



Review

Single drop microextraction—Development, applications and future trends

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ABSTRACT

Single drop microextraction (SDME) has emerged over the last 10–15 years as one of the simplest and most easily implemented forms of micro-scale sample cleanup and preconcentration. In the most common arrangement, an ordinary chromatography syringe is used to suspend microliter quantities of extracting solvent either directly immersed in the sample, or in the headspace above the sample. The same syringe is then used to introduce the solvent and extracted analytes into the chromatography system for identification and/or quantitation. This review article summarizes the historical development and various modes of the technique, some theoretical and practical aspects, recent trends and selected applications.

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1. Brief history of development

The use of a single drop as a collector of analytes in analytical chemistry can be traced to the work of Dasgupta in the mid-1990s. Dasgupta's group first developed methods involving a liquid droplet as a gas sampling interface to extract substances such as ammonia and sulfur dioxide from the air [1]. A silica capillary tube was used to support a water droplet which was used to collect gaseous analytes followed by in-line spectrophotometric analysis. Dasgupta's group subsequently developed a drop-in-drop miniaturized solvent extraction system in which they extracted sodium dodecylsulfate as an ion-pair with methylene blue into a microdrop of chloroform [2]. They employed a peristaltic pump flow manifold and an optical fiber-based absorbance detector.

Cantwell's group was the first to develop single drop microextraction techniques directly compatible with chromatographic analysis. In their first paper [3], Jeannot and Cantwell used a Teflon rod with a spherical recess to hold an 8- μ L drop of octane immersed in a stirred aqueous solution. They called this approach "solvent microextraction" (SME). After extraction, the rod was removed, and a gas chromatography (GC) syringe was used to sample and inject a portion of the octane solution into a GC. In their second paper [4], they demonstrated for the first time the direct use of the GC syringe needle for both suspension of the extracting solvent and injection into the GC. Stirring rate and stirring time were primarily investigated to develop equilibrium and kinetic models for the process.

He and Lee introduced the notion of using the GC syringe needle as a microseparatory funnel in a technique they called dynamic liquid-phase microextraction [5]. Rather than suspending the drop of organic solvent in the solution from the syringe, they contained the solvent within the syringe needle and drew aqueous phase into and out of the syringe repeatedly. This technique required high precision repeated movement of the syringe plunger, but offered improved organic solvent "drop" stability since the drop was protected within the needle.

Ma and Cantwell successfully demonstrated three-phase single drop microextraction with the use of an "unsupported" liquid membrane and an aqueous microdrop of acceptor solution [6]. A Teflon ring near the top of the sample vial held the organic membrane phase in place, and an aqueous acceptor phase was suspended within the organic layer using a high-performance liquid chromatography (HPLC) syringe. With this approach, large enrichment factors could be realized using relative short extraction times for ionizable analytes.

Liu and Lee developed a continuous flow microextraction technique compatible with GC analysis [7]. Polyetheretherketone (PEEK) tubing was used to continuously pass aqueous sample solution through an extraction chamber, and a HPLC valve was used to inject 1–5 μ L of organic solvent into the flowing stream. After reaching the tubing outlet, the organic solvent drop remained attached to the PEEK tubing while aqueous phase continued to flow. A separate GC syringe was used to sample and inject a portion of the solvent phase after extraction.

Extension of SDME to headspace (HS) analysis was developed in the early 2000s independently by Przyjazny et al. [8,9], Jeannot and colleagues [10] and Vickackaite and colleagues [11]. Common high-boiling organic solvents such 1-octanol or *n*-hexadecane were found to be suitable for the determination of volatile or semivolatile analytes. HS-SDME allowed for greater drop stability, avoided problems of drop contamination or loss from "dirty" sample matrices, and in some cases provided for faster extraction rates compared to direct immersion methods.

The early development of SDME has been reviewed in 2002 [12], and a more recent review from 2007 focuses mainly on applications during the first decade of development [13]. A com-

prehensive book detailing the theory and practice of SDME and other solvent microextraction methods (including hollow fiber and dispersive liquid-liquid methods) is also recently available [14]. The purpose and scope of this review will be to provide a useful description of the development and advantages/disadvantages of the different modes of SDME, and to summarize new developments, future trends and selected current applications. More exhaustive literature reviews on the subject have been presented elsewhere [12–14]. Other "liquid-phase" microextraction techniques such as dispersive liquid-liquid microextraction (DLLME), hollow fiber techniques, and directly suspended droplet/solidification techniques are described in other articles in this issue and will not be addressed here.

2. Important theoretical aspects of SDME

2.1. Overview

Transport of analyte molecules from aqueous sample solution to the microdrop is generally limited by slow diffusion rates of the analyte molecules in the condensed (aqueous and/or organic) phases. While temperature and solvent viscosity play a role in these rates of diffusion, the primary mode of rate enhancement is reduction of the distance over which the diffusion must occur. Thus, samples are normally agitated via magnetic stirring, mechanical vibration or syringe plunger motion to increase the amount of convective mixing or interfacial contact area, and therefore reduce the diffusion distance. The time required to reach equilibrium in SDME can be anywhere from seconds to hours, depending on the degree of agitation, phase volumes, interfacial contact area and equilibrium distribution constant. Thus, to avoid excessive analysis times, SDME is often performed under non-equilibrium (kinetically controlled) conditions.

Even in cases where distribution equilibrium is attained in SDME, it is important to note that the extraction is rarely exhaustive. (A significant fraction of the total analyte normally remains in the aqueous (sample) phase at equilibrium.) This is a consequence of the small organic (or receiver) to aqueous (sample) volume ratio employed in SDME, similar to what is encountered in solid-phase microextraction (SPME). In some cases, only a negligible amount of analyte is removed from the sample solution, which can be advantageous in studying speciation and avoiding perturbation of sample-phase equilibria. In any case, whether equilibrium is attained or not, calibration is normally based on aqueous-phase standards which are extracted under identical conditions to the unknown sample, with or without the aid of internal standards.

2.2. Two-phase SDME

In a study of the uptake of gaseous ammonia by an aqueous drop [1], Liu and Dasgupta proposed a radial diffusion model in which the drop was assumed to be spherical and stagnant. Experimental data and visual observation suggested that convection was present within the drop, enhancing the rate of extraction. Diffusion of gaseous NH_3 , establishment of equilibrium at the interface, and protonation of NH_3 were all assumed to be fast relative to transport of NH_4^+ within the aqueous drop.

Jeannot and Cantwell proposed a general model for equilibrium and mass transfer in a two-phase liquid-liquid microextraction system [3,4]. The equilibrium concentration of analyte in the organic phase ($C_{o,eq}$) was shown to be

$$C_{o,eq} = KC_{w,eq} = \frac{KC_w^0}{1 + KV_o/V_w} \quad (1)$$

where K is the equilibrium distribution constant, $C_{w,eq}$ is the equilibrium concentration in the aqueous (water) phase, C_w^0 is the initial

concentration in the water phase, and V_o and V_w are the organic and water phase volumes, respectively. (There is negligible depletion of the sample solution when the second term in the denominator is much smaller than 1.) A general kinetic model which fit experimental data well showed that concentration (C_o) versus time (t) data follows the following first-order model:

$$C_o = C_{o,eq}(1 - e^{-kt}) \quad (2)$$

The rate constant, k , was shown to increase with increasing interfacial contact area, enhanced convection (particularly within the aqueous phase), smaller volumes of both phases, and smaller equilibrium distribution constant. On the other hand, at equilibrium, larger phase volumes and distribution constant result in more moles in the organic phase, according to Eq. (1).

Jeannot and Cantwell also applied SDME to the determination of free (unbound) progesterone in the presence of a binding protein [15]. A small phase ratio ensured negligible depletion of free progesterone from the sample solution, and the authors were able to accurately measure the binding constant of the steroid molecule to bovine serum albumin. Transport of both free and bound forms of progesterone to the interface also resulted in an enhanced rate of extraction.

2.3. Dynamic SDME

In the dynamic technique [5], He and Lee assumed instantaneous equilibrium between the aspirated circulating aqueous sample plug and the film of organic solvent on the inside wall of the syringe needle with each aspiration cycle. Extraction into the organic “plug” within the needle was assumed to be negligible compared with the film. With each new aspiration cycle, it was assumed that the aqueous plug and organic film mixed fully with the respective bulk solutions. A linear relationship between amount of analyte extracted and the number of aspiration cycles resulted.

2.4. SDME with back-extraction (aqueous–organic–aqueous)

SDME with back-extraction is a two-step process in which an ionizable solute is first extracted into an organic layer, followed by extraction and trapping into a second aqueous layer whose pH results in ionization of the solute. For example, Ma and Cantwell [6] started with amine solutes in pH 13 buffer which ensured they were deprotonated (neutral) and extractable into the organic layer. The second aqueous (receiver) drop was buffered at pH 2.1 to protonate (ionize) and therefore trap the amine in the receiver drop. The enrichment factor was maximized by using as small a receiver drop volume as possible compared to the donor phase volume, and followed similar first-order kinetics as Eq. (2). In their model, Ma and Cantwell assumed steady-state behavior of the analyte in the intermediate (organic) phase.

2.5. Headspace SDME (aqueous–headspace–organic)

In headspace SDME (or direct immersion two-phase SDME with a headspace above the sample), the headspace is always a “compartment” for analyte molecules. For maximum sensitivity, therefore, the headspace volume should always be minimized to reduce the amount of analyte present there. At equilibrium, it makes no difference how the three phases are “arranged” (*i.e.* whether the solvent drop is immersed in the aqueous solution or in the headspace), but the kinetics of the extraction process and other processes (*e.g.* rate of solvent evaporation) are significantly affected.

The equilibrium concentration in the organic drop for headspace SDME is given by a modified version of Eq. (2) that includes parti-

tioning into the air phase:

$$C_{o,eq} = \frac{K_{ow}C_w^0}{1 + (K_{aw}V_a/V_w) + (K_{ow}V_o/V_w)} \quad (3)$$

This equation incorporates both an air–water distribution constant (K_{aw}) and an overall organic–water distribution constant (K_{ow}). A very small air (headspace) volume, V_a , or very small K_{aw} reduces Eq. (3) to Eq. (1), the two-phase case.

Theis et al. [10] showed that headspace SDME rate data for extraction of simple aromatic compounds followed the same first-order behavior as seen in two-phase systems (Eq. (2)). Furthermore, it was apparent that enhanced extraction rates could be achieved by increasing the degree of convection in both the aqueous solution and the organic drop.

A kinetic calibration method was developed by Ouyang et al. for headspace SDME [16]. They pre-loaded the extracting solvent with a known amount of a standard. During the extraction process, this standard was desorbed from the extracting drop while analyte was absorbed. Matrix effects were automatically corrected since they affected the desorption and adsorption processes similarly.

Mohammadi and Alizadeh described some equilibrium and kinetic aspects of “dynamic headspace organic solvent film microextraction” in 2006 [17]. In this technique, a portion of the headspace is repeatedly drawn into and out of the needle which contains organic solvent. When the headspace is drawn into the needle, a film of organic solvent forms along the inside walls of the needle, and analytes can equilibrate between the headspace and this solvent film. They showed a linear dependence of the extraction efficiency on the number of sampling cycles, and also showed the importance of fast plunger motion in maximizing the rate of extraction.

Fiamegos and Stalikas studied some theoretical aspects of in-drop derivatization and mass transfer in headspace SDME using low-volatility aldehydes [18]. They found that the chemical reaction rate was either slower than, or comparable to the diffusion rate into the drop. Also, they determined that diffusion within the drop was the slow mass transfer step.

A steady-state kinetic model for headspace SDME was proposed by Schnobrich and Jeannot in 2008 [19]. Benzene, toluene, ethylbenzene and xylene (BTEX) were used as model analytes, and it was shown that the headspace moles remained low and steady, particularly for the analytes with the largest octanol–water partition coefficients. Neither the water–air nor the air–organic distribution process was at equilibrium during the course of the extraction suggesting that neither of these processes alone is rate-limiting for these relatively volatile compounds. Thus, it is important to generate convection in both the water and organic phases if possible.

3. A closer look at various SDME modes

At present, there are seven different modes of solvent microextraction that fall under the category of single drop microextraction. They can be classified into either two-phase or three-phase techniques, depending on the number of phases co-existing at equilibrium. This classification is depicted in Fig. 1. Two-phase modes include direct immersion (DI), continuous flow (CF), drop-to-drop (DD), and directly suspended droplet (DSD), while three-phase modes consist of headspace (HS), liquid–liquid–liquid (LLL), and a combination of LLL and DSD first introduced just recently [20]. The frequency of use of various SDME modes, shown in Fig. 2, is almost evenly divided between two-phase (DI, CF, DD and DSD at 52%) and three-phase (HS and LLL at 48%) modes. By far the most commonly used modes of single drop microextraction are headspace (41% of all described SDME procedures) and direct immersion (38%), most likely due to their simplicity and inexpensive equipment needed for implementation, but also because they were the first solvent

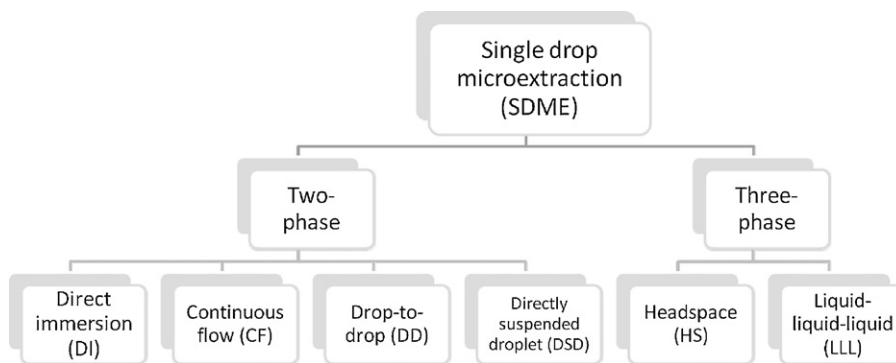


Fig. 1. Single drop microextraction (SDME) classification.

microextraction procedures described in the literature. The other five modes have found limited use, either as a result of additional equipment required, such as a pump (CF), or applicability limited to a small group of analytes (e.g. LLLME is used mostly for ionizable compounds), or because they do not offer any significant advantages compared to the more common modes.

In order to improve the rate of mass transfer, headspace and direct immersion SDME can be performed in a dynamic mode, in which not only the donor phase (sample), but also the acceptor phase (extracting solvent) is in motion (see Sections 2.3 and 4.6 for more detail). Two variants of dynamic SDME are used, unexposed-drop and an exposed-drop. In the unexposed-drop (or in-syringe) method, the solvent is withdrawn, along with 1–3 μL of sample liquid or headspace, into the syringe, held for a specified time (dwell time), and the sample expelled. This process is repeated for 30–90 cycles. The extract is then analyzed. In the exposed-drop method, the extracting solvent drop is exposed to the sample at the needle tip for a specified time and then withdrawn into the needle, held for a specified time, and expelled out to the needle tip again. The sample is not withdrawn into the syringe, however. The unexposed-drop method was first developed by He and Lee, using manual manipulation of the syringe plunger movement [5,21]. This was followed by the use of a syringe pump to improve reproducibility of the plunger movement [22]. The method

was further automated, controlling plunger movement with a variable speed motor, by Saraji [23] and by Mohammadi and Alizadeh [17], by controlling the motor movement with computer software. Full automation of both the exposed-drop and unexposed-drop methods was finally achieved by Ouyang et al. using a commercial computer-interfaced autosampler to control solvent uptake, plunger speed, dwell time, and syringe injection [24].

The two most common SDME modes – direct immersion and headspace solvent microextraction – have somewhat different general fields of applicability, although there are groups of analytes that have been determined by using both these techniques as a sample preparation step. Since in direct immersion SDME the microdrop of an extracting solvent is in direct contact with an aqueous sample (Fig. 3), the solvent must be immiscible with water, which implies the use of nonpolar or very slightly polar solvents. An exception to this rule is the use of ionic liquids as extracting solvents (see Section 5). Consequently, this mode is best suited for the separation/enrichment of nonpolar or moderately polar volatile and semivolatile analytes from relatively clean matrices, such as tap water or groundwater. Since volatile compounds are best preconcentrated by headspace SDME, the preferred use of direct immersion mode is for semivolatile compounds. Examples include organochlorine pesticides [25–31], phthalates [32–35], or drugs [36–45]. In general, the extracting solvent used in direct immersion SDME is volatile, e.g. hexane or toluene, which makes this mode directly compatible with gas chromatography. Consequently, GC has been the predominant final determination technique used in conjunction with direct immersion SDME, accounting for over 62% of analytical procedures described in the literature.

Other final determination methods are also employed. For example, HPLC (over 21% of DI-SDME analytical procedures) can

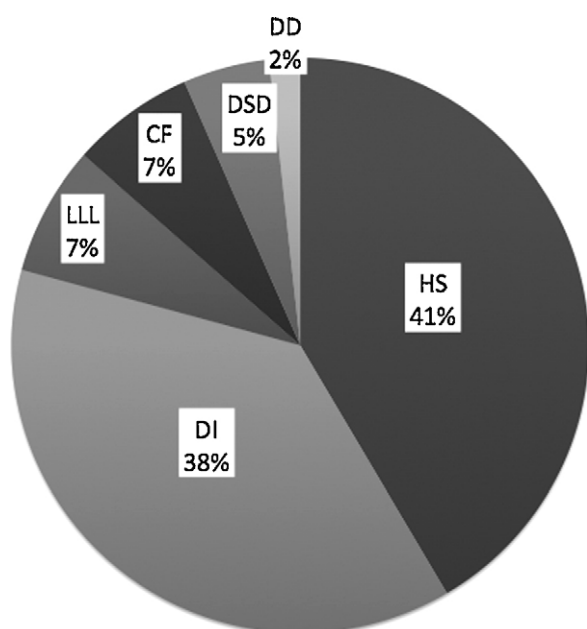


Fig. 2. Frequency of use of various SDME modes.

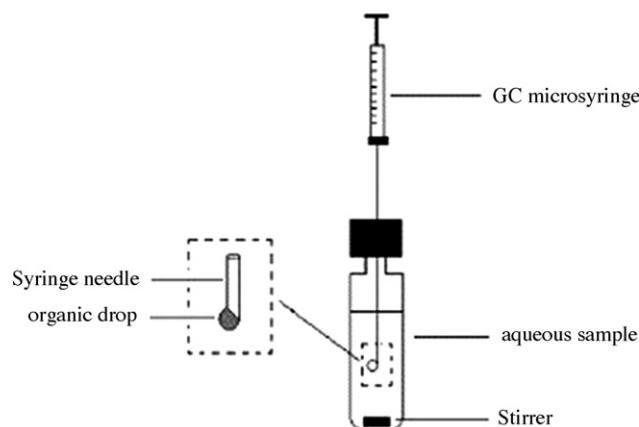


Fig. 3. Direct immersion (DI) SDME. Reprinted from: [13], Copyright (2007), with permission from Elsevier.

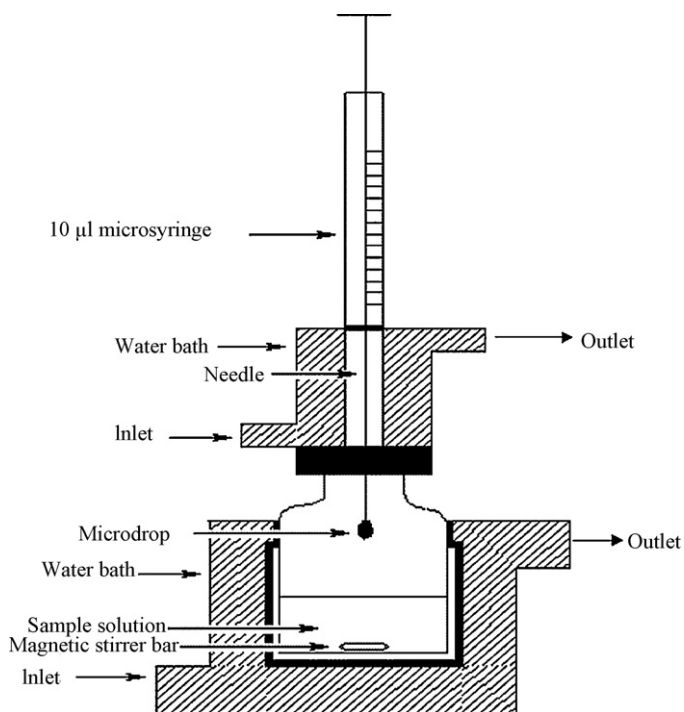


Fig. 4. Headspace (HS) SDME including solvent cooling. Reprinted from: [166], Copyright (2004), with permission from Elsevier.

be used for the analysis of polar semivolatiles, such as phenols. In this case, however, solvent replacement may be necessary unless an ionic liquid was used as the extracting solvent. Solvent replacement involves gentle evaporation of the original extracting solvent, followed by redissolving the residue in a solvent compatible with the HPLC mobile phase or in the mobile phase itself.

Another final determination technique that is being increasingly used in combination with direct immersion SDME is atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry (AP-MALDI-MS). If direct immersion SDME is employed for the separation/enrichment of inorganic species, such as metal ions, following their derivatization, then atomic absorption spectrometry or inductively coupled plasma-mass spectrometry are often used for their final determination. The major advantages of direct immersion SDME are simplicity of equipment used, at least in its static version, and low cost. In the simplest implementation, the equipment used includes an extraction vial with a septum cap, a stir bar, a magnetic stirrer, a microsyringe, and a small volume of extracting solvent. Disadvantages of DI-SDME are mostly related to the ease of dislodgment of the microdrop hanging from the tip of the microsyringe needle during the extraction process, which limits the rate of agitation of sample solution and the type of sample matrix to relatively clean (no solid particles present). Typical stirring rates in direct immersion SDME are no more than 1000 rpm unless specially modified needle tips are employed [46], which allows higher stirring rates, up to 1700 rpm. The need for vigorous agitation and/or dynamic mode of extraction is a consequence of slow mass transfer in liquid–liquid systems due to small diffusion coefficients in liquids. This slow mass transfer results in longer extraction times in DI-SDME compared to other single drop microextraction modes.

Headspace SDME (Fig. 4) is the sample preparation method of choice for volatile and semivolatile compounds, both polar and nonpolar. Sample matrices that are complex and/or dirty or contain solids do not interfere with analyte separation/enrichment. In addition to liquid samples (typically aqueous matrices), gaseous [47,48] and solid matrices [49] are also amenable to this mode. The scope of HS-SDME includes a wide variety of analytes, since

there are virtually no restrictions on extracting solvents used other than low volatility. Thus, examples of analytes often extracted by headspace SDME include trihalomethanes [50–53], BTEX hydrocarbons [9,10,16,17,33,54–56], volatile organic compounds [57–77], and inorganic and organometallic species [33,78–87], the last group often being derivatized prior to extraction. Headspace mode is often applied to extract polar volatile compounds, such as aldehydes [18,88–93], following or concurrently with their derivatization. At the same time, HS-SDME has been used to extract such semivolatile compounds as polycyclic aromatic hydrocarbons [33,76,94–96], polychlorinated biphenyls [76], phenols [33,97–101], and chlorophenols [97,98]. Both nonpolar and polar extracting solvents are common; the latter include ionic liquids [33,50,51,54,94,99,101–105] and aqueous solutions [47,48,69,81–85,96,98,100,106–109] or even pure water [110]. The use of water-based extracting solvents in headspace SDME is interesting since it totally eliminates the use of organic solvents. In addition to sample cleanup, analyte enrichment is also possible with pH control, analogous to the extraction with back-extraction approach in LLLME.

The most popular extracting solvents in headspace SDME are 1-octanol, hexadecane, dodecane, and decane. Since headspace SDME is a three-phase system, equilibration times may in some cases be longer than in direct SDME as a result of two equilibria involved: sample–headspace and headspace–extracting solvent. However, extraction times in headspace SDME can be reduced substantially by increasing the headspace capacity, *i.e.* the amount of analyte contained in the headspace. The headspace capacity, which is equal to the product of headspace (air) volume, V_a , and air–water distribution constant, K_{aw} , can be maximized by increasing either K_{aw} or V_a value, or both. If the amount of analyte extracted into the organic phase is small compared to headspace capacity (less than 5%), then analyte extraction takes place almost exclusively from the headspace. This results in rapid extraction, taking only several minutes, since the diffusion coefficients in the gaseous phase are much larger than those in the liquid phase (by about four orders of magnitude).

The most common final determination technique used in combination with headspace SDME is by far gas chromatography, which accounts for over 75% of all analytical procedures incorporating HS-SDME. High-performance liquid chromatography is a distant second (close to 10%), with atomic absorption spectrometry and capillary electrophoresis accounting for 5% and 3.5%, respectively.

In its simplest implementation, headspace SDME uses the same setup as direct immersion mode except that the microdrop of organic solvent hanging from the tip of a microsyringe is not immersed into an aqueous sample, but remains in the headspace above the sample. This setup has been modified in some procedures by using temperature control of sample and extracting solvent. In order to accelerate the rate of mass transfer of analytes from the sample to the headspace and to increase the amount of analytes transferred to the headspace, it is desirable to raise sample temperature. At the same time, however, elevated temperatures tend to decrease the organic solvent–headspace distribution constant, resulting in lower sensitivity of the determination. The loss of sensitivity can be avoided if the extracting solvent is cooled while the sample is heated (Fig. 4). However, this approach significantly complicates the experimental setup; therefore, it should be used only for ultra trace analyses or for highly volatile analytes with low solvent–headspace distribution constants.

Drop-to-drop solvent microextraction [111–115] is a miniaturized version of direct immersion SDME. It was first introduced by Wu in 2006 [115]. In this mode, both the sample and organic solvent volumes are in the order of microliters (Fig. 5). This approach is recommended if available sample volumes are small (*e.g.* blood). Drop-to-drop microextraction has two prominent features. Firstly,

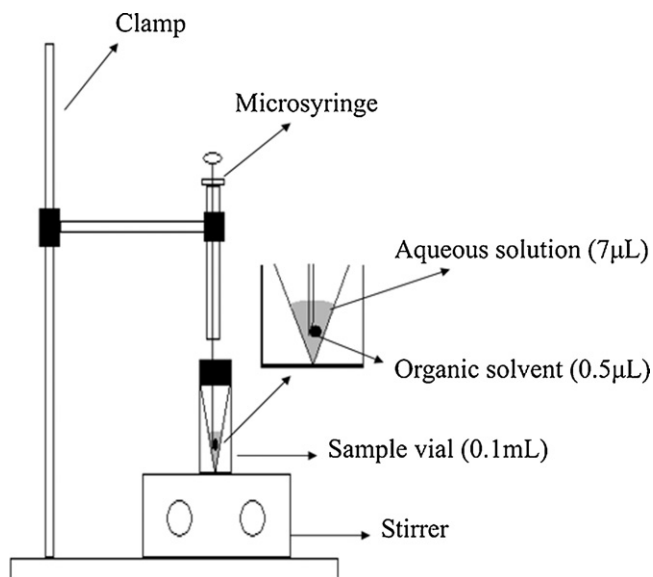


Fig. 5. Drop-to-drop (DD) SDME. Reprinted with permission from: [115], Copyright 2006 American Chemical Society.

as a result of small sample and solvent volumes, equilibrium between the sample and the solvent is established quickly due to a large value of the rate constant, k (see Section 2.2). Consequently, the sample does not have to be stirred, which further simplifies the experimental setup. Secondly, because the phase ratio, V_o/V_{aq} , is relatively large (see Eq. (1)), the enrichment factor is small, so that the main advantage of this mode, other than the small sample volume, is selectivity, which is provided by an extensive sample cleanup. Typical applications of drop-to-drop solvent microextraction include extraction of trimeprazine from 8 μL of urine and blood of rats using 0.6 μL of toluene [111], and extraction of quinine from 30- μL samples of urine and plasma with 2 μL of *m*-xylene [114].

Another two-phase SDME mode that deserves mention is continuous flow microextraction [7,116–123], in which a drop of solvent fully and continuously makes contact with a fresh and flowing sample solution (Fig. 6). The drop can be held at the tip of PEEK tubing which is immersed in a continuously flowing sample in the extraction chamber. Alternatively, a microsyringe can be used to hold a microdrop of the extracting solvent. The presence of both diffusion and convection results in the high extraction efficiency

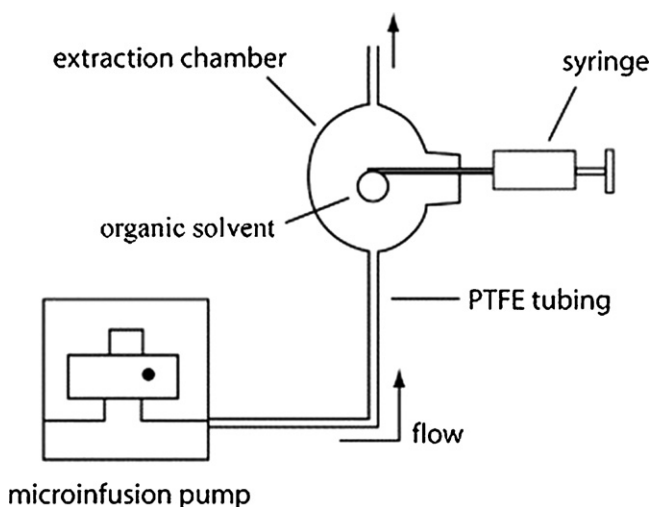


Fig. 6. Continuous flow (CF) SDME. Reprinted from: [123], Copyright (2007), with permission from Elsevier.

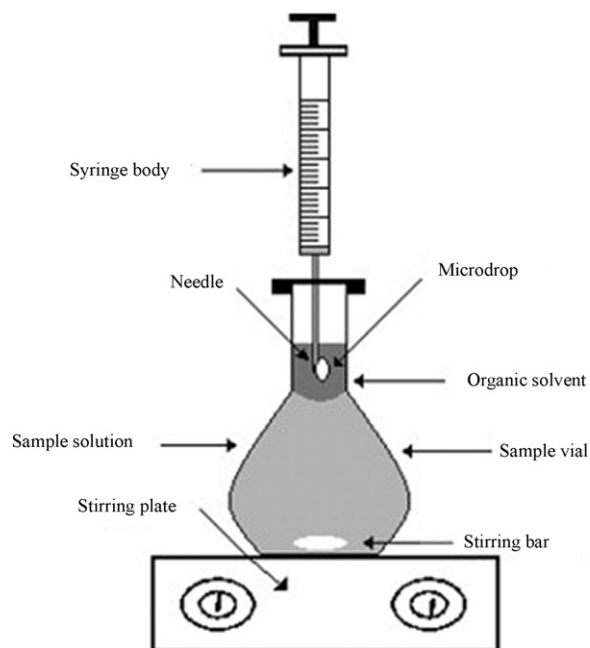


Fig. 7. Liquid-liquid-liquid (LLL) microextraction. Reprinted from: [167], Copyright (2008), with permission from Elsevier.

and rapid establishment of equilibrium between the sample and the extracting solvent. Most procedures making use of continuous flow microextraction are limited to extraction of nonpolar or slightly polar semivolatiles, such as pesticides [117,118], polycyclic aromatic hydrocarbons [123], or aromatic compounds [7,120–122], owing to the fact that only nonpolar extracting solvents are stable in the flowing system and the extent of their dissolution in the flowing sample is small. The second shortcoming of this mode is the need for additional equipment, such as a microinfusion pump. Finally, a direct comparison of continuous flow and static direct immersion SDME has proved the latter to yield superior detection limits and precision [120,124].

Liquid-liquid-liquid microextraction is a three-phase mode which is best suited for the extraction of hydrophilic organic compounds, mostly polar semivolatiles, such as phenols, fatty acids or amines. It is a miniaturized form of extraction with back-extraction. In this mode, analytes are extracted from an aqueous sample to an organic solvent and simultaneously back-extracted from the organic solvent to the acceptor solution, usually a few microliters of an aqueous solution at the appropriate pH (Fig. 7). The organic solvent is therefore an interface between the two aqueous solutions. In order to achieve analyte isolation and enrichment, the acid-base properties of the analytes are used. For acidic analytes, the pH of the donor solution (sample) is adjusted to a low value so that ionization of the analytes is suppressed and they can be extracted as neutral species into the organic solvent. At the same time, the pH of the acceptor solution is maintained at a high value to promote ionization of the analytes. This way, the analytes are converted into ionic species which are excluded from the liquid organic membrane and therefore accumulate in the acceptor solution. In practical implementation, a Teflon ring [125] or a small volumetric flask are used for LLLME, which makes the experimental setup very simple. An organic solvent forms a layer on top of sample and a microdrop of the acceptor solution is immersed into the organic solvent layer. An organic solvent used in liquid-liquid-liquid microextraction must be immiscible with water and have a density lower than water. Since the extract in LLLME is an aqueous solution, this mode is directly compatible with reverse-phase HPLC and capillary electrophoresis, and these two techniques of final determination

have been used exclusively in analytical procedures. Most common applications of LLLME include extraction of drugs from physiological fluids or water [6,126–130] and aromatic amines or phenols from water [125,131–132].

A recent modification of liquid–liquid–liquid microextraction avoids the use of a microsyringe as supporting device [20]. Instead, a large aqueous droplet is freely suspended at the top-center position of a layer of immiscible organic solvent, which is placed on top of a stirred aqueous sample. According to the authors, this configuration improves mass transfer and results in a reduced equilibration time.

4. Experimental parameters affecting DI-SDME and HS-SDME

There are to date approximately 600 research and application papers dealing with solvent microextraction, and the single drop modes account for more than half of these publications [14]. With this large available database, it is possible to identify the important parameters that affect the rates and efficiencies of SDME extractions. Each of the factors listed below will be briefly addressed here.

1. Analyte properties (volatility, polarity and ionization)
2. Extraction solvent properties
3. Extraction solvent purity
4. Syringe
5. Drop volume
6. Agitation
7. Ionic strength (salting out effect)
8. Temperature
9. Sample volume and headspace volume
10. Automation

4.1. Analyte properties

As discussed in preceding sections, the properties of the analyte and the matrix it is in will determine whether direct immersion (DI-SDME) or headspace (HS-SDME) extraction is appropriate. Thus, one must consider the volatility (boiling point), ionization (for acids and bases) and polarity of the analyte and matrix. These properties in turn affect two very important parameters, the organic extracting solvent/water distribution constant (K_{ow}) and the air/water distribution constant (K_{aw}). A detailed discussion of these important parameters can be found in the literature [133]. HS-SDME is appropriate for most polar and nonpolar, lower molecular weight, volatile and semivolatile compounds. Direct immersion (DI-SDME) extraction is appropriate for nonpolar or moderately polar higher molecular weight, semivolatile chemicals. Highly polar chemicals may need to be derivatized to ensure recovery, especially when the matrix is aqueous. Examples of typical applications for HS and DI were described in Section 3.

4.2. Solvent properties

A common misconception is that there are only a limited number of useful solvents that can be used for solvent microextraction. In fact, more than 2 dozen solvents have been successfully used for various SME modes, and this does not include solvent combinations and solvents such as water with extraction enhancers (complexing agents, derivatizing agents and pH control) [14]. There are some important restrictions on the selection of a particular extracting solvent, however. When extracting from an aqueous solution, the solvent needs to be water immiscible. The solvent needs to have a boiling point high enough that it will not evaporate, but also

appropriate for the chromatographic system. It needs to have a high enough viscosity to cling onto the tip of a syringe needle, but not so viscous that the diffusion rate of analyte into the drop affects extraction time significantly. The intermolecular attraction characteristics of the solvent must also be compatible with the analyte being extracted. The most important interaction types are London dispersion forces (van der Waals forces), permanent dipole–dipole interactions, and hydrogen bonding. Thus, 1-octanol has been a popular solvent for SDME, since it has all three interaction capabilities. Furthermore, it has a relatively high-boiling point, relatively low water solubility and moderate viscosity. Traditional separatory funnel extraction solvents, such as diethyl ether, methylene chloride, chloroform and ethyl acetate, on the other hand, are not useful as extracting solvents for SDME because they are too volatile and too water-soluble. These types of solvents have been used successfully in a limited number of cases, however, as solvent modifiers, added to nonpolar, water-immiscible solvents such as toluene [32]. This is a fruitful area for future research.

Compatibility of an extracting solvent with the analytical method is also crucial. As indicated in Section 3, the most widely used instrumentation for SDME extract analysis is GC. In general, volatile analytes are extracted with higher boiling solvents, such as tetradecane or 1-octanol. The extract must be injected using an inlet split of 10/1 to 50/1 to obtain sharp, resolved peaks. Semivolatile analytes are extracted with lower boiling solvents, such as *o*-xylene, using splitless injection.

If reverse-phase HPLC is used, samples extracted with water-immiscible solvents such as toluene, must be exchanged or diluted with a solvent compatible with HPLC such as acetonitrile [118,134]. As an alternative, an ionic liquid [94,103,104,135] or water containing a modifier solvent can be used with reverse-phase HPLC [96] or capillary electrophoresis [100] directly. A nonpolar solvent could also be used directly for normal phase HPLC.

4.3. Extracting solvent purity

Solvent purity is one of the most important factors in SDME, especially when analyzing very dilute solutions. Commercial high purity solvents may contain impurities that will interfere with analyte analysis. These impurities may include solvent analogs, such as xylene present in toluene, or oxidation products, such as aldehydes and alcohols in decane. It may be necessary, for trace analysis, to doubly vacuum-distill a solvent and then store it in a freezer. Standard high grade solvents may be appropriate for higher concentration solutions or if impurities do not interfere with the analysis. Trace impurities can be useful, however, since they can be used as internal standards in the solvent. The concentration of the internal standard does not have to be known precisely, just present at a constant level for a series of analyses. The standard, which can also be added to the solvent, is used to monitor the integrity of the drop. This is especially important when using an autosampler, to ensure that the drop is not lost during sampling. The standard can also be used to account for small sample-to-sample variations in drop size and solvent wicking on the needle.

4.4. Syringe

The most effective syringe for SDME is a standard GC microsyringe. The drop must cling to the tip, without wicking up the exterior of the syringe needle. This requires a maximum needle tip surface area. The standard Hamilton #2 curved bevel syringe tip provides the greatest surface area, and approximately 90–95% of the drop can be withdrawn into the syringe following the extraction. A straight edge bevel GC syringe allows only 80–85% withdrawal and an HPLC syringe very little withdrawal unless a drop size less than 0.5 μ L is used. If HPLC analysis is used, the drop

must be diluted with additional solvent in a sample vial and/or exchanged with an HPLC-compatible solvent and then an HPLC syringe used for injection.

4.5. Drop volume

As indicated in the theory section, the amount of analyte extracted increases with drop volume. Unfortunately, a maximum drop volume for a standard syringe needle is 2–3 μL . A drop size larger than 3 μL is unstable and the drop may fall off the needle, especially when using direct immersion SDME. (The hollow fiber approach, which is outside the scope of this review, is discussed elsewhere in this issue, and is one solution to this problem.) It must be remembered that even high-boiling organic solvents have some volatility and many water-immiscible solvents actually have finite water solubility. As a consequence, a portion of the drop will evaporate and/or dissolve in the sample matrix, especially when elevated extraction temperatures, long extraction times and vigorous agitation of the sample are used. When using direct immersion extraction, the sample may contain salts or soluble macromolecules which would be harmful to the analytical instrumentation used. If a 1- μL drop was used and 1 μL of liquid were withdrawn into the syringe, chances are good that the solvent would be contaminated with the sample matrix. Even when using headspace SDME, the volume of the solvent withdrawn into the syringe may be variable, due to loss of solvent by evaporation/solubility and wicking onto the surface of the needle. Difficulties with drop size variations and solvent wicking are minimized if the drop size used is 0.2–0.5 μL larger than the amount of solvent withdrawn into the syringe following extraction. At high extraction temperatures (50–80 °C), it may be necessary to increase this value.

4.6. Agitation

As indicated earlier, sample agitation is important for reducing extraction time. Three sample agitation methods are available: stirring, vibration and vortexing. Stirring, using a magnetic stir bar, is effective with stirring rates of 300–600 rpm for DI-SDME and 500–1000 rpm for HS-SDME. The limitations of higher stirring rates are the dislodgement of the drop by the sample solution or splashing when using headspace. Vibration and vortex stirring, used with some autosamplers, are also effective, with the limitation that the agitation cannot occur while the drop is exposed at the needle tip. Sample agitation before extraction can lead to reproducible results, especially for HS-SDME, but these techniques are most effective when a computer-controlled autosampler is used, along with dynamic sampling, so the solvent is contained within the syringe needle during the agitation.

Agitation of the extracting solvent can also lead to decreased extraction time (though not extraction efficiency), since a rate-limiting step in the extraction process is often transfer of the analyte from the surface into the bulk of the drop. This is facilitated by continuous renewal of the drop surface. As discussed in Section 2.2, stirring the sample during direct immersion SDME may result in convection within the drop, but dynamic extraction has also been shown to significantly decrease extraction time [22]. In dynamic extraction, reproducibility and extraction efficiency depend on exact repetition of several factors: number of cycles, sample volume drawn into the syringe, extracting solvent volume, plunger speed, dwell time at maximum plunger withdrawal, and exposure time (or dwell time) for the exposed drop in contact with the sample. Plunger movement must be precise and at an optimal rate for 30–90 repetitions. While this can be done manually or with a mechanical device, accurate repetitive sampling requires the use of a computer-controlled autosampler [136].

4.7. Ionic strength (salting out effect)

Salting out is a time-tested technique for increasing extraction efficiency, especially for moderately polar and low molecular weight volatile chemicals. High ionic strength can also decrease the solubility of the extracting solvent. The effect of ionic strength on analyte water solubility and thus the K_{ow} and the Henry's constant (K_{aw}) is exponential. It is thus best to accurately weigh added salt and to use a near, but not saturated concentration of salt. Saturated salt solutions may contain undissolved particles which can dislodge the drop in DI-SDME. If halide exchange is a concern, anhydrous sodium sulfate may be used in the same weight/volume concentrations as sodium chloride. Sodium sulfate must be used with caution, however, since at high concentrations it can crystallize as the hydrate.

Adding salt is not beneficial for increased extraction of nonpolar semivolatile analytes, such as the PAHs, which have K_{ow} values greater than 1000. Addition of salt may, however, be useful for minimizing drop loss when DI-SDME is used.

There are a few reported cases where addition of salt is detrimental in DI-SDME [28,137,138]. This may be due to changes in the viscosity or surface tension of the water sample.

4.8. Temperature

As previously indicated, temperature control is important in SDME extractions, especially for headspace extractions. The organic solvent/water distribution constant (K_{ow}) is only weakly affected by temperature, but the air/water constant (K_{aw}) and the organic solvent/air constant (K_{oa}) are strongly dependent on temperature. Typically, for nonpolar analytes, increasing the temperature of the water solution (or solid matrix) increases headspace concentration. However, some moderately polar analytes become much more water-soluble at elevated temperatures and headspace concentrations therefore decrease with increasing temperature. In addition, solubility in the extracting solvent may decrease with increasing temperatures, decreasing the efficiency of extraction. Therefore, a compromise extraction temperature must be found, especially when extracting samples containing multiple analytes, and extraction time must be minimized to decrease the temperature effect on the drop. One solution to the decreased solubility of analyte in the drop at elevated temperatures is to use a chilled syringe needle, which can be accomplished with a laboratory built [52] or commercial device.

4.9. Sample volume and headspace volume

Many researchers have employed relatively large (5–30 mL) volumes of aqueous sample and large headspace volumes (ranging up to 80% of the vial volume). SDME theory clearly shows that this can be counterproductive, since the maximum amount of analyte extracted is dependent on the K_{ow} and K_{aw} values and larger samples require longer extraction times. In general, aqueous sample volumes of 1–4 mL are optimal for analytes with K_{ow} values less than 1000. Analytes with large K_{ow} values, such as the halogenated pesticides and the PAHs, will yield greater extracted amounts with increased sample sizes up to 30–40 mL. Larger volumes, of course, will require much longer extraction times.

Theoretical calculations further indicate that the headspace, for both DI-SDME and HS-SDME, should be kept to a practical minimum to maximize extraction efficiency. For a 2-mL vial, a sample size of 1–1.5 mL and a headspace of 0.5–1 mL are appropriate. For a 4-mL vial a sample size of 3 mL and a headspace of 1 mL are appropriate. The headspace should be no larger than necessary to allow the drop to be suspended over the stirred sample for HS-SDME

and only enough to avoid sample contact with the septum cap for DI-SDME.

4.10. Automation

Very good accuracy and reproducibility can be achieved by a skilled analyst using manual DI-SDME and HS-SDME extraction. However, when analyzing large numbers of samples or using dynamic extraction, a computer-controlled autosampler is a necessity. The autosampler can perform all steps of DI-SDME and HS-SDME, including agitation, temperature control, syringe plunger movement, cleaning and injection, with accuracy and reproducibility approaching that of a skilled analyst. Several automated procedures involving direct immersion [24,41,136] and headspace SDME [16,17,24,136] have been developed.

5. New developments and trends

A new era for solvent microextraction began in 2003 with the introduction of ionic liquids as extracting solvents [94]. Since then, a considerable number of published analytical procedures made use of ionic liquids, including direct immersion [33,135,139–146] and headspace SDME [33,50,51,54,99,101–105]. Ionic liquids have many unique properties, such as negligible vapor pressure, excellent thermal stability, and high viscosity, which allow the use of stable large drops, thus increasing extraction yield. Their polarity is adjustable through selection of the appropriate cations and anions. Consequently, their miscibility with water and organic solvents, viscosity, and extractability of organic analytes are tunable, which accounts for their versatility and may result in a much larger use in the future. Until recently, the use of ionic liquids in analytical procedures involving solvent microextraction has been limited to HPLC, capillary electrophoresis, and spectroscopic techniques as the final determination methods as a result of their nonvolatility. However, some recent papers describe several approaches which make ionic liquids compatible with gas chromatography. One of them involves the use of a removable interface enabling direct introduction of the ionic liquid extract into a GC–MS system while preventing the ionic liquid from entering the column [50,51,54,139] (Fig. 8). The second approach employs a commercially available thermal desorption system to thermally desorb analytes from ionic liquids and introduce them into the GC [102]. In the third approach, analytes are desorbed from the ionic liquid in the injection port of gas chromatograph, and the ionic liquid is then drawn back into the microsyringe [101]. These approaches may significantly extend the applicability of ionic liquids in single drop microextraction.

So far, the two major areas of application of solvent microextraction have been environmental (61%) and clinical & forensic (21%) analysis. The scope of applications has been recently extended to include more solid samples, particularly plants and their parts, by the addition of a preliminary step prior to microextraction proper. A novel technique, called hydrodistillation–headspace solvent microextraction, used primarily to isolate essential oils from plants and their parts, couples water extraction with solvent microextraction [62,63,67,68,147–149]. In this technique, a small amount of plant material (typically 0.7–4 g) is mixed with water and subjected to hydrodistillation. A microdrop of a high-boiling solvent is suspended in the headspace of a hydrodistilling sample. This arrangement results in a short extraction time (10–20 min, including refluxing), and consumes a small amount of plant material.

Microwave distillation is another novel preliminary step suitable for samples of plant material, combining microwave heating and dry distillation at atmospheric pressure [60]. Plant material is placed in a microwave reactor without any water or organic solvent

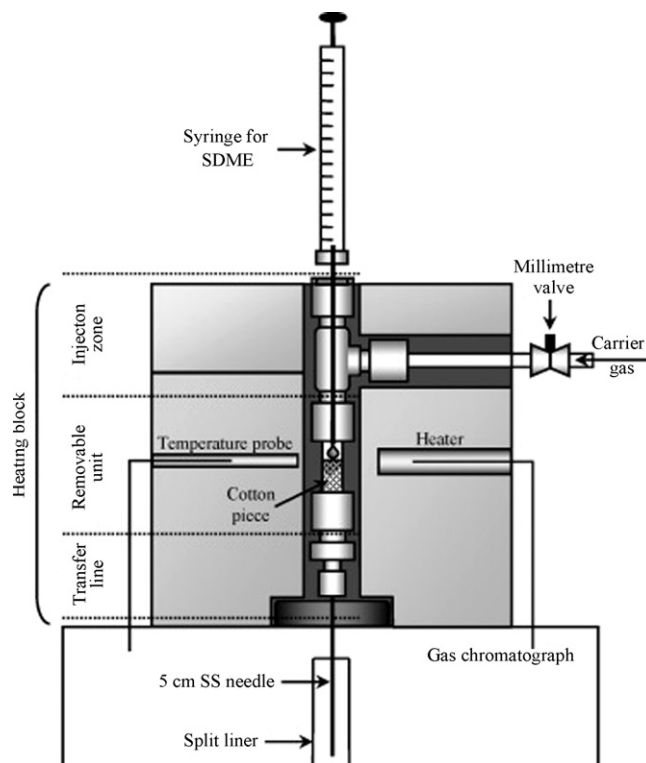


Fig. 8. Interface used for ionic liquid based SDME combined with gas chromatography. Reprinted from: [54], Copyright (2008), with permission from Elsevier.

added. The heating of the water present in plant cells results in their disruption and release of essential oils that are evaporated by the water of the plant material and extracted by headspace SDME. A cooling system outside the microwave oven condenses the distillate. Compared to traditional alternatives, microwave distillation offers reduced extraction time and energy savings.

The applicability of SDME has been further extended by converting those analytes, which cannot be directly extracted by SDME, into extractable species through derivatization reactions. These include, among others, inorganic species (metal ions, anions) and highly polar volatile organic compounds. Derivatization reactions employed in solvent microextraction have been reviewed recently [150,151], and are discussed in a monograph on solvent microextraction published in October 2009 [14]. The extension of analytical procedures involving SDME to inorganic species seems to be particularly attractive. Examples include derivatization followed by microextraction of heavy metal ions by direct immersion [143–145,152–156], continuous flow [157], or headspace SDME [85]; microextraction of derivatized inorganic anions, such as periodate, iodate, bromate, iodide, bromide, cyanide, and sulfide [107,109,158–159], or neutral species, including iodine, nitric oxide, chlorine, and ammonia [78,160–162].

Every new analytical procedure involving solvent microextraction has to be optimized by adjusting extraction parameters, including sample volume, headspace volume (in three-phase mode), organic solvent type and volume, agitation conditions, temperature, pH, extraction time, and sample ionic strength, in such a way as to maximize extraction yield. Most method development procedures described in the literature have used the *one-variable-at-a-time* approach, where just one parameter is varied and all the other parameters are kept constant. This approach is inefficient and requires a large number of experiments. Recently, however, a number of solvent microextraction procedures have been optimized using experimental design. The predominant designs involved simultaneous design making use of response surface

methodology (RSM). Prior to applying the RSM, screening experiments were carried out to find which experimental variables influenced the response significantly, with factorial designs being most common. Experimental design has been applied in optimizing direct immersion [25,32,135,163] and headspace SDME [47,83,104,126,149,163–165].

Solvent microextraction is a highly versatile sample preparation method not only because it can be used for practically all classes of analytes, but also because it is compatible, directly or after solvent replacement, with a wide range of final determination techniques. Gas chromatography is by far the most common final determination technique used in combination with single drop microextraction, followed by high-performance liquid chromatography. GC is the preferred technique for the separation and determination of volatile and semivolatile analytes, while HPLC is the method of choice in the assays involving nonvolatile analytes, such as ionizable analytes. Gas chromatography is compatible with direct immersion SDME, in which nonpolar volatile organic solvents are typically employed. HPLC and capillary electrophoresis are directly compatible with liquid–liquid–liquid microextraction and with headspace SDME using highly polar solvents, ionic liquids or aqueous solutions. HPLC and CE can also be coupled with direct immersion SDME following solvent replacement. For organic analytes, atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry has also been used with SDME, since the small extracting solvent volume is compatible with MALDI-MS.

Following solvent microextraction, inorganic analytes, such as metal or metalloid ions, are typically detected by one of the spectroscopic techniques: UV/vis spectrophotometry, spectrofluorimetry, electrothermal or flame atomic absorption spectrometry, and inductively coupled plasma (ICP)—optical emission spectroscopy or ICP-mass spectrometry. Recently, miniaturized modes of two common spectroscopic techniques coupled with SDME have been reported. The first one involved the determination of acid labile sulfide fraction in water by microvolume turbidimetry following headspace SDME of hydrogen sulfide [109]. The second procedure used fiber optics-based cuvetteless CCD-array microspectrophotometry to determine thiols, chlorine, ammonia, and iodine following direct immersion or headspace SDME [78].

6. Conclusions

The trend toward miniaturization of sample preparation methods has resulted in the development of several techniques which may be described as solvent microextraction (SME). Single drop microextraction modes constitute a significant part of SME. They use much smaller volumes of organic solvents than classical liquid–liquid extraction, permit automation and higher sample throughput, and provide high extraction efficiency. Several areas of growth in the number of SDME applications can be predicted, including environmental, clinical and forensic. Further commercialization and automation of SDME equipment and procedures is likely. The advantages of automated SDME procedures include improved precision, increased sample throughput due to reduction of extraction times when using dynamic SDME, and unattended operation.

A more expanded use of ionic liquids can be anticipated, owing to their unique properties and compatibility with a variety of final determination techniques. Ionic liquids can prove especially valuable for the extraction of strongly polar analytes.

Single drop microextraction can find use in most sample preparation procedures, with analytes ranging from volatile organic compounds, through polar and nonpolar semivolatiles, to ionic compounds and metal ions, as long as suitable solvents and equip-

ment are available. In its simplest implementation, manual direct immersion or headspace mode, no equipment is needed other than that already available in any analytical laboratory.

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