RESEARCH PAPER

Matrix compatibility of typical sol–gel solid-phase microextraction coatings in undiluted plasma and whole blood for the analysis of phthalic acid esters

Xiao-Wei Zhang[1] · Yao-Juan Chu[1] · Yu-Hao Li[1] · Xiu-Juan Li[1]

Abstract

Sol–gel materials have been widely used for solid-phase microextraction (SPME) coatings due to their outstanding performance; in contrast, sol–gel SPME coatings have seldom been used for in vivo sampling. The main reason is that their matrix compatibility is unclear. In order to promote the application of this type of coating and accelerate the development of in vivo SPME, in this study, the matrix compatibility of several typical sol–gel coatings was assessed in plasma and whole blood using phthalic acid esters as analytes. The service life of five kinds of sol–gel coatings was among 20–35 times in undiluted plasma, while it was 27 times for a homemade commercial polydimethylsiloxane coating, which indicates good matrix compatibility of sol–gel coatings in untreated plasma. The sol–gel hydroxy-terminated silicone oil/methacrylic acid fiber achieved the highest extraction ability among all of the fibers, and it was tested in pig whole blood. It could be continuously used for at least 22 times, demonstrating good potential for in vivo sampling. Subsequently, a direct-immersion SPME/gas chromatography–flame ionization detection method was established for the determination of 5 phthalic acid esters in blood. Compared with other methods reported in the literature, this method is rapid, simple, sensitive, and accurate, and does not need expensive instruments or tedious procedures. A simulation system of animal blood circulation was constructed to verify the practicability of sol–gel SPME coatings in animal vein sampling. The result illustrated the feasibility of that coating for in vivo blood sampling, but a more accurate quantification calibration approach needs to be explored.

Keywords Solid-phase microextraction · Sol–gel coatings · Matrix compatibility · Blood · In vivo solid-phase microextraction · Phthalic acid esters

Introduction

In vivo sampling is currently an important research topic in the field of analytical chemistry [1, 2]. It is non-invasive or less invasive toward a subject, and allows for real-time monitoring of samples including living systems in natural environments, and consequently can reflect more accurately the organismal physiological and biochemical processes under the nature condition [1]. It is especially necessary for unstable compounds and unstable status [3]. Moreover, in vivo sampling allows multiple samplings of the same system over time, which reduces the interorganism variation and saves cost [4]. In recent years, solid-phase microextraction (SPME) is becoming a promising in vivo sampling technique owing to its ultrasimplified workflow, miniaturized extraction assembly, nonlethal sampling characteristic, minor disturbance to living organisms, and many other advantages [1–4]. It has been applied for in vivo sampling of animals, insects, plants, microorganism, and cells [5]. Although significant progress has been made, the application of in vivo SPME still faces challenges including the development of matrix-compatible coatings and the establishment of quantitative calibration methods [6, 7].

Blood is the most commonly used biological fluid in clinical diagnosis. During the analysis, complex matrix compositions, enzymatic reactions, possible instability of some

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Xiao-Wei Zhang and Yao-Juan Chu contributed equally to this paper.

✉ Xiu-Juan Li
  lixiujuan@mail.hzau.edu.cn; lixj78@126.com

1   Key Laboratory of Environment Correlative Dietology
    (Ministry of Education), College of Food Science
    &Technology, Huazhong Agricultural University,
    Wuhan 430070, China

analytes, pH variations, storage conditions, and the addition of anticoagulants may affect the integrity of samples [6]. Therefore, whole blood tests are more clinically persuasive than plasma and serum tests. In vivo blood SPME sampling eliminates the blood draw step, extracts a little analytes in a short time, and reduces the risk of analyte degradation or transformation during sample pretreatment [8]. Accordingly, in contrast to in vitro whole blood analysis, the result of in vivo blood sampling is more accurate and more convincing. This technique is especially precious for small animals and unstable compounds. However, during direct sampling of whole blood, coating fouling often occurs due to the presence of proteins, blood cells, and other substances [9]. Therefore, matrix-compatible SPME coatings are required to provide satisfactory recoveries and good repeatability in this complex matrix. This is the first problem to be solved for in vivo blood SPME sampling.

Up to now, some biocompatible materials, such as polydimethylsiloxane (PDMS) [10, 11], polypyrrole [8, 12–15], poly(ethylene glycol) [16, 17], polyacrylonitrile (PAN) [9, 18–22], fluoropolymers [23], chitosan [24, 25], collagen [26], and sol–gel coatings [27–31], have been reported as extraction phases for direct-immersion SPME (DI-SPME) for the determination of different analytes in blood, serum, and plasma. However, only a few coating chemistries, such as polypyrrole [12–15], poly(ethylene glycol) [16, 17], PAN [19], and collagen [26], are used for in vivo blood sampling; even though, various drawbacks have hindered their further implementation [6, 32].

Among those extraction phases, PAN-based coatings are the most widely used materials for whole blood analysis due to their anti-fouling performance and amenability to various coating geometries/supports/tunable sorbent chemistry, but they still have some limitations. PAN-based SPME coatings are mainly used for solvent-assisted desorption, and are incompatible with thermal desorption conditions during gas chromatography (GC) analysis due to the thermal degradation of PAN [23]. Polypyrrole and poly(ethylene glycol)-based coatings also have the same limitation. PDMS-based coatings are capable of withstanding typical thermal desorption temperatures, but they usually present bleeding peaks, and may swell when in contact with certain solvents [6, 23]. Furthermore, PDMS-based coatings are mainly used for extraction of nonpolar substances.

Sol–gel technology is widely used for preparing SPME coatings [33, 34]. Numerous significant advantages of sol–gel coatings are reported, namely, easy preparation, high thermal and chemical stability, long lifetime, high surface area, various monomers, possibility to design the material structure, and property by varying the proportion of the sol–gel solution [34]. Furthermore, sol–gel functional hybrid materials are nontoxic and noninjurious to living system and have been widely used for biomedical applications such as scaffolds and matrices for tissue repair and regeneration, nanocarriers for delivering biomolecules, and diagnostic purposes [35]. All of these advantages show the great potential of sol–gel coatings in in vivo SPME application.

So far, the sol–gel coatings have been used for serum and plasma samples for various purposes. Zilfidou et al. developed a fabric phase adsorption extraction membrane coated with a sol–gel adsorbent, and it had a reusability of at least 30 times for extraction of 5 antidepressant drug residues from human serum [27]. Sol–gel coatings also have excellent reusability in more complex plasma samples. Mu et al. developed a sol–gel aptamer SPME fiber to capture adenosine from undiluted human plasma, and the fiber could be used over 20 cycles without significant loss of efficiency [28]. El-Beqqali et al. reported a molecularly imprinted sol–gel tablet for micro-solid-phase extraction of methadone in human plasma, and each tablet could be used for twenty times [29]. When the plasma is diluted, longer lifetime could be obtained. Moein et al. prepared a needle trap utilizing a molecularly imprinted sol–gel xerogel for on-line microextraction of bilirubin from five times diluted plasma, and the needle could be used for approximately one hundred extractions [30]. Recently, a novel SPME fiber based on a capillary glass tube coated with magnetic $Fe_3O_4/Cu_3(BTC)_2$ metal organic framework nanocomposites was prepared using sol–gel matrix as dispersant and adhesive [31]. The fiber was successfully applied for determination of non-steroidal anti-inflammatory drugs in real samples including human urine, serum, plasma, and tablet formulation. These studies illustrate the matrix compatibility of sol–gel SPME coatings in serum and plasma; however, within our knowledge, there is few literature reported on the use of sol–gel SPME fibers for whole blood sampling.

In order to investigate the matrix compatibility of sol–gel SPME coatings, and promote the development of in vivo SPME sampling, in this study, several sol–gel SPME coatings were carefully investigated in plasma and whole blood with phthalic acid esters (PAEs) as model analytes. PAEs are a class of compounds that have been extensively used as plasticizers in different applications. They enter human body and animals through a variety of ways, causing great threats and harm to their lives and health [36]. The implementation of pre- and post-desorption rinsing procedures was significant to extend the coating lifetime. After that, a direct-immersion solid-phase microextraction-gas chromatography (DI-SPME/GC) method was established for analyzing PAEs in whole blood. Finally, a simulation system of animal blood circulation was constructed to explore the applicability of sol–gel coatings for sampling in animal veins, which is the upfront work of in vivo sampling of living animals.

Materials and methods

Chemicals, standard solutions, and samples

Analytical standards (purity greater than 98%) of dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), and di (2-ethylhexyl) phthalate (DEHP) were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China), and detailed information of these analytes is given in Table S1 (Supplementary information). Trifluoroacetic acid (TFA, 99%), n-vinylpyrrolidone (NVP), divinylbenzene (DVB), butyl methacrylate (BMA), and methacrylic acid (MAA) were purchased from the same company. Methanol, acetonitrile, toluene, and acetone were analytically pure and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydroxy-terminated silicone oil (OH-TSO), poly(methylhydrosiloxane) (PMHS), tetraethoxysilane (TEOS), and 3-(trimethoxysilyl)propyl methacrylate (KH-570) were obtained from Hubei Wuhan University Organic Silicone New Material Co., Ltd. (Wuhan, China). Sylgard 184® (PDMS prepolymer and curing agent) was purchased from Dow Corning (Midland, MI, USA). Hydrophilic-lipophilic balanced (HLB) particles were synthesized according to Vasiljevic et al. [37]. Ultrapure water was obtained from a Millipore-Q ultrapure water system (Massachusetts, USA). Stainless steel wire (80 μm o.d.) was purchased from Jinsanshun Stainless Steel Wire Co., Ltd. (Dongguan, China).

A mixture of stock solution containing DMP (5 mg mL^{-1}), DEP (2 mg mL^{-1}), DBP (1 mg mL^{-1}), BBP (1.5 mg mL^{-1}), and DEHP (10 mg mL^{-1}) was prepared by dissolving the above-mentioned analytical standards in methanol. Mixed standard working solutions were prepared by serial dilution of the stock solution with methanol. The stock solution was stored at -18 °C, while all of the working solutions were stored at 4 °C.

Fresh whole pig blood (EDTA-K$_2$ as anticoagulant) was obtained from a slaughter house in Wuhan. All experimental protocols were approved by the Animal Ethics Committee of Huazhong Agricultural University. Plasma was isolated from fresh whole blood by centrifugation (3000 r min^{-1} for 10 min at 4 °C). The whole blood was maintained at 4 °C for a maximum of 1 week and plasma was kept frozen at -80 °C until use. Before using, the sample was brought to room temperature.

Apparatus and chromatographic conditions

The experiments were performed on an SP-6890A capillary GC system (Shandong Lunan Ruihong Chemical Engineering Instrument Co., Ltd., Tengzhou, China) equipped with a capillary splitless injector and a flame ionization detector (FID). A personal computer equipped with a chromatopac model N2000 (Hangzhou Mingtong Technology Co., Ltd., Hangzhou, China) was used to process chromatographic data. Compounds were separated on an SE-54 capillary column (30 m × 0.32 mm × 0.33 μm, Lanzhou ATECH Technologies Co., Ltd., Lanzhou, China). The oven temperature programming was as follows: initial temperature, 100 °C; increased to 280 °C at 15 °C min^{-1}, held for 6 min. The temperatures of the injector and the detector were set at 280 °C. Nitrogen (99.999%) was used as carrier gas and kept at a linear velocity of 12–15 cm s^{-1} for all of the analyses.

Identification of the extracted analytes in blood was performed in an Agilent 6890 N GC coupled to an Agilent 5979B mass selective detector with a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies). The carrier gas was helium at a flow rate of 1.2 mL min^{-1}. A split/splitless injector was used in the splitless mode, and the injector temperature was 280 °C. The mass detector operated in the electron impact mode at 70 eV in a range from 45 to 550 amu, and the ion source temperature was set at 230 °C. The oven temperature programming was the same as GC–FID. Peaks were identified by comparing their mass spectra to the NIST 05 library (matching quality higher than 90%), and further identified using authentic standards.

A magnetic stirrer DF-101S (Zhengzhou Greatwall Scientific Industrial and Trading Co., Ltd., Zhengzhou, China) was employed for stirring the sample during extraction. 24G disposable venous indwelling needles were purchased from Shandong Haidike Medical Products Co., Ltd. (Heze, China). Sterile syringes were purchased from Wuhan Hengkang Medical Instrument Co., Ltd. (Wuhan, China). BT100 peristaltic pump (including PTFE tube, 0.8 mm i.d., 3.8 mm o.d., and 40 cm of length) was attained from Shanghai Qingpu Huxi Instrument Factory (Shanghai, China).

Preparation of SPME fibers

See Supplementary information.

DI-SPME procedure

Eight grams of whole blood (or plasma) and a magnetic stir bar were added into a 10-mL glass vial. The vial was then sealed and put in the magnetic stirrer for 15 min incubation at 37 °C with a stirring rate of 1000 r min^{-1}. DI-SPME was conducted for 40 min at the same condition. After extraction, the fiber was rinsed for 10 s and gently dried with Kimwipe tissue. Desorption was carried out for 15 min at 280 °C, which was followed by a wash step and subsequent cleaning with Kimwipe tissue being soaked in acetone. The rinsing

and washing conditions are described in Table S2 (Supplementary information). For sampling of the diluted sample, a portion of plasma was thoroughly mixed with four portions of ultrapure water, and 8 g of this mixture was submitted to DI-SPME.

Simulation of in vivo SPME sampling

In order to verify the practicability of sol–gel SPME coatings for animal vein sampling, a simulation system of animal blood circulation was constructed (Fig. 1).

A peristaltic pump (1) set at a flow rate of 10 mL min^{-1} functioned as an artificial heart [38], and a PTFE tube acted as an artificial vein. Fresh anticoagulated pig blood (8 g) was placed in a matrix reservoir (2) and the temperature was controlled at 37 °C in a water bath. A venous indwelling needle (3) was implanted into the simulated vein. During sampling, a 1-mL disposable sterile syringe (4) was inserted into the heparin cap to manually push and pull the blood at a sampling rate of 1.4 mL min^{-1}, and a self-made SPME device (5) was also inserted for extraction of 5 min. Rinsing and desorption steps were performed using the same conditions as described for the optimized procedure.

Quantitative calibration for DI-SPME/GC method

Calibration samples were prepared by spiking an appropriate volume of different standard solutions into whole blood. Spiked blood samples were incubated overnight in a refrigerator at 4 °C to permit protein binding and then extracted in DI-SPME mode under the optimized conditions. A plot of the responses versus the concentrations of analytes was then developed, and the unknown concentration initially present in the sample was calculated by extrapolation, which was the x-intercept in the plot.

Recoveries were obtained by standard addition method. Three levels of known quantities of PAEs were spiked to pig whole blood. Each of the samples was analyzed by the developed DI-SPME/GC method, and its concentration was then calculated according to the above linear standard curves. The recovery value was obtained by the ratio of the calculated concentration to the spiked one.

Quantitative calibration for in vivo SPME/GC method

The sampling rate calibration method was used for quantitative calibration [1]. The concentration of analytes in the sample matrix (C_0) can be obtained according to Eq. (1).

$$C_0 = {}^n\!/R_s t \tag{1}$$

where R_S is the sampling rate for an analyte, and n is the amount extracted by the SPME fiber at time t. Before the quantification of C_0, the sampling rate for a targeted analyte was pre-determined using the same equation where C_0 was obtained by DI-SPME/GC method, and n was obtained by liquid injection of a series of standard solutions with different concentrations of analytes. And then, the calculated R_S was used for quantification of other samples.

Results and discussion

More and more SPME coatings are prepared via sol–gel technology because of their outstanding properties [33]. The OH-TSO coating represents the basic chemical structure of sol–gel coatings, and its main chemical composition is polydimethylsiloxane, just like the commercial PDMS coating. It has good extraction ability to nonpolar analytes. DVB is often added to enhance the extraction capacity to

Fig. 1 Schematic of the flow system for animal blood circulation (**a**) and in vivo sampling (**b**)
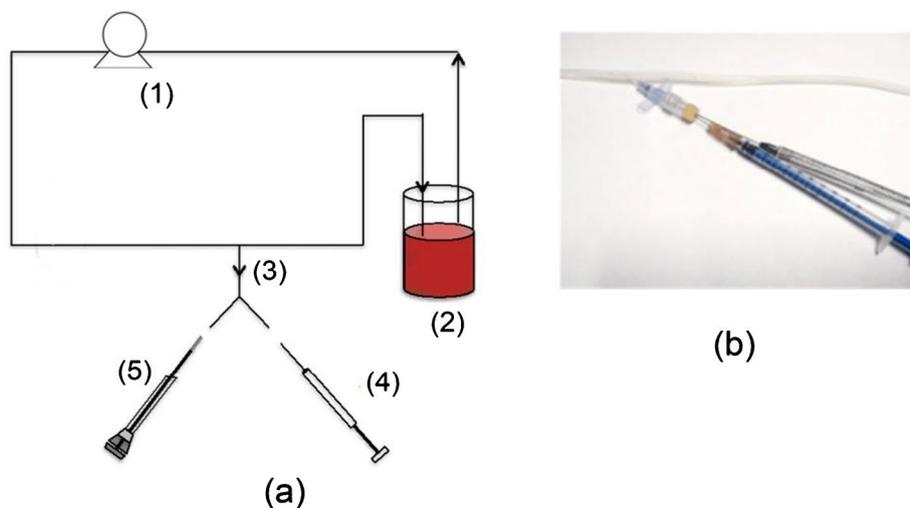
unsaturated compounds through π-π interaction. MAA is a paramount functional monomer for sol–gel coatings and molecularly imprinted coatings to improve the extraction efficiency to polar compounds through hydrogen bonding and dipole–dipole interactions. BMA is less polar than MAA, but its film-forming ability is better than that of MAA, and then it is often used as a substitute for MAA. HLB is a good material to cover a lot of analytes regardless of polarity [39]. Consequently, these five kinds of sol–gel coatings were selected to investigate their matrix compatibility in blood. The preparation and characteristics of these fibers were well described previously [40–42].

PAEs are one of the most frequently detected persistent organic pollutants in the environment. Several of them have been shown to cause negative health effects to animals and humans and their use is restricted in most countries. PAEs are a class of compounds with similar structure, but exhibit a large variety of physicochemical properties due to different carbon chains. Table S1 (Supplementary information) shows the physicochemical properties of five analytes tested in this paper. The logarithm of the octanol–water partition coefficients (log K_{OW}) shows a wide range, from 1.60 for the rather hydrophilic DMP to 7.60 for the very hydrophobic DEHP, which covers the logK_{OW} of most of analytes for GC instrument [39]. In addition, the solubility also varies greatly, from 4000 to 0.27 mg L^{-1}. In the structure, PAEs compounds contain unsaturated benzene ring, polar ester group, and nonpolar alkyl group, which are the most typical groups for organic compounds. As a result, these PAEs are selected as model analytes to investigate the performance of the sol–gel coatings.

Exploration of pre-desorption rinsing and post-desorption washing conditions of sol–gel OH-TSO coating in blood and plasma

Previous research has shown that a cleaning procedure including a rapid rinsing of the fiber after extraction and a post-desorption washing after desorption plays an important role in protecting SPME coatings from fouling as a result of direct exposure to complex matrices [43, 44]. Following these recommendations, the rinsing and washing conditions, summarized in Table S2 (Supplementary information), were investigated based on the sol–gel OH-TSO coating.

As shown in Fig. S1 (Supplementary information), methods 1–3 did not give good results. The amounts extracted increased at first, and then decreased dramatically. At the beginning, the unpolymerized components in the coating might be eluted and some absorption sites were released, and thereby the extraction capacity of the coating increased. However, as the number of extraction times increased, blood cells, proteins, and other substances in whole blood adhered to the surface of the coating, and were further solidified on

the surface during the high-temperature desorption, which consequently decreased the extraction capacity. Fig. S1 (Supplementary information) declared that the cleaning procedures of these methods could not effectively remove the fouling, but method 3 showed some positive effect on the cleaning because the peak areas declined more slowly than those using the other two methods. Acetone probably cleared away some of the residues since it demonstrated excellent capacity to remove the fouling characterized by high content of sugar, fat, and proteins [43, 44]. Unfortunately, the whole blood sample in the laboratory was used up, and it was difficult to obtain due to the occurrence of African swine fever and COVID-19. The subsequent cleaning procedure had to be investigated in plasma preserved in an ultracold storage freezer.

As listed in method 4 (Table S2, Supplementary information), the post-desorption washing time was extended to 3 min, and simultaneously, the plasma was diluted with 4 times of ultrapure water to reduce matrix interference and guarantee good repeatability during the test. Most of the compounds showed acceptable repeatability during the first 10 extractions. Except DEHP, the RSD of the peak areas of other analytes was less than 7%. The relative amounts extracted by the fiber of the other nine extractions were between 83.92 and 115.86% compared to the first one. However, the extraction capacity declined significantly after 20 consecutive extractions (Fig. S1d). After each post-desorption washing, the coating was gently wiped using acetone-soaked Kimwipe tissue. Fig. S2 (Supplementary information) showed that most of the debris attached to the coating surface was cleaned. In method 5, a mixture of water and 0.1 mol L^{-1} hydrochloric acid solution was used for washing. Under this condition, the fiber showed satisfactory repeatability during 20 extractions with RSD from 4.7 to 7.8% except DEHP.

Lifetime and extraction ability of sol–gel coatings in undiluted plasma

In the above experiments, the OH-TSO coating showed acceptable repeatability in diluted plasma with method 5. Considering the possible potency of method 5 in undiluted plasma, Fig. S3 (Supplementary information) evaluated 52 consecutive extractions in undiluted matrix. During the first 35 extractions, the amounts extracted of 5 PAEs did not change a lot, and the RSD ($n = 35$) for each analyte varied within 4.4–19.6%. After 52 consecutive extractions, the fiber was still good for extraction of DEP and DBP, with a RSD of 9.9% and 11.1%, respectively. The lifetime of OH-TSO coating in undiluted plasma is longer than that of the aptamer sol–gel SPME coating [28] and the molecularly imprinted sol–gel tablet coating [29], but shorter than that of the C18–PAN thin-film coating [9]. The latter could be

Table 1 The lifetimes of 6 coatings in undiluted plasma and the RSDs of their consecutive extractions of 5 PAEs

Coating	Extraction numbers	RSD (%)				
		DMP	DEP	DBP	BBP	DEHP
OH-TSO	35	19.61	11.44	4.74	4.40	16.45
OH-TSO/MAA	28	7.01	9.03	7.70	14.80	14.30
OH-TSO/MAA/HLB	25	23.10	13.69	6.73	10.49	20.66
OH-TSO/DVB	21	13.08	8.93	4.56	10.04	15.83
OH-TSO/DVB/BMA	20	19.47	13.32	19.74	13.21	26.34
Self-made PDMS	27	26.57	32.33	12.38	21.73	26.57

used at least 70 times for human plasma analysis. Due to the incompatibility with thermal desorption, the PAN-based coatings are desorbed by solvents. During the solvent desorption process, substances attached to the surface of the coating can be further removed, thereby extending the lifetime of the coating. In this study, PAEs were desorbed at high temperature, which caused the impurities to bind more firmly to the coating surface, thereby affecting its reusability.

In order to investigate the matrix compatibility of other sol–gel SPME coatings in undiluted plasma, 3 commonly used sol–gel fibers were prepared, namely OH-TSO/MAA, OH-TSO/DVB, and OH-TSO/DVB/BMA fibers. Given the good anti-matrix interference performance of PDMS materials, PDMS probes were also coated and used as a comparison. Recently, SPME extraction phase made of HLB particles immobilized with PAN was used because of its excellent extraction ability to a wide range of analytes [37, 39], and therefore, OH-TSO/MAA/HLB fiber was prepared

and applied to plasma matrix. The results for those coatings are summarized in Table 1.

The lifetime is defined according to the RSD of a group of consecutive extractions (below or around 20%) and the extraction capacity (the extraction capacity does not decrease dramatically). As is shown, the OH-TSO coating had the longest lifetime in undiluted plasma. The service life of OH-TSO/MAA coating was 28 times in plasma, and the surface of the coating was smooth after being wiped with acetone-soaked Kimwipe tissue. Besides, the repeatability was better than that of the OH-TSO coating. The surface of OH-TSO/MAA/HLB fiber was relatively rough because HLB particles were blended in the coating. After being wiped by the tissue, the coating became thinner, the extraction capacity decreased, and the service life decreased. The sol–gel OH-TSO/DVB fiber was not easy to be coated, and the surface was not as smooth as those of the OH-TSO/MAA and OH-TSO coatings, which affected its repeatability and lifetime.

Fig. 2 Comparison of the extraction capability of different coatings to 5 PAEs in plasma. Spiked concentration: 125 μg kg^{-1} for each PAEs. Extraction and desorption conditions: extraction time, 30 min; extraction temperature, 37 °C; desorption temperature, 280 °C; desorption time, 10 min; cleaning condition, method 5. Different letters indicate that there is a significant difference in the groups ($p < 0.05$), and the same letter indicates that there is no significant difference ($p > 0.05$)
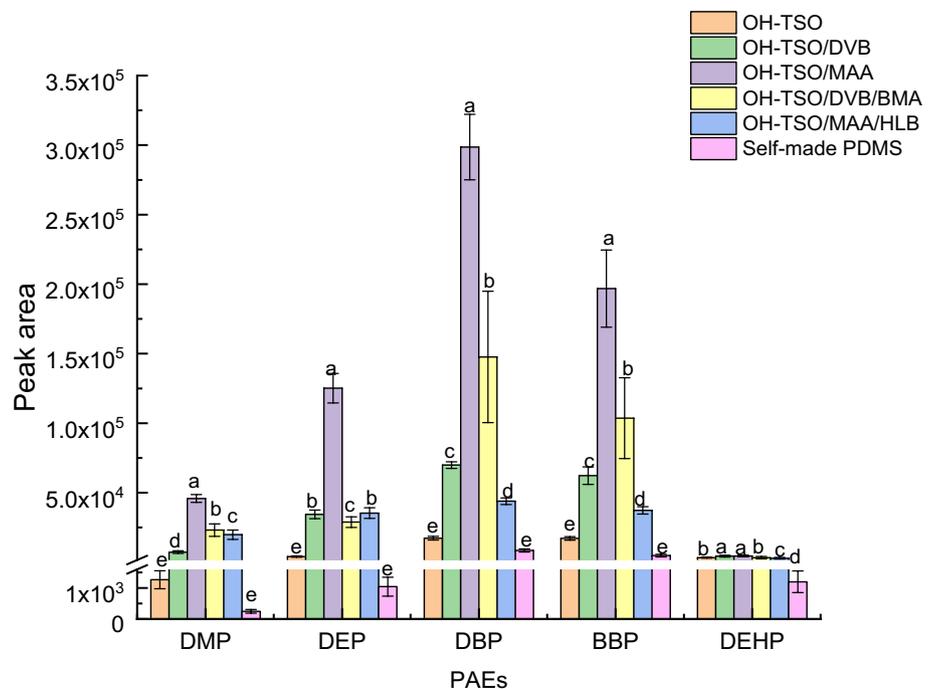
Table 2 The linear ranges, coefficients of determination (R^2), precision (RSD), LOD, and LOQ

Analytes	Linear range ($\mu g\ kg^{-1}$)	R^2	LOD[a] ($\mu g\ kg^{-1}$)	LOQ[b] ($\mu g\ kg^{-1}$)	RSD[c] (%, $n=5$)
DMP	1.25–62.5	0.9989	0.655	1.250	10.29
DEP	0.5–25	0.9990	0.233	0.500	9.06
DBP	0.25–12.5	0.9994	0.025	0.084	8.74
BBP	0.375–18.5	0.9969	0.170	0.375	14.20
DEHP	2.5–125	0.9986	0.728	2.426	9.05

[a]The LOD and [b]LOQ were obtained based on the standard deviation (δ) of the responses in six replicated blank extractions and the slope (S) of the calibration curve according to the equations LOD $= 3\ \delta/S$ and LOQ $= 10\ \delta/S$

[c]Spiking level: 6.25 $\mu g\ kg^{-1}$ for DMP, 2.5 $\mu g\ kg^{-1}$ for DEP, 1.25 $\mu g\ kg^{-1}$ for DBP, 1.875 $\mu g\ kg^{-1}$ for BBP, and 12.5 $\mu g\ kg^{-1}$ for DEHP

As for the sol–gel OH-TSO/DVB/BMA fiber, the coating was structurally loose and easy to fall off, and as a result, the reusability was not good. The service life of the homemade PDMS coating was 27 times, but the repeatability was poor, and the RSDs of 4 PAEs were all higher than 20%.

The applications of sol–gel materials in biomedical field [35] suggest their excellent matrix compatibility in blood, which means that the materials are less likely to hurt themselves as well as the blood components. At the same time, sol–gel technology gives the coating a three-dimensional network structure and nano-scale pores, which can isolate biological macromolecules from the surface. DVB, BMA, and other ingredients are usually added to improve the extraction performance of the coating, but it seems that they do not contribute much to the service life of the coating, which may be related to many factors. On the one hand,

PAEs require long extraction time and high desorption temperature; on the other hand, these components are small molecules, their polymerization conditions are unclear, and their thermal stability is also poorer than that of PDMS, OH-TSO, and other silylating agents. Generally speaking, the sol–gel coated fibers showed acceptable matrix compatibility in undiluted plasma.

Figure 2 compares the extraction capacity of different coatings. As expected, the extraction capacity of sol–gel OH-TSO coating was higher than that of the self-made PDMS due to the outstanding properties of sol–gel technology. MAA, DVB, BMA, and HLB all could enhance the extraction capacity to PAEs. OT-TSO/MAA fiber achieved the highest extraction capacity owing to the strong polarity of MAA. The HLB coating was unexpectedly poor and later

Table 3 Recoveries and precision (RSD) of PAEs in spiked whole blood samples

Analytes	Spiking levels ($\mu g\ kg^{-1}$)	Recovery (%)[a]	RSD (%, $n=3$)
DMP	2.5	96.28	6.58
	12.5	97.73	9.93
	62.5	100.92	6.18
DEP	1	104.19	10.99
	5	108.67	15.85
	25	99.85	9.97
DBP	0.5	119.89	7.18
	2.5	93.91	2.48
	12.5	99.73	3.52
BBP	0.75	82.93	6.46
	3.75	108.71	15.08
	18.75	101.02	5.08
DEHP	5	115.96	11.84
	25	86.01	13.43
	125	100.09	10.87

[a]A t-test was applied for statistical processing; the results indicate that there is no difference between the spiked concentration and the measurement result ($p > 0.05$)
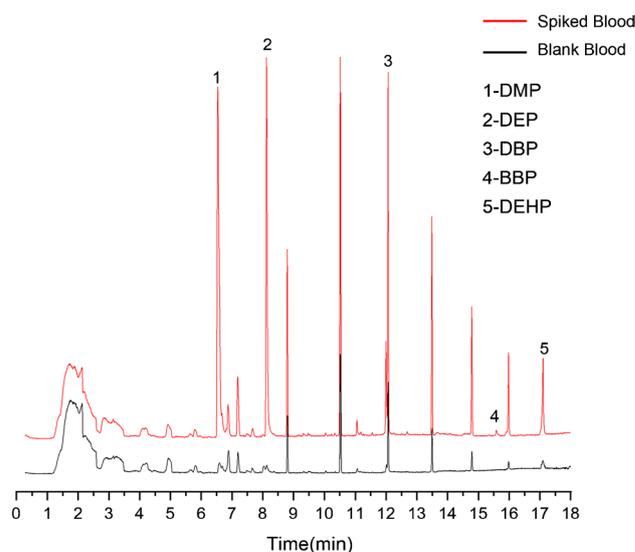

Fig. 3 Chromatograms of 5 PAEs in spiked and unspiked whole blood by DI-SPME/GC–MS. Spiked concentration: DMP, 62.5 $\mu g\ kg^{-1}$; DEP, 25 $\mu g\ kg^{-1}$; DBP, 12.5 $\mu g\ kg^{-1}$; BBP, 18.75 $\mu g\ kg^{-1}$; DEHP, 125 $\mu g\ kg^{-1}$. Extraction and desorption conditions: extraction time, 30 min; extraction temperature, 37 °C; desorption temperature, 280 °C; desorption time, 10 min; cleaning condition, method 5

Table 4 Comparison of the proposed DI-SPME/GC-FID method with other methods reported in literatures for determination of PAEs in blood

Methods	Sample pretreatment	Linear range	LOD	R^2	RSD (%)	Ref
Dispersive magnetic SPE-GC/MS	Plasma was mixed with HCl (37% w/w) and TFA to precipitate proteins. Afterwards, the resultant solution was vortexed and then centrifuged. The supernatant was separated and diluted with ultrapure water	0.5–200 g L^{-1}	0.08–0.15 g L^{-1}	0.9977–0.9993	2.50–9.50	[46]
SPE-GC/MS	Serum was loaded onto and passed through the cartridge. After washing with 5% acetonitrile aqueous solution, the analytes were eluted with acetonitrile and collected in clean glass tubes. The eluent was evaporated to dryness under a gentle stream of nitrogen at 45 °C. The residue was reconstituted for analysis in acetonitrile	5–1000 ng mL^{-1}	2.3–13 ng mL^{-1}	0.9869–0.9973	5.2–13.4	[47]
Dispersive liquid–liquid microextraction-GC/MS	0.2 mg trichloroacetic acid removes proteins from 10 mL plasma and yields 7 mL supernatant. Then the supernatant was extracted with 750 μL acetonitrile containing 10 μL chlorobenzene. This solution (pH = 5) was centrifuged for 5 min at 4500 rpm. After centrifuging, the dispersed fine droplets of extracting solvent sedimented at the bottom of the test tube. The volume of the sedimented phase was determined by a 100 μL microsyringe. 2 μL of the sedimented phase was removed by a 10 μL microsyringe and injected into GC	50–1000 ng mL^{-1}	1.5–2.5 ng mL^{-1}	0.996–0.998	3.2–4.1	[48]
DI-SPME-GC/MS	The serum lyophilized content of Moni-Trol vials was reconstituted with water and homogenized at room temperature. After reconstitution, 2 mL samples were spiked with phthalate ester standard solution. After 1 h of contact time, samples were pretreated by adding 0.5 mL of acetonitrile. Extraction was performed by direct-immersion SPME	30–4000 ppb	15 pg μL^{-1}	0.978–0.993	< 4	[49]
DI-SPME-GC/FID	Anticoagulated whole blood was directly put to SPME	0.25–125 μg kg^{-1}	0.025–0.728 μg kg^{-1}	0.9968–0.9994	8.74–14.20	Present method

it was found that the particles were too large. After the particles were ground to a size of 1–5 μm, the coating had better extraction efficiency (the data were not shown in this paper).

Lifetime of sol–gel OH-TSO/MAA coating in whole blood

Due to its excellent performance to PAEs in plasma, OH-TSO/MAA fiber was tested in whole blood, and the results are given in Fig. S4 (Supplementary information). The OH-TSO/MAA fiber could be continuously used for 22 times in whole blood with RSD between 7.27 and 14.84% for 5 PAEs. After cleaning, the coating surface was still smooth and clean under the microscope. Recently, a new SPME coating, namely fluoropolymer/hydrophilic–lipophilic balance, was developed and it could be used for 15 consecutive extractions in whole blood samples [23]. The comparison demonstrates the good matrix compatibility of OH-TSO/MAA fiber in whole blood, indicating its application prospect as probes

Table 5 Comparison of the concentrations of *C* obtained by the two sampling methods

Analytes	Spiking levels (μg kg^{-1})	C (μg kg^{-1})		P^a
		In vivo SPME	DI-SPME	
DMP	1.25	15.74 ± 0.67	1.51 ± 0.35	< 0.05
	6.25	17.12 ± 0.34	7.81 ± 1.90	< 0.05
	25	21.52 ± 1.52	20.45 ± 2.56	> 0.05
	41.67	32.44 ± 3.80	31.83 ± 5.02	> 0.05
DEP	0.5	1.69 ± 0.47	0.75 ± 0.56	> 0.05
	2.5	2.53 ± 0.19	2.53 ± 0.48	> 0.05
	10	6.92 ± 0.82	8.73 ± 1.05	> 0.05
	16.67	14.35 ± 0.95	16.20 ± 2.24	> 0.05
DBP	0.25	2.08 ± 0.11	0.31 ± 0.14	< 0.05
	1.25	2.97 ± 0.26	1.64 ± 0.44	< 0.05
	5	5.31 ± 0.22	4.74 ± 0.49	> 0.05
	8.33	7.32 ± 0.67	8.10 ± 0.71	> 0.05
BBP	0.375	3.55 ± 0.36	0.39 ± 0.47	< 0.05
	1.875	3.41 ± 0.13	2.25 ± 0.77	> 0.05
	7.5	5.69 ± 0.58	5.00 ± 0.69	> 0.05
	12.17	8.25 ± 0.70	9.49 ± 1.60	> 0.05
DEHP	2.5	47.83 ± 1.49	2.16 ± 0.22	< 0.05
	12.5	51.45 ± 3.11	13.04 ± 2.91	< 0.05
	50	57.98 ± 1.84	40.45 ± 3.54	< 0.05
	83.33	77.44 ± 3.84	76.73 ± 6.81	> 0.05

^{a}A *t*-test is applied for statistical processing; $p < 0.05$ indicates that there is a significant difference in the groups, and $p > 0.05$ indicates that there is no significant difference

for blood sampling. Nonetheless, measures should be taken to further improve the lifecycles of sol–gel SPME coatings.

Establishment and evaluation of DI-SPME/GC-FID method for PAEs in whole blood using sol–gel OH-TSO/MAA fiber

In order to further study the performance of sol–gel coatings in whole blood, a DI-SPME/GC-FID method was established to detect PAEs in blood using sol–gel OH-TSO/MAA fiber after several parameters associated with SPME efficiency were optimized (Fig. S5, Supplementary information).

As shown in Table 2, the linear ranges were achieved in μg kg^{-1} levels with good coefficients of determination for all analytes. The precision, expressed as RSD, gave satisfactory outcomes, ranging from 8.74 to 14.20%. The LODs were between 0.025 and 0.728 μg kg^{-1}. The method was used to analyze PAEs in unspiked pig blood sample. The result showed that the sample was positive and the concentrations were 0.46 μg kg^{-1} for DMP, 0.84 μg kg^{-1} for DEP, 0.41 μg kg^{-1} for DBP, and 1.61 μg kg^{-1} for DEHP. BBP was not detected. And then,

recovery tests were performed in order to study accuracy. As shown in Table 3, the recoveries of five PAEs in the spiked blood were between 82.93 and 119.89% with RSD ≤ 15.85%, revealing the good accuracy and repeatability of the method, and meet the FDA criteria of bioanalytical validation guidelines [45]. In order to confirm the result, both a spiked sample and an unspiked sample were analyzed by GC–MS. Figure 3 shows the chromatograms. In the blank sample, both of the two small peaks at the retention time of peak 1 are DMP, the small peak on the left of peak 2 is diethyltoluamide, and the interfering peak on the left of peak 3 is octadecamethylcyclononasiloxane (from the column coating). They are difficult to be separated from the analytes, and cause influence on the recovery, but have little effect when the concentrations of analytes are high.

Table 4 compares the sample pretreatments and analytical performance of the proposed DI-SPME/GC-FID method and other methods reported in literature for determination of PAEs in blood samples. The proposed method is rapid, simple, sensitive, and does not need expensive instruments or tedious procedures. The LODs were similar with or rather lower than those obtained by the reported methods.

The potential of sol–gel coatings for in vivo SPME/GC

SPME is a non-exhaustive extraction technique in which only a small part of analytes is removed from the sample matrix. Therefore, the results of SPME need to be calibrated so as to get the accurate concentrations. The existing calibration methods of SPME have been summarized and discussed in other document [1]. Thereinto, the sampling rate calibration approach is widely used for in vivo sampling [1, 7]. It assumes that within a linear model of an extraction process, the rate of mass transfer (or sampling rate) remains constant throughout the duration of sampling, and the relationship between the concentration of target analyte in the sample matrix and the extracted amount of analyte can be expressed by Eq. (1). The sampling rates for targets can be pre-determined under laboratory conditions, and then can be directly used for quantification during in vivo analysis.

Figs. S6 and S7 (Supplementary information) investigate the sampling conditions, and the R_s were obtained and presented in Table S3 (Supplementary information).

Four levels of PAEs standard solutions were spiked to whole blood samples. After overnight incubation at 4 °C, the samples were analyzed by DI-SPME/GC method and in vivo SPME/GC method, respectively. As shown in Table 5, for the two high spiking levels, the detected values by in vivo sampling were close to the spiked ones, but for the low spiking levels, the method was noneffective. However, the DI-SPME sampling was always effective for all of the concentrations. According to the current research, R_s may be related to the

concentrations of analytes in the sample. The concentration of R_s calculated (Table S3, Supplementary information) was between the two high concentrations in Table 5, and the concentrations obtained by these rates were more accurate. Follow-up studies will be carried out for more accurate quantification for in vivo sampling.

Conclusion

It's the first time to systematically and comprehensively assess the matrix compatibility of sol–gel coatings in blood samples. The model analytes are PAEs. The extraction time is long and the desorption temperature is high. These factors are not conducive to service life evaluation. Even so, the lifetime of these fibers was similar or longer than other fibers reported in literature, indicating their good matrix compatibility in blood. The successful application of these coatings in whole blood and artificial veins demonstrates the great potential of sol–gel SPME coatings in in vivo sampling, which is a very important technique for animal research. In the long run, it is necessary to further extend the service life of the coatings, and at the same time, it is necessary to develop and improve more effective quantitative correction methods for in vivo sampling.

Declarations

Ethics approval All experimental procedures were approved by the Animal Ethics Committee of the Huazhong Agricultural University.

Conflict of interest The authors declare no competing interests.

Animal welfare All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The blood samples used in this work were obtained from a slaughter house that provides pork for supermarkets. As this blood was not collected specifically for this study, this work was exempted from IACUC approval.

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