the robustness of the PAN-based coatings prepared on the new support was evaluated through multiple extractions from plasma and whole blood. Although there are cases where single-use samplers are certainly required, other analytical applications might be more flexible, and reusable SPME devices are a most convenient and cost-effective option. To validate the suitability of the new device for multiple use applications, SPME-HLB-PBT samplers made on flat support (with and without over-coating) were arranged in a Concept 96-compatible fashion, as shown in Figure 1D. Given that these devices were prepared by coating a PBT surface that was subsequently cut into smaller pieces, an initial assessment of inter-thin-film reproducibility was carried out. As can be seen in Table S8 (Supporting Information), RSD values below 7 and 3.5 % (n=6) were found for SPME devices with and without PAN over-coating, respectively. In addition to carrying out an assessment of inter-device reproducibility, SPME-HLB-PBT samplers were physically inspected by

taking microscope pictures of the coatings and of the interface between the coating and the plastic support (Figure S7, Supporting Information). The obtained images of the lateral view of the devices confirm that good attachment was achieved between the PAN-based coatings and the PBT surface. It is worth highlighting that even after undergoing a cutting process, the SPME coating remained firmly adhered to the polymeric material. Regarding reusability, no statistical differences were observed between recovery values obtained in the first and twentieth trials performed in plasma (Figure 3). In the case of whole blood, propranolol and stanozolol showed a slightly lower recovery during the first extraction when compared to all subsequent experiments. This observed outcome may be due to the extra layer of PAN applied to the HLB coating which, in addition to slowing down the extraction kinetics, might require that the overcoated HLB coating receive more extensive conditioning in comparison to coatings without this over-coating; however, this effect was observed only for the two less polar drugs tested. Overall, the obtained results suggest that using PBT as a substrate may provide the same coating robustness and stability obtained with the use of etched stainless steel.

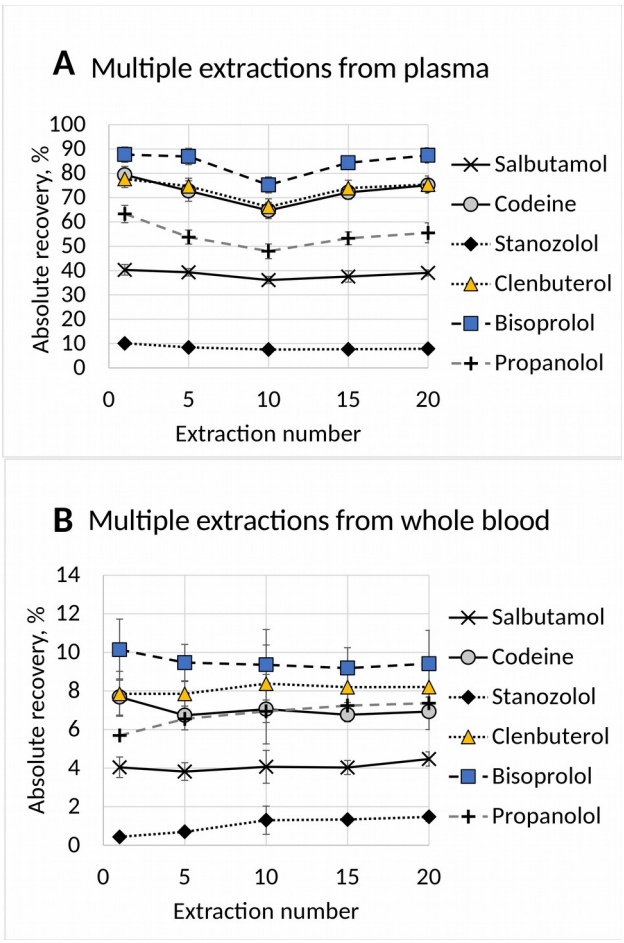

Figure 3. Evaluation of the stability and robustness of the HLB-PAN coating applied on PBT support. Consecutive extractions were performed from plasma (A) and whole blood (B) spiked at 70 ng mL^{-1} and mixed with 1 M phosphate buffer in a 9:1 ratio (sample:buffer) (n=6). Extractions from whole blood were carried out using HLB thin-films with PAN-over coating.

Conclusions

In this work, the suitability of PBT as a support to manufacture SPME devices that could be potentially employed as disposable samplers was demonstrated. Robust PAN-HLB coatings, free of background interferences and able to stand exposure to organic solvents for long periods of time, were attained by following a modified coating preparation procedure. Since no etching

with hydrochloric acid was required, a greener manufacturing process with a reduced production of chemical waste was possible. Rewarding figures of merit were found when these SPME samplers were used for quantitative analysis of multiple doping substances in urine, plasma, and whole blood. LOQ values determined in urine met or were below the MRPL requirements set by WADA. In the case of plasma and whole blood, LOQ levels were in the low ng mL^{-1} which is within the range of expected concentrations in such matrices.[48] Absence of an absolute matrix effect in extracts obtained from the three tested biofluids proved that the SPME-HLB-PBT devices provided satisfactory sample clean-up. Indeed, whole blood analysis was facilitated by utilizing rounded SPME-HLB-PBT devices together with the implementation of multiple washing steps and vortex agitation conditions. HLB-PAN was demonstrated to be a more easily wettable coating than C18-PAN, which may be an important criterion to consider when developing disposable devices for spot sampling. The high stability of the biocompatible coating applied on PBT permitted the cutting of smaller portions from an already large coated piece, which may be convenient in cases where tailor-made sizes are needed. Although these devices were developed considering the concept of single-use, they can be re-used in applications where carryover at trace levels does not represent a serious concern. Overall, the introduction of alternative materials, such as polymeric substrates, may represent an important opportunity for new advances in the commercialization and acceptance of SPME in the bioanalytical field. Besides traditional fiber and flat thin-film configurations, the development of other SPME geometries may be facilitated by integrating new materials as supports, and by taking advantage of technologies such as 3-D printing to create innovative samplers.

Associated content

Supporting information

Additional information as noted in the text. This information is available free of charge via the internet at http://pubs.acs.org/.

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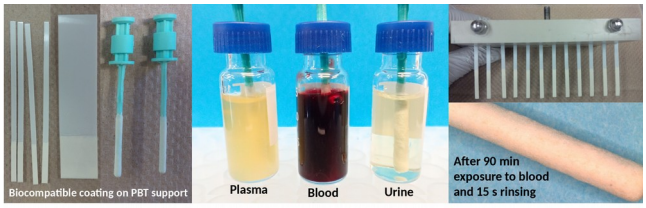
Biocompatible coating on PBT support
Plasma
Blood
Urine
After 90 min
exposure to blood
and 15 s rinsing