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Review

# Novel stir bar sorptive extraction methods for environmental and biomedical analysis

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**Abstract**

Stir bar sorptive extraction (SBSE) is sample preparation technique that involves the extraction and enrichment of organic compounds from a liquid sample. The technique is based on the principle of sorptive extraction. A large amount of extraction phase is coated on a stir bar. An analyte is extracted into the extraction phase, based on its octanol–water partitioning coefficient and the phase ratio. Recently, various methods involving SBSE were developed in order to further facilitate analysis and improve sensitivity. In this review, we focused on the novel methods that involve SBSE with in situ derivatization, SBSE with in situ de-conjugation, thermal desorption (TD) in the multi-shot mode and TD with in tube derivatization method. Those methods were applied successfully to the trace analysis of environmental and biological samples and extremely low detection limits were achieved.


*Keywords:* Stir bar sorptive extraction (SBSE); In situ derivatization; In situ de-conjugation; Multi-shot mode; In tube derivatization; Water; Urine; Blood

## Contents



## 1. Introduction

Analytical methods generally require an extraction and enrichment step before an analyst can perform the chromatographic separation and detection of trace organic compounds in aqueous matrices. During the extraction and enrichment step,

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the trace solutes are isolated from the matrix and concentrated to enable their identification or quantification. In environmental, biomedical and other types of analyses, the analyst uses a variety of extraction and enrichment techniques, including liquid–liquid extraction (LLE), solid-phase extraction (SPE), liquid–gas extraction (such as purge-and-trap) and liquid–gas equilibrium (such as static headspace (HS)).

Miniaturization has become one of the dominant trends in analytical chemistry. Typical examples of miniaturization techniques for sample preparation include micro-liquid–liquid extraction [1,2] (or in-vial liquid–liquid extraction [3]), disc-cartridge SPE [4,5], on-line SPE [6,7], fiber-in tube SPE [8], solid-phase microextraction (SPME) [9], in tube SPME [10], solid-phase dynamic extraction (SPDE) [11], microextraction in a packed syringe (MEPS) [12], liquid phase microextraction (LPME) [13], single drop microextraction (SDME) [14], solvent bar microextraction [15] and membrane assisted extraction (MAE) [16]. Such techniques, when used in combination with state-of-the-art analytical systems, can result in faster analysis, higher sample throughput, lower solvent consumption and less manpower per unit sample while maintaining or even improving sensitivity. In particular, the reduction of solvent consumption in analytical laboratories is expected to contribute significantly to the reduction of analytical costs. In most instances, miniaturized sample preparation techniques can also be automated and coupled on-line to the analysis. The on-line coupling of extraction and analysis, whereby the entire extract is transferred to the analytical system, results in higher sensitivity and lower potential analyte loss. Moreover, the analyst can use smaller sample volumes.

In the past, analytical chemists gave much attention to solvent-free sample preparation techniques that are based on sorptive extraction using gum base. Those techniques include SPME [9] and stir bar sorptive extraction (SBSE) [17]. Sorptive extraction has proven to be an interesting and environmentally friendly alternative to liquid extraction. In sorptive extraction, the analytes are extracted from the matrix (mostly aqueous) into a non-miscible liquid phase. In contrast to extraction with adsorbents in which the analytes are bound to the active sites on a surface, not only the surface area but also the total amount of the extraction phase is important in sorptive extraction. The most widely used sorptive extraction phase is polydimethylsiloxane (PDMS). This phase is well known as a stationary phase in gas chromatography (GC), is thermo stable, can be used over a wide temperature range (220–320 °C), and has interesting diffusion properties. The extraction with PDMS can therefore be compared with micro-liquid–liquid extraction. After the extraction, the solutes can be introduced quantitatively into the analytical system by thermal desorption (TD) or liquid desorption (LD). The former process has high sensitivity because the entire extract can be analyzed by TD and GC. On the other hand, the latter process can be applied to high performance liquid chromatography (HPLC) [18–28] or capillary electrophoresis (CE) [28,29], and high polarity or thermally labile compounds can be analyzed.

Baltussen et al. [30] have reviewed the principles and applications of sorptive extraction, and David et al. have reviewed the principles and applications of the SBSE technique [31]. The main difference between SPME and SBSE is the much larger volume of PDMS used in the latter, which results in higher recoveries and higher sample capacity. However, since the PDMS phase is a non-polar liquid phase, it is preferable that the polarity of the analyte be low. Relatively high polarity compounds are not well recovered.

Recently, various methods involving SBSE were developed in order to further facilitate analysis and improve sensitivity. In this review, we focused on the novel methods that involve SBSE with in situ derivatization, SBSE with in situ de-conjugation, TD in the multi-shot mode and TD with in tube derivatization method. Those methods were applied successfully to the trace analysis of environmental and biological samples.

## 2. Theory

In the mid 1980s, different research groups [32–35] investigated the extraction of organic compounds from an aqueous or gas phase using open-tubular traps coated with thick PDMS films. However, practical limitations, such as low sample capacity and small breakthrough volumes, have limited the applicability of the PDMS-coated open-tubular traps. Some 10 years ago, Arthur and Pawliszyn developed a microextraction method based on PDMS sorption and called it SPME [9]. Because of its simplicity and performance, SPME created a lot of interest in sorptive extraction techniques. The advantages of sorptive extraction include predictable enrichment, the absence of displacement effects, inertness and rapid TD at mild temperatures. In SPME, however, the amount of extraction phase (e.g. the amount of PDMS-coated on the fiber) is very small. For a typical 100 μm PDMS fiber, which is the most widely used fiber, the volume of the extraction phase is approximately 0.5 μl. Consequently, the extraction efficiency for solutes that are partially water soluble is quite low [36]. For very polar compounds, however, competition can occur between the aqueous phase, the SPME fiber, the glass wall of the extraction vessel, and the surface of the polytetrafluoroethylene stir bar used to stir samples [37,38].

Based on these observations, a new extraction technique called SBSE was developed. Stir bars were coated with a layer of PDMS and used to stir aqueous samples, thereby extracting and enriching solutes into the PDMS layer [17]. Although the extraction phase in SBSE is the same as that in SPME, its amount is 50–250 times larger. After extraction, the solutes are thermally desorbed and analyzed by GC in a similar manner to SPME. Alternatively, the analytes can be desorbed by LD. Therefore, the basic principles of SPME and SBSE are identical.

Sorptive extraction is, by nature, an equilibrium technique, and for water samples, the extraction of solute from the aqueous phase into the extraction phase is controlled by the partitioning coefficient of the solute between the silicone phase and the aqueous phase. Recent studies have correlated this partitioning coefficient with the octanol–water distribution coefficient ($K_{o/w}$). Although not exactly correct, $K_{o/w}$ gives a good indication of whether and how well a given solute can be extracted with SPME or SBSE.

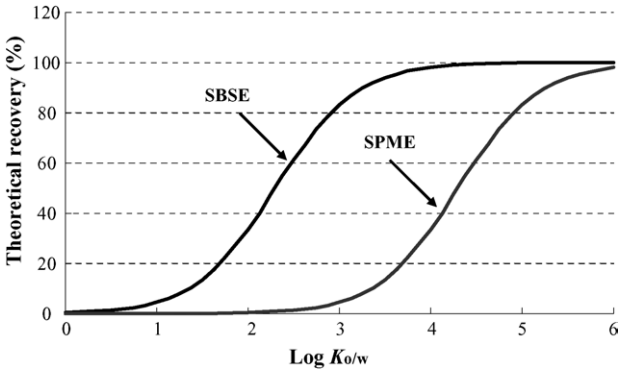


Fig. 1. Theoretical recoveries of SBSE and SPME. Theoretical recoveries for solutes as a function of the octanol–water partitioning coefficient $K_{o/w}$ for SBSE (10 ml sample, 50 μl PDMS phase) and SPME (10 ml sample, 0.5 μl PDMS phase).

However, it is very important in this respect to realize that sorptive equilibrium is also dependent upon the phase ratio ($\beta$), and thus the amount of PDMS applied. This relationship is shown in Eq. (1).

$$K_{o/w} \doteq K_{PDMS/w} = \frac{C_{PDMS}}{C_w} = \left[\frac{m_{PDMS}}{m_w}\right]\left[\frac{V_w}{V_{PDMS}}\right] = \beta\frac{m_{PDMS}}{m_w} \quad (1)$$

The distribution coefficient between PDMS and water ($K_{PDMS/w}$) is defined by the ratio of the concentration of a solute in the PDMS phase ($C_{PDMS}$) to the concentration of the solute in water ($C_w$) at equilibrium. This ratio is equal to the ratio of the mass of the solute in the PDMS phase ($m_{PDMS}$) to the mass of the solute in the aqueous phase ($m_w$) multiplied by $\beta$ (where $\beta = V_w/V_{PDMS}$). The theoretical recovery, which is expressed as the ratio of the extracted amount of solute ($m_{PDMS}$) to the original amount of solute in water ($m_0$, with $m_0 = m_w + m_{PDMS}$), is thus dependent upon $K_{PDMS/w}$ and $\beta$, as described in Eq. (2).

$$\frac{m_{PDMS}}{m_0} = \frac{\left[\frac{K_{PDMS/w}}{\beta}\right]}{1 + \left[\frac{K_{PDMS/w}}{\beta}\right]} \quad (2)$$

Using this equation, the analyst can calculate the theoretical recovery for a solute with a known partition coefficient and a given $\beta$. From Eq. (2), it is also clear that the extraction efficiency is increased with increasing $K_{PDMS/w}$. Because $K_{PDMS/w}$ is similar to $K_{o/w}$, the analyst can predict the extraction efficiency. Besides $K_{PDMS/w}$, $\beta$ (volume of sample/volume of PDMS) is also important. The larger the amount of PDMS is, the larger $\beta$ is and the higher the extraction efficiency is. In addition, Bicchi et al. have reported that $\beta$ is an important parameter at the recovery and the equilibrium time [39]. When $\beta$ is large, the recovery is decreased and the equilibrium time is extended. On the other hand, when $\beta$ is small, a high recovery and a short equilibrium time are obtained. Therefore, in SBSE, it is important to increase the amount of PDMS as much as possible.

Fig. 1 shows the influence of $K_{o/w}$ and $\beta$ on the theoretical recovery. In SPME, the volume of PDMS is approximately 0.5 μl. This results in low recoveries for solutes with low $K_{o/w}$ values, e.g. less than 10,000. In SBSE, 25–125 μl of PDMS coating is used. Consequently, the sensitivity is increased by a factor of 50–250. The theoretical recovery reaches 100% for solutes with $K_{o/w}$ values lower than 500 ($\log P$ greater than 2.7). The theoretical recoveries can be calculated for a given sample volume, selected stir bar dimensions, and a solute using KowWIN software (Syracuse Research Corp., Syracuse, New York, USA), which is based on a $\log K_{o/w}$ calculator.

## 3. SBSE tools

Twister PDMS-coated stir bars are available from Gerstel GmbH (Mülheim an der Ruhr, Germany). These stir bars have three essential parts. The first and innermost part is a magnetic stirring rod, which is necessary for transferring the rotating movement of a stirring plate to the liquid sample. The second part is a thin glass jacket that covers the magnetic stirring rod. The third and outermost part is the layer of PDMS sorbent into which the analytes are extracted. The glass layer is essential for the construction of a high-quality stir bar as it effectively prevents the decomposition of the PDMS layer, which is catalyzed by the metals in the magnetic stirring rod. Recently, a novel approach that applied sol–gel technology to the coating material for the stir bar was reported [40]. In addition, an SBSE device was prepared using alkyl-diol-silica (ADS) restricted access material (RAM) as the coating layer [27]. Moreover, PDMS rod extraction as novel, simple and inexpensive approach to absorptive extraction of compounds from environmental samples was reported [41]. It is expected that the SBSE technique using a novel sorptive phase would have wide-ranging applications.

## 4. Instrumentation

In contrast to SPME, in which desorption takes place at the inlet of a gas chromatograph, SBSE is used in combination with a TD system. Because a larger amount of an extraction phase is used, the desorption process for SBSE is slower than that for an SPME fiber, and thus desorption combined with cold trapping and reconcentration is required. The entire process is automated, and two systems are available commercially: the TDS-A classic TD system and a specially designed Twister desorption unit (both from Gerstel). The systems can be mounted on a gas chromatograph equipped with a CIS-4 programmed-temperature vaporizing inlet (Gerstel). The programmed-temperature vaporizing injector is used as a cryotrap for cryogenic refocusing of the thermally desorbed analytes. Temperatures as low as −150 °C are used together with liquid nitrogen cooling. Both systems allow fully automated control of all desorption, trapping and injection conditions, including temperatures, flows and split or splitless modes. On the other hand, in the HPLC analysis, it was able to use conventional HPLC system.

## 5. Methods

### 5.1. Stir bar sorptive extraction

Stir bar sorptive extraction of a liquid sample is performed by placing a suitable amount of sample in a headspace vial or

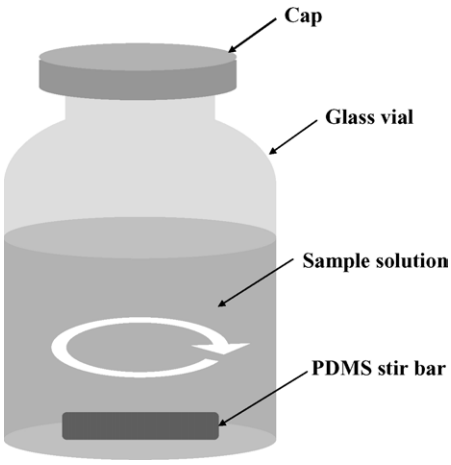


Fig. 2. Schematic of SBSE set-up.

a container. A PDMS-coated stir bar is added and the sample is stirred for 30–240 min (Fig. 2). The extraction time is controlled kinetically; it is determined by the sample volume, the stirring speed and the stir bar dimensions, and must be optimized for a given application [39]. Optimization is normally accomplished by measuring analyte recovery as a function of the extraction time. The optimum conditions are obtained when no additional recovery is observed even when the extraction time is increased further. Some methods have been reported to improve the recovery of the analyte [42–44]. The recovery of a high polarity analyte is improved when sodium chloride is added to the sample [42,43]. On the other hand, the adsorption of low polarity analyte to the surfaces of the glass and the sample matrix is suppressed by using such organic solvents as methanol [43,44]. After extraction, the stir bar is removed and very gently wipe with lint-free tissue to remove water droplets.

Two desorption methods are known. One is TD [17] and the other is LD [18]. In the former, a PDMS-coated stir bar is placed inside an empty glass TD tube. In some cases, we recommend rinsing the stir bar lightly with distilled water to remove adsorbed proteins or other sample components. This step prevents the formation of non-volatile materials during the TD step. Rinsing does not cause solute loss because the sorbed solute is present in the PDMS phase. Finally, the solute is thermally desorbed. The desorption temperature is application-dependent and primarily determined by the volatility of the solute; it is typically between 150 and 300 °C. Desorption is accomplished within 5–15 min under 10–50 ml $min^{-1}$ helium flow. As an alternative to TD, the analyst can use LD. Sampling can also be performed in the headspace of a liquid or a solid sample, the so-called head space sorptive extraction (HSSE) method [45–52], and LD is used [18–29,53–55]. Approximately 100–200 μl of an organic solvent, such as methanol and acetonitrile, is used for the desorption. To perform the desorption, the stir bar is extracted at room temperature or by ultrasonication. The desorption time is approximately 5–10 min. In the case of LD, the sample is subjected to conventional HPLC [18–28], CE [28,29] or GC with large-volume injection (LVI) [53–55].

### 5.2. SBSE with in situ derivatization

Since the PDMS phase is a non-polar liquid phase, it is preferable that the polarity of the analyte be low. Relatively high polarity compounds, such as phenolic compounds, are not well recovered. Therefore, SBSE with in situ derivatization, wherein derivatization and SBSE are performed at the same time, was developed [48,56–69]. The derivatization of a phenolic hydroxyl group with acetic acid anhydride (AA) [56–68], a carboxyl group with ethyl chloroformate (ECF) [56–58] and carbonyl compounds with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) [48] has been reported. In addition, the determination of organotin in water sample by SBSE with in situ derivatization using sodium tetraethylborate ($NaBET_4$) has been reported [69]. As shown in Eq. (2), when $\log K_{o/w}$ is increased, the theoretical recovery is increased. Moreover, in the analysis by GC, decreasing the polarity leads to improved sensitivity. As a result, SBSE with in situ derivatization has high sensitivity. In addition, the derivative of an analyte with high $\log K_{o/w}$ reaches equilibrium (full equilibration) rapidly, whereas the analyte with low $\log K_{o/w}$ reaches equilibrium slowly [62–64]. Therefore, SBSE with in situ derivatization can be accomplished at a shorter time compared with conventional SBSE.

Many studies have used the acylation of a phenolic hydroxyl group with acetic anhydride. The common protocol for SBSE with in situ acylation is as follows. A sample is added into a vial. Then, sodium carbonate or potassium carbonate is added in order to adjust the pH of the sample to basic. Acetic anhydride as the derivatization agent is added. Ito et al. have reported that the pH and volume of acetic anhydride are important parameters in SBSE with in situ acylation [67]. A PDMS-coated stir bar is added and the sample is stirred for 30–240 min. After extraction, the stir bar is removed, and very gently wiped with lint-free tissue to remove water droplets. Then, it is subjected to TD–GC–MS.

Fig. 3 shows the chromatograms of such phenolic xenoestrogens as 2,4-dichlorophenol (2,4-DCP), 4-*tert*-butylphenol (BP), 4-*tert*-octylphenol (OP), 4-nonylphenol (NP), pentachlorophenol (PCP) and bisphenol A (BPA) standard solution (100 pg $ml^{-1}$) subjected to SBSE with in situ derivatization, as well as the chromatograms of the same subjected to SBSE without derivatization. An increase in sensitivity was observed in the former [65].

### 5.3. SBSE with in situ de-conjugation

Many compounds are metabolized into gluconic acid or sulfate conjugate in the human body. In order to determine such compounds in biological samples, SBSE is performed prior to de-conjugation [56–62,70]. However, the de-conjugation process is tedious and time-consuming. Recently, we developed SBSE with in situ de-conjugation, wherein SBSE and de-conjugation are performed at the same time [71]. As a result, the operation time was successfully shortened.

The common protocol for SBSE with in situ de-conjugation is as follows. A biological sample, such as urine or plasma, is added to a vial. Since the enzymatic activity is susceptible to

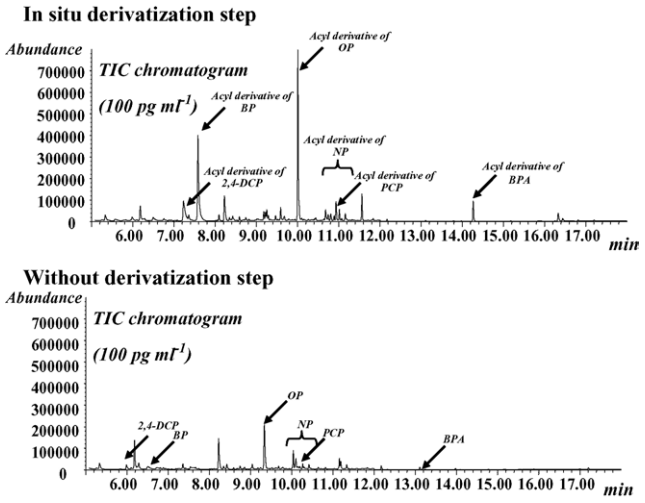


Fig. 3. Comparison of chromatogram of phenolic xenoestrogens subjected to SBSE with in situ derivatization with that subjected to SBSE without derivatization, For SBSE with in situ derivatization: 10 ml of phenolic xenoestrogen standard solution (100 pg $ml^{-1}$) was added into a headspace vial. Then, sodium carbonate (53.0 mg) and sodium hydrogen carbonate (42.0 mg) for pH adjustment (pH 10.5), and acetic anhydride (200 μl) as the derivatization agent were added. The stir bar was added and the vial was crimped with a Teflon-coated silicone septum. SBSE was performed at room temperature for 90 min while stirring at 1000 rpm. After the extraction, the stir bar was easily removed, rinsed with purified water, dried with lint-free issue and placed inside a glass TD tube. The TD tube was then placed inside the TD unit. The stir bar was thermally desorbed in the TD system, and this was followed by GC–MS. For SBSE without derivatization: the same procedure was performed except that no derivatization agents were added.

pH, a suitable buffer solution is added. Then, β-gluconidase is added for the de-conjugation. A PDMS-coated stir bar is added and the sample is stirred for 30–240 min. After extraction, the stir bar is removed, and very gently wiped with lint-free tissue to remove water droplets. Then, it is subjected to TD–GC–MS.

### 5.4. TD in the multi-shot mode

In general, after the pretreatment with SBSE, one PDMS-coated stir bar is thermally desorbed in the TD system, and this is followed by GC–MS. On the other hand, the simultaneous TD of five stir bars at a maximum can be carried out in the "multi-shot" mode (Fig. 4). By carrying out simultaneous TD of two or more stir bars, high-sensitivity analysis can be achieved [63]. In Eq. (2), when the sample volume is increased, the recovery is decreased in the case of the single-shot mode and the amount of analyte that is extracted into the PDMS phase is decreased. In addition, an increase in the volume of the sample means an increase in $\beta$, and the extraction time is extended [39]. When

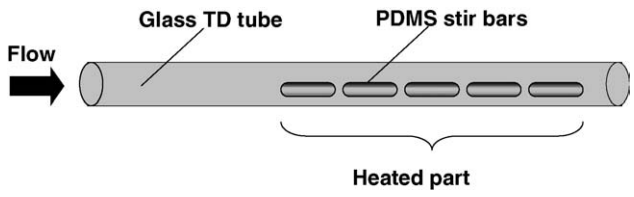


Fig. 4. Schematic of glass TD tube for TD in the multi-shot mode.

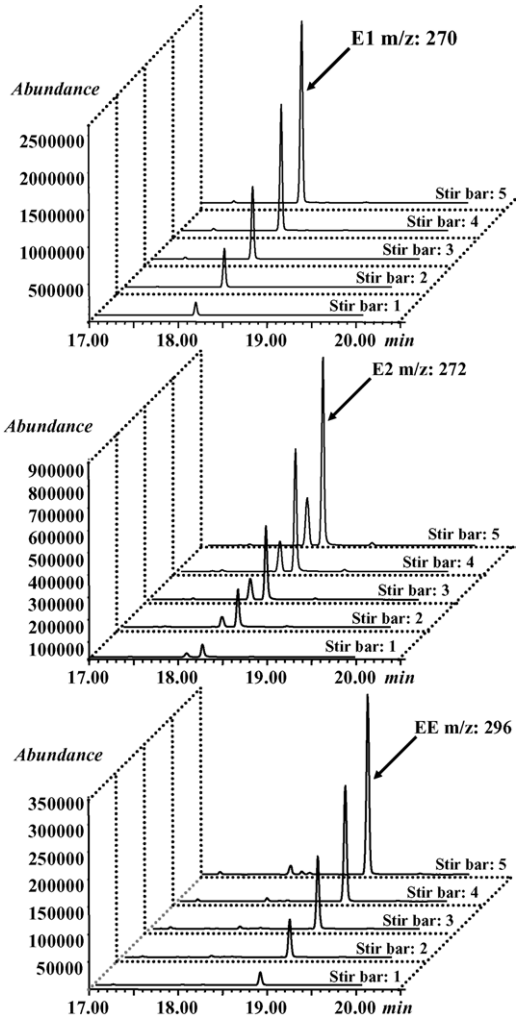


Fig. 5. Chromatograms of acyl derivatives of estrogens (10 ng $ml^{-1}$) subjected to SBSE with in situ derivatization and TD–GC–MS in the single- or multi-shot mode. One PDMS-coated stir bar, sodium carbonate (106.0 mg) as the pH adjustment agent (pH 11.5), and acetic anhydride (100 μl) as the derivatization agent were added to each of the five standard solutions (10 ng $ml^{-1}$, 10 ml) and stirring was performed for 2 h at room temperature (25 °C) in a glass vial. Then, the stir bars were simultaneously subjected to TD–GC–MS.

the multi-shot mode is used, $\beta$ is not increased. As a result, the extraction time is not extended and the recovery is not decreased. Therefore, use of the multi-shot mode improves the sensitivity. We have reported the trace analysis of natural and synthetics estrogens, such as estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE), in river water sample, which involves SBSE with in situ derivatization followed by TD–GC–MS in the multi-shot mode [63]. SBSE with in situ derivatization is performed after adding stir bars to each of the approximately 10 ml water samples (10 ng $ml^{-1}$). The simultaneous TD of one to five stir bars is performed in the multi-shot mode. When the number of stir bars is increased, higher peak responses are obtained (Fig. 5). Moreover, it is possible to subject some stir bars that have been pretreated with different methods to GC–MS at the same time. Ochiai et al. have reported that TD of two PDMS stir bar was carried out at the same time after two different SBSE methods were performed so called "dual SBSE method" [72].

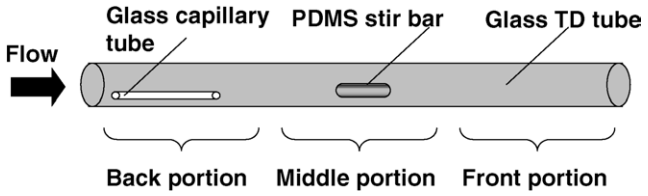


Fig. 6. Schematic of glass TD tube for TD with in tube derivatization.

As a result, the screening method is expected to have wide applications.

The outline of TD in the multi-shot method is shown below. Two or more SBSEs of a liquid sample are performed by placing a suitable amount of the sample in a headspace vial or a container. A PDMS-coated stir bar is added and the sample is stirred for 30–240 min. After extraction, the stir bar is easily removed with forceps, rinsed with purified water, and dried with lint-free issue. Two or more stir bars are placed inside a glass TD tube in the multi-shot mode. Then, the glass TD tube is placed inside the TD unit. The stir bar is thermally desorbed in the TD system, and this is followed by GC–MS.

### 5.5. TD with in tube derivatization

Because SBSE with in situ derivatization involves derivatization in a water sample, the silylation agent that can derivatize various functional groups is limited. Therefore, there is a limitation in the derivatization of the functional groups. To solve this problem, TD with in tube derivatization, in which the target compound is derivatized during TD from the PDMS-coated stir bar, was developed [73]. As a result of examining various silylation agents, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was chosen because of its high volatility, and TD with in tube derivatization was achieved. Because BSTFA is able to derivatize various functional groups, the application of TD with in tube derivatization to the measurement of various analytes is expected.

The outline of TD with in tube derivatization is shown below. SBSE of a liquid sample is performed by placing a suitable amount of sample in a headspace vial or a container. A PDMS-coated stir bar is added and the sample is stirred for 30–240 min. After extraction, the stir bar is easily removed with forceps, rinsed with purified water, dried with lint-free issue and placed inside a glass TD tube. Then, a glass capillary tube filled with BSTFA is inserted into the back portion of the glass TD tube (Fig. 6). Moreover, in order to improve the stability of the derivatization reaction, quartz wool is added to the glass TD tube, and this method is called “quartz wool assisted (QWA) in tube silylation” [64]. The glass TD tube is placed inside the TD system where the stir bar is thermally desorbed and subjected to GC–MS thereafter.

## 6. Quantification in SBSE

Quantification in SBSE can be performed in different ways, and the selection of the method is mainly dictated by the complexity of the sample. For example, both external standardization and internal standard addition can be used for tap water because there are no matrix effects that contribute to the equilibrium. For samples in which the matrix effects contribute to the equilibrium, environmental water and biological fluids, different methods can be used, including single-level calibration with a standard having a concentration that is similar to the estimated concentration and prepared in a blank matrix to compensate for matrix effects, internal standard addition of deuterated or $^{13}$C-labeled target solutes, and standard addition at three to six concentrations. The first method requires a blank sample to compensate for the matrix effects; however, strange as it may seem, these samples are often difficult to obtain. For the second approach, labeled standards are commercially available for only a few solutes, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs) and endocrine disrupting chemicals (EDCs). The last method is by far the easiest to use in a routine environment and human biological analysis.

## 7. Applications

### 7.1. Environmental analysis

SBSE has been applied successfully in environmental analysis. Its main advantage is that it can be applied to volatile organic compounds (VOCs) and semi-volatile compounds. When used in combination with LD and HPLC, it can even be applied to non-volatile compounds. The compounds are extracted and enriched depending upon their octanol–water partitioning coefficient. Table 1 summarizes the SBSE methods used in environmental analyses. Successful applications include volatile aromatics [17,38], halogenated solvents [17,38], PAHs [18–21,43,44,67,73], PCBs [75], organochlorine pesticide (OCPs) and organophosphorus pesticide (OPPs) or insecticides [76], pyrethroid pesticides [55], odorous compounds [77,78], organotin compounds [79] and EDCs [53,54], such as alkylphenols (APs) [54,66,73,79], BPA [54,62,65,66], chlorophenols (CPs) [60,65,68] and estrogens [63,64].

In the case of VOC analysis, the SBSE–TD–GC–MS [17] and HSSE–TD–GC–MS [38] methods enable measurement with the limit of detection (LOD) at the sub ng $l^{-1}$ level. In the case of PAH analysis, because PAHs have high boiling points, the SBSE–LD–HPLC–FD method is mainly used, in which LOD is of the 0.1–1 ng $l^{-1}$ level [18–21]. On the other hand, the SBSE–TD–GC–MS method enables measurement with LOD at the sub ng $l^{-1}$ level [21,43,44,64]. In addition, Ito et al. have reported the determination of hydroxyl PAHs (OH-PAHs) by SBSE with in situ derivatization followed by TD–GC–MS (LOD = 0.27–25 ng $l^{-1}$) [67]. Vercauteren et al. have reported the determination of organotin in water sample by SBSE with in situ derivatization followed by GC–ICP–MS with LOD of the 0.1 pg $l^{-1}$ (ppq) level [69]. Popp et al. have reported the determination of PCBs in water sample with the SBSE–TD–GC–MS method (LOD = 0.05–0.15 ng $l^{-1}$) [75]. The simultaneous determination of 64 pesticides in river water samples with the SBSE–TD–GC–MS method, in which LOD is 0.2–20 ng $l^{-1}$, was reported by Nakamura and Daishima [42]. In the analysis of odorous compounds, such as 2-methylisoborneol (MIB),

Table 1
Overview of SBSE for environmental applications

| Analyte | Sample | Limit of detection | Method | Derivatization | Desorption | Instrument | References |
|---|---|---|---|---|---|---|---|
| VOCs | Water | Sub ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [17] |
| VOCs | Water | Sub ng $l^{-1}$ | HSSE | – | TD | TD–GC–MS | [38] |
| PAHs | Water | 0.2–2 ng $l^{-1}$ | SBSE | – | LD | HPLC–ED | [18] |
| PAHs | Water | 0.4–5 ng $l^{-1}$ | SBSE | – | LD | HPLC–ED | [19] |
| PAHs | Water | 0.1–1.2 ng $l^{-1}$ | SBSE | – | LD | HPLC–ED | [20] |
| PAHs | Water | 0.3–2 ng $l^{-1}$ | SBSE | – | LD | HPLC–ED | [21] |
| PAHs, OCPs, PCBs | Water | 0.05–1 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [21] |
| PAHs, pesticides | Water | – | SBSE | – | TD | TD–GC–MS | [43] |
| PAHs | Water | 0.1–2 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [44] |
| PAHs | Seawater | Sub ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [74] |
| OH-PAHs | Water | 0.27–25 ng $l^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [67] |
| Qrganotin compounds | Water | 0.1–1.2 ng $l^{-1}$ | SBSE with in situ derivatization | Ethylation | TD | TD–GC–ICPMS | [69] |
| PCBs | Water | 0.05–0.15 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [75] |
| Pesticides | Water | – | SBSE | – | TD | TD–GC–MS | [39] |
| Pesticides | River water | 0.2–20 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [42] |
| OPPs | Water | 0.8–15.4 ng $l^{-1}$ | SBSE | – | TD | TD–GC–AED | [76] |
| Pyrethroid pesticides | Water | 1.0–2.5 ng $l^{-1}$ | SBSE | – | LD | LVI–GC–MS | [55] |
| MIB, geosmin | Water | 0.091–0.18 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [77] |
| MIB, chloroanisole, geosmin | Water | 0.1–1 ng $l^{-1}$ | SBSE | – | TD | TD–GC–MS | [78] |
| EDCs | Water | 0.01–0.24 ng $l^{-1}$ | SBSE | – | LD | LVI–GC–MS | [53] |
| EDCs | Water | 0.025–0.400 ng $l^{-1}$ | SBSE | – | LD | LVI–GC–MS | [54] |
| OP, NP | River water | 0.002–0.02 ng $ml^{-1}$ | SBSE | – | TD | TD–GC–MS | [79] |
| BPA | River water | l–5 pg $ml^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [62] |
| APs and BPA | River water | 0.1–3.2 ng $l^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [66] |
| APs | River water | 0.2–10 pg $ml^{-1}$ | TD with in tube derivatization | Silylation | TD | TD–GC–MS | [73] |
| CPs | River water | l–2 pg $ml^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [60] |
| Phenols | Water | 0.1–0.4 ng $l^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [68] |
| Phenolic xenoestrogens | River water | 0.5–5 pg $ml^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [65] |
| Estrogens | River water | 0.2–5 pg $ml^{-1}$ | SBSE with in situ derivatization<br>TD in the multi-shot mode | AA | TD | TD–GC–MS | [63] |
| E2 | River water | 0.l–0.5 pg $ml^{-1}$ | SBSE with in situ derivatization<br>TD with in tube derivatization<br>TD in the multi-shot mode | AA, silylation | TD | TD–GC–MS | [64] |

geosmin and trichloroanisole, with the SBSE–TD–GC–MS method, LODs below 1 ng $l^{-1}$ were reported [77,78]. The simultaneous determination of sub μg $l^{-1}$ EDCs in water sample by using SBSE and LD followed by GC–MS with LVI was reported [53,54]. In addition, phenolic xenoestrogens, such as NP, OP, BPA and CPs, in water sample were determined by SBSE or SBSE with in situ derivatization followed by the TD–GC–MS method (LOD = sub ng $l^{-1}$ level) [60,62,65,66,68,79]. Moreover, the determination of APs in river water by SBSE and TD with in tube derivatization followed by GC–MS was reported (LOD = 0.2–10 pg $ml^{-1}$) [73]. On the other hand, the simultaneous determination of estrogens, such as E1, E2 and EE, in river water sample by using SBSE with in situ derivatization and TD in the multi-shot mode followed by GC–MS was reported (LOD = 0.2–5 ng $l^{-1}$) [63]. In addition, SBSE with in situ derivatization and TD with in tube derivatization followed by GC–MS in the multi-shot mode were used in an ultrahigh-sensitivity analysis of E2 in river water sample (LOD = 0.1 ng $l^{-1}$) [64].

### 7.2. Biomedical analysis

SBSE can also be applied to the determination of organic compounds in biological fluids. Table 2 shows the applications of different SBSE methods in biomedical analysis. Different types of solutes have been extracted from serum, plasma and urine samples, including drugs of abuse [56], Terpenes and sesquiterpenes [56], steroids [56], nicotine [56], fatty acids [56], phenols [56], barbiturates and benzodiazepines [57], prescription drugs [58], caffeine [80] and its metabolites [27], PCBs [81], di(2-ethlyhexyl) phthalate (DEHP) [56], 1-hydroxylpyrene [56,59], phenolic xenoestrogens [60–62,70,71] and tuberculostearic acid (TBSA) [82].

Tienpont et al. have developed a protocol for the determination of analytes in urine or blood samples by means of the SBSE method [56–58]. Urine samples can be extracted directly or after enzymatic hydrolysis. In situ derivatization (AA or ECF) can be used as well. Blood samples, including serum and plasma, bile fluid and sperm, must be diluted with water or a buffer solution prior to extraction.

On the other hand, the determination of 1-hydroxylpyrene [56,59] or phenolic xenoestrogens [60–62,70] in human biological samples by using SBSE or SBSE with in situ derivatization has been reported (LOD = sub ng $l^{-1}$). Moreover, we have reported the determination of NP glucuronide in human urine samples by SBSE with in situ de-conjugation [71]. Stopforth et al. have reported a rapid method for the detection of TBSA in sputum sample [82].

Table 2
Overview of SBSE for biomedical applications

| Analyte | Sample | Limit of detection | Method | Derivatization | Desorption | Instrument | References |
|---|---|---|---|---|---|---|---|
| Drugs of abuse | Urine | – | SBSE with in situ derivatization | AA, ECF | TD | TD–GC–MS | [56] |
| Terpenes and sesquiterpenes | Urine | – | SBSE | – | TD | TD–GC–MS | [56] |
| Steroids | Urine | – | SBSE | – | TD | TD–GC–MS | [56] |
| Nicotine | Urine | – | SBSE | – | TD | TD–GC–MS | [56] |
| Fatty acids | Urine | – | SBSE with in situ derivatization | ECF | TD | TD–GC–MS | [56] |
| Phenols | Urine | – | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [56] |
| Barbiturates and benzodiazepines | Urine | 1 μg l$^{-1}$ (SCAN), 10 ng l$^{-1}$ (SIM) | SBSE with in situ derivatization | AA, ECF | TD | TD–GC–MS | [57] |
| Prescription drugs | Urine, blood | – | SBSE with in situ derivatization | AA, ECF | TD | TD–GC–MS | [58] |
| Caffeine, theophylline | Whole blood | 0.06–0.4 μg ml$^{-1}$ | SBSE | – | TD | TD–GC–MS | [80] |
| Caffeine and metabolites | Plasma | 25 ng ml$^{-1}$ | RAM–SBSE | – | LD | HPLC–UV | [27] |
| PCBs | Sperm | Sub pg ml$^{-1}$ | SBSE | – | TD | TD–GC–MS | [81] |
| DEHP | Plasma | 0.3 μg l$^{-1}$ | SBSE | – | TD | TD–GC–MS | [56] |
| 1-Hydroxypyrene | Urine | 20 ng l$^{-1}$ (SCAN), 0.2 ng l$^{-1}$ (SIM) | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [56] |
| 1-Hydroxypyrene | Urine | 2 ng l$^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [59] |
| OP, NP | Urine, plasma | 0.004–0.04 ng ml$^{-1}$ | SBSE | – | TD | TD–GC–MS | [70] |
| NP glucuronide | Urine | 0.2 ng ml$^{-1}$ | SBSE with in situ de-coniugation | – | TD | TD–GC–MS | [71] |
| BPA | Urine, plasma, saliva | 20–100 pg ml$^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [62] |
| Chlorophenols | Urine | 10–20 pg ml$^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [60] |
| Phenolic xenoestrogens | Urine | 10–50 pg ml$^{-1}$ | SBSE with in situ derivatization | AA | TD | TD–GC–MS | [61] |
| TBSA | Sputum | 0.2 ng ml$^{-1}$ | SBSE | – | TD | TD–GC–MS | [82] |

## 8. Conclusions

A number of novel SBSE methods have been developed and can be used to determine trace organic compounds in aqueous matrices, including water and biological samples. We have obtained sensitivities lower than 1 ng l$^{-1}$ in environment analysis and 1 μg l$^{-1}$ in biomedical analysis, depending upon the solute ($\log K_{o/w}$), sample volume, stir bar dimensions and GC–MS sensitivity. In addition, the SBSE method is widely applied also to the food analysis [22,23,26,28,29,47,48,72,83–87]. Moreover, the application of SBSE is tried to the field of chemical ecology [88]. In the future, it is expected that the SBSE technique with novel method would have wide ranging application advances to various fields.

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